Summary Basis for Regulatory Action

Date:	August 17, 2022
From:	Jakob Reiser, PhD, Chair of the Review Committee, Office of Tissues and Advanced Therapies (OTAT), Division of Cellular and Gene Therapies (DCGT)
BLA STN:	BL 125717/0
Applicant:	bluebird bio, Inc.
Submission Receipt Date:	September 20, 2021
Action Due Date:	August 19, 2022
Proper Name:	betibeglogene autotemcel
Proprietary Name:	ZYNTEGLO
Indication:	Treatment of adult and pediatric patients with β-thalassemia who require regular red blood cell (RBC) transfusions

Recommended Action: The Review Committee recommends approval of this product.

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Director, Office of Tissues and Advanced Therapies

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Acting Director, Office of Compliance and Biologics Quality

Discipline Reviews	Reviewer / Consultant - Office/Division
CMC • CMC Product (OTAT/DCGT and OCBQ/DBSQC) • Facilities review (OCBQ/DMPQ) • Establishment Inspection Report (OCBQ/DMPQ and OTAT/DCGT) • QC, Test Methods, Product Quality (OCBQ/DBSQC)	Jakob Reiser, PhD, CBER/OTAT/DCGT Anna Kwilas, PhD, CBER/OTAT/DCGT Tal Salz, PhD, CBER/OTAT/DCGT Brian Stultz, MS, CBER/OTAT/DCGT Michael (Brad) Strader, PhD, CBER/OVRR/DBPAP Andrew Timmons, PhD, CBER/OTAT/DCGT Massoud Motamed, PhD, CBER/OTAT/DCGT Seth Schulte, MS, CBER/OCBQ/DBSQC Claire Wernly, CBER/OCBQ/DBSQC Esmeralda Alvarado Facundo, PhD, CBER/OCBQ/DBSQC Varsha Garnepudi, PhD, CBER/OCBQ/DBSQC Most Nahid Parvin, PhD, CBER/OCBQ/DBSQC Tao Pan, PhD, CBER/OCBQ/DBSQC Wei Wang, PhD, CBER/OCBQ/DMPQ Carolina Panico, MD, PhD, CBER/OTAT/DCGT
Pre-license Inspection	Wei Wang, PhD, CBER/OCBQ/DMPQ Jakob Reiser, PhD, CBER/OTAT/DCGT Anna Kwilas, PhD, CBER/OTAT/DCGT Christian Lynch, CBER/ORO/DROP
Clinical • Clinical (OTAT/DCEPT) • Postmarketing safety epidemiological review (OBPV/DPV) • BIMO	Karl Kasamon, MD, CBER//OTAT/DCEPT Firoozeh Alvandi, MD, CBER/OBPV/DPV/PB Colonious King, CBER/OCBQ/DIS
Statistical • Clinical data (OBPV/DB) • Non-clinical data	Jiang Hu, PhD, CBER/OBPV/DB
Non-clinical/Pharmacology/Toxicology • Toxicology (OTAT/DCEPT) • Developmental toxicology (OTAT/DCEPT) • Animal pharmacology	Melek Sunay, PhD, CBER/OTAT/DCEPT
Clinical Pharmacology	Xiaofei Wang, PhD, CBER/OTAT/DCEPT
Labeling • Promotional (OCBQ/APLB) • Carton/Containers (OTAT/DRPM and OTAT/DCGT)	Benjamin Cyge, CBER/OCBQ/DCM/APLB Cara Pardon, MS, CBER/OTAT/DRPM Mona Badawy, CBER/OTAT/DRPM Jakob Reiser, PhD, CBER/OTAT/DCGT Anna Kwilas, PhD, CBER/OTAT/DCGT

	Tal Salz, PhD, CBER/OTAT/DCGT Brian Stultz, MS, CBER/OTAT/DCGT Andrew Timmons, PhD, CBER/OTAT/DCGT
Other Review(s) not captured above categories, for example: • Consults • Devices • Software • Human Factors • FONSI	Oluchi Elekwachi, PharmD, CBER/OCBQ/DCM/APLB Zhaobo (Felix) Fan, PhD, CDRH/OSEL Caroline Pinto, PhD, CDRH/OSEL Tianjiao Dai, PhD, CBER/OBPV/DB
Advisory Committee Summary	Advisory Committee Meeting was held on June 9-10, 2022,

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1. Introduction

bluebird bio, Inc. submitted a Biologics License Application (BLA), STN BL 125717, for betibeglogene autotemcel (beti-cel). The proprietary name is ZYNTEGLO. ZYNTEGLO is an autologous hematopoietic stem cell-based gene therapy indicated for treatment of adult and pediatric patients with β-thalassemia who require regular red blood cell (RBC) transfusions. β-thalassemia is a rare disease manifested by chronic, severe anemia, accompanied by life-threatening complications. Despite currently available treatments, a substantial unmet medical need remains.

ZYNTEGLO consists of an autologous CD34+ cell-enriched population that contains the patient's own hematopoietic stem cells (HSCs) transduced ex vivo with the BB305 lentiviral vector (LVV) encoding $\beta^{A\text{-}T87Q}$-globin. ZYNTEGLO is supplied frozen in 20 mL fluoro-ethylene-propylene bags as a suspension for intravenous infusion. Each bag contains between 2×10^6 and 20×10^6 cells/mL (1.7 to 20×10^6 CD34+ cells/mL), frozen in approximately 20 mL of cryopreservation solution. The minimum dose is 5.0×10^6 CD34+ cells/kg patient weight.

Each patient undergoes HSC mobilization with granulocyte colony stimulating factor (G-CSF) and plerixafor in combination, followed by apheresis to harvest the cells. The collected cells are shipped to the manufacturing site where CD34+ cells are selected and then transduced with BB305 LVV to manufacture ZYNTEGLO. After myeloablative conditioning and ZYNTEGLO infusion, transduced HSCs engraft in the bone marrow and differentiate to reconstitute the hematopoietic system, including red blood cells (RBCs) that contain HbA^{T87Q} to treat the patient's β-thalassemia.

This document summarizes the basis for regular approval of ZYNTEGLO. Data from 41 subjects from two adequate and well controlled Phase 3 studies, Studies HGB-207 and HGB-212, provide the primary evidence of safety and effectiveness in this BLA. The recommendation for approval is based on demonstration of transfusion independence in adult and pediatric patients with all genotypes of transfusion-dependent thalassemia (TDT). The major risks of treatment with ZYNTEGLO include delayed platelet engraftment and thrombocytopenia.

The review team recommends regular approval of this BLA with a safety post-marketing requirement (PMR) consisting of a post-marketing observational study to assess the long-term safety of ZYNTEGLO, including risk of insertional oncogenesis, a PMR for extractable and leachable testing on the final container, and eight post-marketing commitments (PMC) related to product quality assessment.

2. Background

Beta (β)-thalassemia is a group of inherited hemoglobinopathies caused by mutations in the β-globin gene leading to reduced or absent expression of β-globin in erythropoietic cells. The resulting non-alpha globin chain imbalance in erythrocyte progenitors causes precipitation of unpaired alpha-globin chains, leading to destruction of erythroid precursors and ineffective erythropoiesis and peripheral hemolysis. Due to resulting

impairment of hepcidin regulation, β-thalassemia leads to increased iron absorption and progressive iron overload.

Transfusion-dependent β-thalassemia (TDT) is a rare hemoglobinopathy associated with life-long anemia requiring frequent red blood cell (RBC) transfusions, and is complicated by organopathy related to iron overload, reduced quality of life, and shortened survival. Allogenic hematopoietic stem cell transplantation (AHSCT) using HLA-matched, related donors results in the best outcomes, but few patients have such donors available. Despite supportive care with transfusions, iron chelators, and luspatercept (an erythroid maturation agent), there continues to be a significant unmet need for patients with this disease.

ZYNTEGLO is a biological product containing genetically modified autologous hematopoietic stem cells (HSCs) transduced with BB305 lentiviral vector (LVV) encoding $\beta^{A\text{-}T87Q}$-globin. $\beta^{A\text{-}T87Q}$-globin expression is designed to correct the β/α-globin imbalance in erythroid cells of patients with β-thalassemia who require regular RBC transfusions and has the potential to increase total hemoglobin (Hb) to normal levels and eliminate dependence on chronic RBC transfusions.

Regulatory History

Regulatory Events / Milestones	Date
1. Initial IND submission (BB-IND 15324)	December 19, 2012
2. IND allowed to proceed	January 17, 2013
3. Fast Track designation granted	January 31, 2013
4. Orphan Drug designation granted (ODD # DRU-2013-3905)	March 18, 2013
5. Breakthrough Therapy designation granted	January 29, 2015
6. Rare Pediatric Disease designation granted (RPD-2018-193)	November 30, 2018
7. Pre-BLA meeting	November 7, 2019
8. BLA submission – final module of rolling BLA received	September 20, 2021
9. BLA filed	November 18, 2021
10. Mid-Cycle Communication	January 18, 2022
11. Pre-license Inspection	February 14 -18, 2022
12. Late-Cycle meeting	May 23, 2022
13. Major Amendment	January 14, 2022
14. Action Due Date	August 19, 2022

3. Chemistry Manufacturing and Controls (CMC)

This biologics license application (BLA) provides an adequate description of the manufacturing process and characterization of betibeglogene autotemcel (beti-cel; ZYNTEGLO). The CMC review team concludes that the manufacturing process, along with associated test methods and control measures, is capable of yielding a product with consistent quality characteristics.

a. Product Quality

To manufacture ZYNTEGLO, autologous hematopoietic progenitor cells obtained by leukapheresis (Hematopoietic Progenitor Cells Apheresis [HPC-A]) are collected from each patient at a Qualified Treatment Center (QTC) for use in the manufacture of the drug product. The apheresis material is shipped to Lonza-Houston, Inc. for (b) (4) drug product manufacturing. CD34+ cells are selected using the (b) (4) and transduced with BB305 LVV to manufacture ZYNTEGLO. To do this, the HPC-A are enriched for cells expressing CD34 by (b) (4) The ZYNTEGLO drug product consists of autologous CD34+ cells transduced with BB305 LVV, suspended in (b) (4) cryopreservation solution containing 5% dimethylsulfoxide (DMSO).

The BB305 LVV is derived from HIV-1 and is replication-incompetent and self-inactivating (SIN). The packaged genomic viral RNA contains no viral genes and less than (b) (4) of the HIV-1 genome. The BB305 LVV encodes $\beta^{A\text{-}T87Q}$-globin, a β-globin derivative that can be distinguished from normal β-globin by reversed phase high performance liquid chromatography (HPLC).

BB305 LVV is manufactured using a third-generation vector design in which the necessary viral genes are expressed from separate plasmids to minimize the risk of generating replication-competent lentivirus. Essential proteins are encoded by three packaging plasmids used in the transfection of HEK293T cells during BB305 LVV production.

Vials of BB305 LVV are stored at ≤-65°C until required for use in ZYNTEGLO manufacturing. After release, the vials may be shipped to a long-term storage facility or to a ZYNTEGLO manufacturing site. Vials are packed in shipping containers containing dry ice to maintain temperatures of ≤-65°C. Temperature monitoring is performed during transit of all BB305 LVV shipments.

Manufacturing Control Strategy

Manufacturing process consistency is assured through (1) raw material and reagent qualification programs, (2) in-process monitoring, (3) in-process control testing (4) lot release and stability testing, (5) manufacturing process validation and continuous process verification, and (6) traceability by using a chain-of-identity system. The critical process parameters and critical quality attributes of ZYNTEGLO were defined through process characterization and validation studies. Methods used to determine product quality were validated except for robustness studies for some tests, which will be resolved through postmarketing commitments (PMCs). Manufacturing and testing

comply with Current Good Manufacturing Practices. The shelf life for the BB305 LVV stored at ≤-65°C is (b) (4) . The shelf life for the ZYNTEGLO drug product stored in the vapor phase of liquid nitrogen at ≤ -140°C is 12 months.

Comparability assessments:

During the review of the BLA, comparability of products which were manufactured at different manufacturing facilities was assessed to enable pooling of clinical data. The Applicant evaluated three different manufacturing processes, Processes 0, 1, and 2, in their clinical development program. Process 2 is the commercial process. Earlier manufacturing processes were not considered comparable to the commercial process. Briefly, three manufacturing facilities were used in the two Phase 3 studies HGB-207 and HGB-212, LHI(b) (4) (commercial site), LHI(b) (4) (reference site), and (b) (4). The comparability assessment that was provided in the initial BLA was incomplete and the acceptance criteria were not adequately justified. During the review period, the Applicant provided a supplemental comparability analysis, with consideration of FDA comments. The supplemental comparability analysis demonstrated equivalence for the majority of critical quality attributes (CQAs), the exceptions being %CD34+ cells for products manufactured at (b) (4) and VCN, %LVV, and $\beta^{A\text{-}T87Q}$-globin protein expression for products manufactured at LHI(b) (4) relative to products manufactured at the LHI(b) (4) reference site. However, FDA determined that the observed differences were not biologically or clinically significant, and the clinical product manufactured with the commercial process was considered comparable across manufacturing sites.

PMR/PMCs

The following issues were identified but could not be resolved during the review cycle. These issues will be resolved through PMR/PMCs by March 30, 2024.

Several unresolved issues were present for the final container (b) (4)), which is not an approved or cleared cryopreservation bag (see 3.e below). To resolve these issues bluebird bio committed to perform (b) (4) testing and (b) (4) testing on the (b) (4) bag to ensure bag integrity. Due to potential safety concerns, bluebird bio will also perform additional testing to support the extractable data provided during the review and leachable testing on the final container as a PMR.

Several issues with the drug product lot release tests submitted in the BLA could not be resolved. These included many tests that were not validated for (b) (4) , such as, (b) (4) assays. The BLA review noted that some samples for lot release tests were taken on the drug substance and not the drug product. bluebird bio will evaluate the sampling process and revise for the following tests: (b) (4) bluebird bio also committed to revise the sterility testing plan.

Additional post-marketing commitments include supplemental (b) (4) data for the drug product and feasibility of a leachable study on the (b) (4) manufacturing bag.

b. Testing Specifications

The analytical methods and their validations and/or qualifications reviewed for the ZYNTEGLO drug substance and drug product were found to be adequate for their intended use.

ZYNTEGLO Specifications are listed below:

Final Commercial ZYNTEGLO Release Specifications

Quality Attribute	Test	Method	Acceptance Criteria
Potency and Strength	Vector Copy Number (VCN)	(b) (4) qPCR	(b) (4)
	% LVV+ Cells	(b) (4) qPCR	(b) (4)
	(b) (4)	(b) (4)	(b) (4)
	(b) (4)	(b) (4)	(b) (4)
	Colony Forming Cells (CFC)	(b) (4)	(b) (4)
			(b) (4)
	$\beta^{A\text{-}T87Q}$-globin Quantitative Protein Expression	(b) (4)	(b) (4) $\beta^{A\text{-}T87Q}$-globin (relative to (b) (4)
Identity	$\beta^{A\text{-}T87Q}$-globin Quantitative Protein Expression		(b) (4)
	(b) (4)	(b) (4)	(b) (4)
Purity and Content	(b) (4)		(b) (4)
	(b) (4)	(b) (4)	(b) (4)
	Total Cell Concentration		2.0E+06 to 20E+06 total cells/mL
	(b) (4)		(b) (4)
Safety	Sterility	(b) (4)	No Growth
	Endotoxin	(b) (4)	(b) (4)
	Mycoplasma	(b) (4)	None Detected
Quality	Appearance	Visual assessment	Colorless to white to red, including shades of white or pink, light yellow, and orange.

c. CBER Lot Release

An exemption has been granted from CBER Lot Release testing, including no requirement for submission of product samples to CBER. The basis for this decision is that ZYNTEGLO is an autologous product; as such, each lot will treat a single patient. Failure of a single lot will have minimal potential impact on public health.

d. Facilities Review / Inspection

Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. Inspection histories and activities for facilities involved in the manufacture of ZYNTEGLO are summarized in the table below.

Manufacturing Facilities for ZYNTEGLO

Name/Address	FEI Number	Waiver or Inspection	Justification and Results
(b) (4) (b) (4) *, BB305 LVV, manufacturing and testing*	(b) (4)	Waived	ORA (b) (4) VAI
Lonza Houston, Inc. 14905 Kirby Drive Houston, TX 77047 USA *beti-cel* (b) (4) *drug product manufacturing and testing*	3013629214	PLI	CBER February 14 -18, 2022 NAI
(b) (4) *Drug product release testing*	(b) (4)	Waiver	ORA (b) (4) VAI
(b) (4) *Drug product release testing*	(b) (4)	Waiver	ORA (b) (4) VAI
(b) (4)	(b) (4)	Waiver	ORA (b) (4)

(b) (4) *Drug product release testing*			NAI
(b) (4) *Drug product release testing*	(b) (4)	Waiver	ORA (b) (4) VAI

CBER conducted a pre-license inspection (PLI) of the Lonza Houston, Inc. facility in February 2022. No Form FDA 483 was issued, and the inspection was classified as No Action Indicated (NAI).

Office of Regulatory Affairs (ORA) performed a surveillance inspection of (b) (4) . All 483 issues were resolved, and the inspection was classified as voluntary action indicated (VAI).

ORA performed a surveillance inspection of (b) (4) . All 483 issues were resolved, and the inspection was classified as VAI.

ORA performed a surveillance inspection of (b) (4) . All 483 issues were resolved, and the inspection was classified as VAI.

ORA performed a surveillance inspection of (b) (4) . No Form FDA 483 was issued, and the inspection was classified as NAI.

ORA performed a surveillance inspection of (b) (4) . All 483 issues were resolved, and the inspection was classified as VAI.

e. Container/Closure System

The container closure system (CCS) for ZYNTEGLO consists of a primary package container (a 20 mL (b) (4) Cryopreservation bag), a secondary package container (a (b) (4) bag), and a tertiary metal package container (cryocassette). The ZYNTEGLO drug product is filled into (b) (4) bag(s) (e.g., 1 bag for 20 mL DP, or 2 bags for 40 mL DP). The (b) (4) bag is sterile and ready-to-use (RTU, manufactured by (b) (4) . (b) (4) Following visual inspection, a product label is applied to the bag, and the product bag is placed inside of a sterile RTU (b) (4) (manufactured by (b) (4) The (b) (4) bag is (b) (4) . The product bag is inserted into a metal cassette that has a label containing both product and patient information. The container-closure integrity (CCI) was tested using a (b) (4) method by (b) (4) to determine that the primary package container is able to maintain the container integrity under normal use, storage, and transportation conditions.

The BLA was missing information about the (b) (4) Cryopreservation bag, which resulted in several PMCs and a PMR.

f. Environmental Assessment

The BLA included a request for categorical exclusion from an Environmental Assessment under 21 CFR 25.31(c). This request and supporting information provided by bluebird bio are acceptable to conclude that ZYNTEGLO poses a negligible risk to the environment or to the general public. There are no significant environmental or public health impacts posed by the product or its manufacturing. Data provided in the BLA indicates that the potential for recombination of the LVV is low. The potential for ZYNTEGLO to persist in the environment is negligible because these cells have stringent nutritional requirements for survival and therefore are not viable in the environment. The FDA concluded that this request is justified, and no extraordinary circumstances exist that would require an environmental assessment.

4. Nonclinical Pharmacology/Toxicology

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 $\beta^{A\text{-}T87Q}$-globin. In vivo POC studies showed that immunodeficient mice administered ZYNTEGLO displayed bone marrow engraftment (BME) and $\beta^{A\text{-}T87Q}$-globin expression.

In vivo pharmacology and toxicology studies were conducted to evaluate the activity and safety of BB305 LVV-transduced murine bone marrow 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 animals 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 or leukemia was within the reported range (15.7-25.3%) for radiation-associated lymphoma in C57BL/6 mice (Will et al. Mol Ther 15:782-791, 2007), and 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 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 ZYNTEGLO. Considering the product characteristics and safety profile, these studies are not warranted.

5. Clinical Pharmacology

The clinical pharmacology review team's recommendation for approval of ZYNTEGLO is based on review of data from the two ongoing Phase 3 studies HGB-207 and HGB-212, with supportive data from Phase 1-2 study HGB-204, and a population pharmacodynamic (PD) study.

Key clinical pharmacology findings are summarized below:

General Pharmacodynamics

- After infusion of ZYNTEGLO, lentiviral vector copy number in peripheral blood (PB VCN) levels increased rapidly over the first few months before reaching a plateau. At Month 6, the median (min, max) PB VCN level in the Phase 3 studies was 1.293 (0.16, 4.52) c/dg (N=37). PB VCN levels generally remained stable as of the data cut-off date for all studies, although high inter-subject variability of PB VCN kinetic profiles was observed.
- HbA^{T87Q} generally increased steadily after administration of ZYNTEGLO and stabilized by approximately Month 6 post-infusion. In the ongoing Phase 3 studies, subjects with TDT had a Month 6 median (min, max) HbA^{T87Q} of 8.74 (0.00, 12.01) g/dL (N = 35). HbA^{T87Q} remained durable with a median (min, max) of 8.80 (0.34, 12.43) g/dL at Month 24 (N = 30), demonstrating sustained expression of the $\beta^{A\text{-}T87Q}$ protein derived from irreversible integration of the $\beta^{A\text{-}T87Q}$-globin gene into long-term HSCs.
- Intrinsic factors, such as genotype, age at baseline, race, sex, and weight at baseline did not impact the steady-state levels and time to steady-state for PB VCN and HbA^{T87Q}.
- Analysis of hemoglobin (Hb) showed that HbA^{T87Q} was the major contributor to unsupported total Hb. The relative contributions of endogenous Hb may differ for individual subjects.
- At Month 6 post-infusion of ZYNTEGLO, the median (min, max) unsupported total Hb levels were 11.55 (8.4, 13.3) g/dL (N=22) and 10.20 (8.5, 13.2) g/dL (N=15) in Studies HGB-207 and HGB-212 respectively. The median unsupported total Hb levels were > 10 g/dL during the observation period of Phase 3 studies.

Dosing Characteristics and Responses

- Both drug product vector copy number (DP VCN) and the percentage of transduced cells in drug product (DP %LVV+ Cells) measure drug product characteristics related to transduction efficiency. Positive non-linear correlative relationship was observed between DP VCN and DP % LVV+ cells: DP %LVV+ cells increased rapidly with the increase of DP VCN at low DP VCN levels, and then plateaued at higher levels of DP VCN after reaching approximately 80% to 90% DP %LVV+ cells. There was a positive correlation between DP %LVV+ cells and PB VCN levels at Month 6 after ZYNTEGLO infusion. Population PD analysis also indicated that DP %LVV+ cells was the most important covariate impacting PB VCN levels.
- There was a significant correlative relationship between DP VCN and HbA^{T87Q} levels at Month 6 after infusion of ZYNTEGLO: HbA^{T87Q} increased gradually with DP VCN at lower DP VCN values, but at higher DP VCN values, HbA^{T87Q} levels plateaued at approximately 8 to 10 g/dL. This observation indicates a feedback regulation of β-globin (or total Hb) levels within erythroid cells to maintain β-globin (or total Hb) levels below certain upper levels.

- There was a significant correlative linear relationship between DP %LVV+ cells and HbA^{T87Q} at Month 6: subjects who received ZYNTEGLO with higher DP %LVV+ cells had higher HbA^{T87Q} at Month 6, compared to subjects who received ZYNTEGLO with lower DP %LVV+ cells.
- There was no correlation between ZYNTEGLO subpopulations and PB VCN and HbA^{T87Q} at post-infusion Months 6 and 24.
- There was no correlation between total CD34+ cell dose and HbA^{T87Q} in peripheral blood at either Month 6 or Month 24 for the *All TDT* pool, indicating that the lowest cell dose evaluated to date was adequate for effective reconstitution of HSCs in treated subjects.
- The targeted AUC range of busulfan evaluated in clinical studies was considered adequate for myeloablation.

Pharmacodynamic Responses and Transfusion Independence (TI)

- PB VCN and HbA^{T87Q}: HbA^{T87Q} increased quickly with the increase of PB VCN at lower PB VCN levels, followed by a HbA^{T87Q} plateau at higher PB VCN levels. This observation suggests the regulation of β-globin levels within erythroid cells and selection of erythroid cells producing $\beta^{A\text{-}T87Q}$-globin during engraftment for balanced α-globin/β-globin ratios.
- HbA^{T87Q} and transfusion independence (TI): Subjects with higher HbA^{T87Q} levels were less likely to need blood transfusion. The median (min, max) HbA^{T87Q} at the time when no blood transfusion was needed was 8.44 (0.75, 13.85) g/dL, and the median (min, max) HbA^{T87Q} at the time that subjects had blood transfusion was 0.88 (0.00, 5.06) g/dL.
- Unsupported total Hb at Month 6 and transfusion independence (TI): All of the 31 subjects who had ≥ 9 g/dL unsupported total Hb at Month 6 achieved TI. Statistically significant association was observed between unsupported total Hb level (≥ 9 g/dL) at Month 6 and TI.

6. Clinical/Statistical

a. Clinical Program

To support licensure of ZYNTEGLO, bluebird bio, Inc. submitted data from an interim analysis of two ongoing Phase 3 studies, two completed Phase 1-2 studies, and one long-term follow-up study. The clinical review team's recommendation for regular approval of ZYNTEGLO is based on review of efficacy and safety data from the Phase 3 studies, HGB-207 and HGB-212, that treated 41 subjects, with supportive safety data from the Phase 1/2 study, HGB-204, which treated 23 subjects. The BLA also included safety data from the Applicant's sickle cell disease and cerebral adrenoleukodystrophy (CALD) clinical programs.

Studies HGB-207 and HGB-212 are ongoing, open-label, multicenter, single-arm studies evaluating the efficacy and safety of ZYNTEGLO at a dose of ≥5 x 10^6 CD34+cells/kg in transfusion dependent subjects. The studies share parallel designs except that HGB-207 includes only subjects with non-β0/β0 genotype, whereas HGB-212 evaluated subjects with the β0/β0 genotype as well as subjects with another genotype that has the same degree of clinical severity as the β0/β0 genotype. Study HGB-207 prospectively evaluated two different age cohorts: Cohort 1 included subjects 12 years of age and

older and Cohort 2 included subjects < 12 of age. Both studies excluded subjects over the age of age 50. The primary efficacy endpoint was transfusion independence (TI), defined as weighted average hemoglobin (Hb) ≥ 9 g/dL without RBC transfusions for ≥ 12 months at any time after ZYNTEGLO infusion. Secondary endpoints included assessment of transfusion reduction, duration of TI, and iron overload and iron chelation parameters. Safety endpoints focused on adverse events, hematopoietic cell recovery, laboratory parameters, and insertional oncogenesis.

The primary efficacy analysis was performed on the efficacy analysis population, comprised of 41 subjects (18 in HGB-212 and 23 in HGB 207) infused with a single dose of ≥ $5x10^6$ CD34+ cells/Kg body weight. The primary efficacy analysis is based on treated subjects who were evaluable for TI as of the data cut-off, defined as those subjects who achieved TI, completed the Month 24 Visit, or did not achieve TI during the study.

Efficacy Results

Of 41 subjects infused with ZYNTEGLO in Phase 3 studies, the median age was 13 years (range 4 - 34); 49% were female; 49% were Asian, 44% White, 5% other, 2% unreported. Treated subjects were followed for a median of 27 months (range 4 - 48).

In Study HGB-207, 20 of 22 TI-evaluable subjects met TI (91%; 95% CI: 71, 99). In Cohort 1, 14 out of 15 TI-evaluable subjects (93%; 95% CI: 68, 100) achieved TI and in Cohort 2, 6 of 7 TI-evaluable subjects (86%; 95% CI: 42.1, 100) achieved TI. In Study HGB-212, 12 out of 14 TI-evaluable subjects (86%; 95% CI: 57, 98) achieved TI.

In the integrated analysis of efficacy, efficacy outcomes were generally consistent across subgroups with respect to genotype and age. All subjects achieving TI remained transfusion-independent to last follow up, with a median duration of TI of 26 months, (range 12-39); and maintained a weighted average Hb during TI of 11.5 gm/dL, (range of 9.3-13.7). There were nine subjects with at least 6 months of follow-up after ZYNTEGLO infusion who either did not achieve TI or were not yet TI-eligible. Four of the subjects who did not achieve TI, had a median 43% (range 3 to 93%) decrease in transfusion volume from 6 months after ZYNTEGLO infusion to last follow-up. Another three subjects with at least six months of follow-up had no transfusions after ZYNTEGLO administration but were not yet TI-evaluable.

In summary, Studies HGB-207 and HGB-212 represent adequate and well-controlled trials that provide substantial evidence of effectiveness of ZYNTEGLO in adult and pediatric patients with transfusion-dependent thalassemia. The results support a traditional approval for ZYNTEGLO.

b. Bioresearch Monitoring (BIMO) – Clinical/Statistical/Pharmacovigilance

Bioresearch Monitoring inspections were performed for the Applicant and three domestic clinical study sites that participated in the conduct of Study HGB-207, and HGB-212. The inspections did not reveal any issues that impact the integrity of the data submitted in this original Biologics License Application (BLA).

c. Pediatrics

The application does not trigger PREA because ZYNTEGLO received orphan drug designation (ODD) prior to the submission of the BLA for the treatment of patients with β-thalassemia who require regular red blood cell transfusions. Per the Pediatric Research Equity Act (PREA) and 21 Code of Federal Regulations (CFR) 314.55(d), ODD products are exempt from pediatric study requirements.

However, as β-thalassemia is typically diagnosed early in life, the Applicant appropriately evaluated pediatric subjects in their clinical development program. The clinical data support the safety and effectiveness of ZYNTEGLO in the pediatric population.

d. Other Special Populations

ZYNTEGLO has not been studied in other special populations.

7. Safety and Pharmacovigilance

The primary safety population included 41 Phase 3 study subjects in the Phase 3 studies, HGB-207 and HGB-212, who received busulfan myeloablation, and ZYNTEGLO at a median dose of 9.4 $x10^6$ CD34+ cells, range 5 - 42.1), by the data cutoff of March 9, 2021. Treatment-emergent adverse events (TEAEs) were defined as all AEs occurring after initiation of ZYNTEGLO administration to Month 24 visit. Subjects were followed for a median of 27 months (range 4 to 48); safety data were collected to data cutoff of March 9, 2021.

Summary of safety findings:

Nonlaboratory adverse events (AEs) from Day 1 to Month 24 reported in ≥ 30% of subjects included mucositis, febrile neutropenia, fever, abdominal pain, musculoskeletal pain, cough, alopecia, epistaxis, and vomiting. Laboratory-based AEs from Day 1 to Month 24 reported in > 95% of subjects included cytopenias (neutropenia, thrombocytopenia, leukopenia, and lymphopenia). The AE pattern is consistent with that seen in the post-myeloablative setting, and likely attributable to conditioning.

No deaths were reported. A total of 32 serious adverse events (SAEs) were reported by 15 subjects. From Day 1 to Month 24, SAEs reported in ≥ 5% of subjects included fever, liver veno-occlusive disease, febrile neutropenia, neutropenia, thrombocytopenia, and stomatitis. These were attributed largely to myeloablative conditioning, except two SAEs of thrombocytopenia that occurred in the context of delayed platelet engraftment, of which the review team considered one as clinically important, which was associated with epistaxis requiring hospitalization.

The review team focused on the following as adverse events of special interest (AESIs): thrombocytopenia with delayed platelet engraftment (PE), replication-competent lentivirus (RCL), and malignancies, especially due to LVV-related insertional oncogenesis.

Thrombocytopenia was observed and the median time to PE was 46 days (range 20 - 94), which is delayed in comparison to outcomes reported after allogeneic HSCT for β-thalassemia. However, delayed engraftment was generally not associated with bleeding events, except for the one SAE noted above.

There was no evidence of LVV-derived RCL following ZYNTEGLO treatment. No malignancy was observed in ZYNTEGLO recipients, and universal screening with integration site analysis (ISA) showed polyclonal reconstitution with no evidence of clonal predominance. Three subjects had relatively frequent LVV integrations which met the oligoclonality criterion using an integration site analysis (ISA) algorithm; however the relative frequency results have remained stable and improved over time, with subjects having stable hematologic laboratory findings. As such, the clinical significance of these findings is unclear.

The review team also focused on clinical safety information submitted in this BLA for the Applicant's SCD and CALD clinical development programs with related LVV-based products.

Two of 49 subjects with sickle cell disease treated with a similar product manufactured with the same LVV as ZYNTEGLO were diagnosed with AML. One subject's leukemia blasts had prominent LVV integrations into VAMP4 gene, though the relationship of the integrations to the development of AML has not been proven. Notably, over 50% of ZYNTEGLO subjects were also reported to have low level integrations into VAMP4; however, these findings were not associated with clinical sequelae. The BLA also included data reporting myelodysplastic syndrome (MDS) with prominent LVV integrations into the oncogene MECOM in two subjects and into PRDM16, a related proto-oncogene, in a third subject treated with a related LVV-based product for cerebral adrenal leukodystrophy (CALD). The review team evaluated these findings and considered the Advisory Committee deliberations (see Section 8 below), and concludes that these findings do not have significant implications for the safety profile of ZYNTEGLO.

In conclusion, the safety profile of ZYNTEGLO largely reflects the requisite myeloablative conditioning, with frequent cytopenias and gastrointestinal symptoms, which resolved. Delayed platelet engraftment with thrombocytopenia is a notable safety finding, although with limited clinical sequalae. Due to insertional oncogenesis risk inherent to lentiviral vectors, a PMR safety study will be required.

8. Labeling

The proposed proprietary name, ZYNTEGLO, was reviewed by the Advertising and Promotional Labeling Branch (APLB) on November 5, 2021 and was found acceptable. CBER communicated the acceptability of the proprietary name to the applicant on December 14, 2021.

The Advertising and Promotional Labeling Branch (APLB) reviewed the proposed Prescribing Information, Patient Package Insert, and package and container labels on August 1, 2022, and found them acceptable from a promotional and comprehension perspective.

9. Advisory Committee Meeting

Considering the first-in-class nature of ZYNTEGLO as well as the complexities related to the potential risk of insertional oncogenesis and hematologic malignancies with ZYNTEGLO, and in light of hematologic malignancies reported after treatment with products manufactured with the same or related LVV for other diseases, an Advisory Committee (AC) Meeting was convened on June 9-10, 2022. The AC was asked to assess the benefit-risk profile of ZYNTEGLO and provide recommendations on testing for insertional oncogenesis.

Acknowledging the potential risks of vector integration and insertional oncogenesis, the AC members discussed the observed findings in the Applicant's clinical development program for sickle cell disease and CALD for similar LVV-based products. The AC members opined that the hematologic malignancies observed in SCD subjects who received a similar LVV-based product are not informative regarding the risk of hematologic malignancy in patients with β-thalassemia who received ZYNTEGLO. The AC members also opined that the pathophysiologic characteristics of SCD may predispose patients to increased risk of hematologic malignancy. Additionally, the AC members considered the Applicant's product for the treatment of CALD to be distinct from ZYNTEGLO, and as such, the MDS cases observed in the CALD clinical program were not informative regarding the risk of insertional oncogenesis with ZYNTEGLO.

Ultimately, the expert panel unanimously concluded that ZYNTEGLO had a favorable benefit-risk profile for the treatment of patients with β-thalassemia.

10. Other Relevant Regulatory Issues

ZYNTEGLO was granted breakthrough therapy designation, and the BLA was reviewed under priority review. The Applicant was also issued a Rare Pediatric Disease voucher.

11. Recommendations and Benefit/Risk Assessment

a. Recommended Regulatory Action

The Applicant provided substantial evidence of effectiveness and reasonable assurance of safety based on two adequate and well controlled clinical investigations.

The review team recommends regular approval of ZYNTEGLO for the treatment of adult and pediatric patients with β-thalassemia who require regular red blood cell (RBC) transfusions.

b. Benefit/Risk Assessment

ZYNTEGLO administration resulted in transfusion independence in 89% of subjects with β-thalassemia who required regular red blood cell (RBC) transfusions. The most important risk identified with ZYNTEGLO is delayed platelet engraftment and slow platelet recovery. This risk is minor compared to the substantial benefit of transfusion independence. Therefore, the overall benefit-risk profile of ZYTEGLO is favorable.

The clinical trial data do not suggest a safety concern that would necessitate a Risk Evaluation and Mitigation Strategy (REMS). However, a PMR safety study will be required to assess the long-term risk of hematologic malignancies related to insertional oncogenesis.

c. Recommendation for Postmarketing Activities

As insertional oncogenesis is a potential risk of treatment with ZYNTEGLO, the Applicant agreed to conduct the following postmarketing study as a safety Postmarketing Requirement.

1. A postmarketing, prospective, multi-center, observational study to assess the long-term safety of betibeglogene autotemcel and the risk of secondary malignancies occurring after treatment with betibeglogene autotemcel. The study will include at least 150 β-thalassemia patients and the enrolled patients will be followed for 15 years after product administration. The study design will include monitoring (at pre-specified intervals) for clonal expansion with adequate testing strategies.

   Final protocol submission: November 30, 2022
   Study completion date: March 31, 2043
   Final study report submission: March 31, 2044

The Applicant agreed to the following CMC Postmarketing Requirement (PMR):

2. A study to justify the sample processing steps in the (b) (4) and provide information to support the identification process used for the extractables study for the (b) (4) bag. Also conduct a leachables study for the (b) (4) bag over the duration of the shelf-life of the product. In addition, submit a toxicological risk assessment.

   The timetable for the PMR study is:
   Draft Protocols Submission: August 31, 2022
   Final Protocols Submission: November 30, 2022
   Extractable Study Completion Date: February 28, 2023
   Interim Study Report Submission: April 30, 2023 (Extractable Study)
   Leachable Study Completion Date: January 30, 2024
   Final Study Report Submission: March 30, 2024 (Leachable and Toxicological Risk Assessment)

The Applicant agreed to the following CMC postmarketing commitments (PMCs):

3. bluebird bio, Inc., commits to qualify a (b) (4) test of their (b) (4) to provide greater (b) (4) assurance of their final drug product and to submit these qualification results as a supplement to their file on or before March 31 of 2023.

   Final Report Submission: March 31, 2023

4. bluebird bio, Inc., commits to establish the sensitivity of (b) (4) method for the (b) (4) bag.

   Final Report Submission: February 28, 2023

5. bluebird bio, Inc., commits to perform the additional (b) (4) assessments of the (b) (4) assays as described in BLA 125717.

   Final Report Submission: June 30, 2023

6. bluebird bio, Inc., commits to add testing of beti-cel cryopreserved drug product (DP) for (b) (4) as described in BLA 125717.

   Final Report Submission: February 28, 2023

7. bluebird bio, Inc., commits to perform a supplemental (b) (4) study of beti-cel assessing the (b) (4) under the intended conditions as described in BLA 125717.

   Final Report Submission: February 28, 2023

8. bluebird bio, Inc., commits to assess the feasibility of (b) (4) (b) (4) The feasibility assessment will include a proposed path forward for completing a leachable study for the (b) (4), including a date the final leachable study report will be submitted to the FDA.

   Final Report Submission: February 28, 2023

9. bluebird bio, Inc., commits to conducting (b) (4) testing following the conditions outlined in (b) (4)," and provide justifications for the test method, results, and conclusions as part of a complete test report.

   Final Report Submission: December 31, 2022

10. bluebird bio, Inc., commits to perform a (b) (4) study to evaluate drug product bag integrity following (b) (4)

    Final Report Submission: December 31, 2022