

**CBER CMC BLA Review Memorandum**

**BLA STN 125717**

**Product Name**

Zynteglo  
betibeglogene autotemcel

**Reviewers**

**Jakob Reiser, Chair OTAT/DCGT/GTIB**  
**Tal Salz, CMC Reviewer OTAT/DCGT/GTB**  
**Andrew Timmons, CMC Reviewer OTAT/DCGT/GTB**  
**Brian Stultz, CMC Reviewer OTAT/DCGT/CTTB**  
**Anna Kwilas, CMC Reviewer OTAT/DCGT/GTB**

1. **BLA#:** STN 125717

2. **APPLICANT NAME AND LICENSE NUMBER**

**Name:** bluebird bio, Inc.

**License Number:** 2160

3. **PRODUCT NAME/PRODUCT TYPE**

Non-Proprietary/Proper Name/USAN: betibeglogene autotemcel

Proprietary Name: Zynteglo

Company codename: LentiGlobin BB305 Drug Substance

UNII Code: MEE8487RTP

NDC Code: NDC73554-3111-1

4. **GENERAL DESCRIPTION OF THE FINAL PRODUCT**

- a. **Pharmacological category:** Autologous Hematopoietic Stem-Cell-Based Gene Therapy
- b. **Dosage form:** Suspension for infusion
- c. **Strength/Potency:** Between  $2 \times 10^6$  and  $20 \times 10^6$  cells/mL (b) (4)
- d. **Route of administration:** Intravenous infusion
- e. **Indication:** Treatment of patients with  $\beta$ -thalassemia who require regular red blood cell (RBC) transfusions

5. **MAJOR MILESTONES**

Initial IND Submission (BB-IND 15324)	December 19, 2012
IND allowed to proceed	January 17, 2013
Fast Track Designation granted	January 31, 2013
Orphan Drug Designation granted (ODD #13-3905)	May 15, 2013
Breakthrough Therapy Designation granted	January 29, 2015
Rare Pediatric Disease designation granted (RPD-2018-193)	November 30, 2018
Pre-BLA Meeting	November 7, 2019
Rolling Submission Nonclinical Module	December 18, 2019
Rolling BLA Submission CMC and Clinical Modules	September 20, 2021
First Committee Meeting	October 15, 2021
Filing Meeting	November 5, 2021
Mid-Cycle Meeting	January 18, 2022
External Late-Cycle Meeting	May 23, 2022
PDUFA action due date (original)	May 20, 2022
Major Amendment Acknowledgement	January 14, 2022
Advisory Committee Meeting	June 9-10, 2022
PDUFA action due date:	August 19, 2022

6. **CMC/QUALITY REVIEW TEAM**

Reviewer/Affiliation	Section/Subject Matter
Brian Stultz, M.S., CBER/OTAT/DCGT/CTTB	BB305 LVV and beti-cel DS: Control of Materials Control of excipients
Andrew E. Timmons, Ph.D., CBER/OTAT/DCGT/CTB	BB305 LVV: Manufacture, Characterization, Control of Drug Substance, Reference Standards, Container Closure System, Stability
Jakob Reiser, Ph.D., CBER/OTAT/DCGT/GTIB	BB305 LVV Manufacturing Process Development, Validation of Analytical Procedures, Batch Analyses
Tal Salz, Ph.D., CBER/OTAT/DCGT/CTB	beti-cel DS/DP: Manufact. Process, Control of Critical Steps and Intermediates, Process Validation and Evaluation, Characterization, Impurities, Container Closure System, Stability, Chain of Identity, Batch Analysis, Control of DP
Anna Kwilas, Ph.D., CBER/OTAT/DCGT/CTB	beti-cel DP: Justification of Specifications, Excipients of Human or Animal Origin, Novel Excipient, Control of DP
Steven Bauer, Ph.D., OTAT/DCGT/CTTB	Consult review for (b) (4)
Elena Gubina, Ph.D., OTAT/DCGT/CTB1	Consult review for (b) (4)
Archana Devi Siddam, Ph.D., OTAT/DCGT/CTB	Consult review for (b) (4)
Guo-Chiuan Hung, Ph.D., OTAT/DCGT/CTB1	Consult review for (b) (4)
Mercy Quagrain, Ph.D., OTAT/DCGT/CTB	Consult review for (b) (4) (b) (4)
Carolina Panico, M.D. Ph.D., OTAT/DCGT/TEB	Consult review for (b) (4)
Bao-Ngoc Nguyen, Ph.D., OTAT/DCGT/TEB	Consult review for (b) (4)
Michael (Brad) Strader, M.Sc., Ph.D., OVRP/DBPAP/LIB	Consult reviews for (b) (4) $\beta^{A18/Q}$ globin expression assay
Tianjiao Dai, Ph.D., OBPV/DB/DNCE	Consult statistical review for non-clinical data

## 7. INTER-CENTER CONSULTS REQUESTED

Reviewer/Affiliation	Section/Topic	In agreement with consult recommendations (Yes/No)

Zhaobo Fan, Ph.D., CDRH/OSEL/DBCMS Caroline Pinto, Ph.D., CDRH/OSEL/DBCMS	(b) (4) bag	Yes
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**8. SUBMISSION(S) REVIEWED**

Date Received	Submission/Amendment number	Comments/Status
December 19, 2019	125717/0	Initial submission (Modules 1, 2 and 4)
September 20, 2021	125717/1	Submission of Modules 1, 2, 3 and 5
November 15, 2021	125717/5	Response to IR #4 (DBSQC) received November 9, 2021 (BB305 LVV purity)
November 15, 2021	125717/6	Response to IR #5 (Clinical) received November 10, 2021 (Safety data related to insertional mutagenesis).
November 29, 2021	125717/8	Response to IR #1 (CMC) received November 19, 2021
November 30, 2021	125717/9	Response to IR #1 (DMPQ) received November 16, 2021 (Manufacturing processes for BB305 LVV and Lenti-D LVV)
January 4, 2022	125717/16	Response to IR #2 (CMC) received December 23, 2021
January 28, 2022	125717/26	Response to IR (CMC) received January 6, 2022 (validation of the quantitative bA-T87Q-globin expression assay for drug product)
February 4, 2022	125717/30	Response to IR #2 (CMC) received December 23, 2021 (unexecuted batch records)
February 11, 2022	125717/35	Response to IR #3 (CMC) dated February 3, 2022
March 4, 2022	125717/44	Response to the Agency's Mid-Cycle Meeting Minutes
April 4, 2022	125717/49	Response to IR #4 (CMC) dated March 25, 2022
April 5, 2022	125717/50	Updated Integration Site Analysis (ISA) Algorithm
April 15, 2022	125717/53	Response to IR #28 (Clinical) dated April 8, 2022 (VAMP4 insertion site)
April 22, 2022	125717/55	Response to IR #2 (DMPQ) dated April 6, 2022 (container closure integrity test)

April 29, 2022	125717/59	Response to CMC-Device IR #1, dated April 20 (performance testing of (b) (4) Cryopreservation bag)
May 4, 2022	125717/61	Response to IR (Clinical) for IND 15905, dated April 29, 2022 (gene expression data of genes within (b) (4) ; WGS data for subject (b) (6)
May 12, 2022	125717/63	Response to CMC IR #3 (DBSQC), dated May 9, 2022 (positive control (b) (4) assay for BB305 LVV)
May 12, 2022	125717/64	Response to IR #2 (Clinical Pharmacology), dated May 2, 2022 (impact of different cell populations such as short-term progenitor cells and true long-term re-populating stem cells on 1) VCN in peripheral blood (PB VCN), and 2) HbA <sup>T87Q</sup> in peripheral blood)
May 19, 2022	125717/66	CMC Information Request CTD Alignment Updates
May 20, 2022	125717/67	Response to IR #5 (CMC) dated May 13, 2022
May 27, 2022	125717/69	Results and Day 4 Batch Records from Process Demonstration Run at Pre-License Inspection of Lonza Houston Inc. (LHI) Pearland
May 27, 2022	125717/70	Clinical Information Amendment – LCM Follow-up Request (Response to elivaldogene autotemcel BLA 125755 Information Request Question 5 received on 21 May 2022 regarding VAMP4 and CALM)
June 17, 2022	125717/73	Response to IR #6 (CMC) dated June 10, 2022 (Comments 1-4 and 7)
June 22, 2022	125717/74	Response to CMC-Device IR #3 dated June 17, 2022 ((b) (4) Cryopreservation bag)
June 24, 2022	125717/75	Response to IR #6 (CMC) dated June 10, 2022 (Comments 5 and 6)
June 28, 2022	125717/83	Response to IR #7 (CMC) dated June 28, 2022
June 30, 2022	125717/79	Response to IR #6 (CMC) dated June 10, 2022 (Comment 8)

July 1, 2022	125717/82	Response to IR #7 (CMC) dated June 28, 2022
July 21, 2022	125717/84	Response to IR #8 (CMC) dated July 15, 2022
July 22, 2022	125717/85	Response to Request for PMCs: DMPQ PMC dated July 18, 2022, CMC PMCs dated July 18 and July 19, 2022
July 22, 2022	125717/86	Response to IRs from February – June 2022 regarding the (b) (4) bag and Request for PMR
July 29, 2022	125717/88	Response to IR #6 (CMC) dated June 10, 2022 (Comment 7) regarding unexecuted batch records
August 1, 2022	125818/89	Response to Informal Telecon held between bluebird bio and FDA 28 July 2022 regarding the PMC language submitted on 22 July 2022
August 3, 2022	125717/92	Response to IR #9 (CMC) dated July 29, 2022
August 4, 2022	125717/93	Response to PMR Protocol Recommendations, dated July 29, 2022
August 5, 2022	125717/94	Follow-up to IR #8 (CMC) dated 15 Jul 2022 (Comment 3) regarding DS Supplemental Comparability Analysis and DS Comparability Studies
August 10, 2022	125717/97	Response to CMC IR (b) (4) PMR dated August 9, 2022
August 10, 2022	125717/98	Response to IR #10 (CMC) regarding Packaging labels, dated August 9, 2022

**9. Referenced REGULATORY SUBMISSIONS (e.g., IND BLA, 510K, Master File, etc.)**

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	Acceptable for LVV manufacturing CMC Reviewer Steven Bauer (CBER/OTAT/DCGT/CTTB)

BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	Suitable for commercial manufacturing CMC Reviewer Elena Gubina (CBER/OTAT/DCGT/GTB1)
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	Information provided is adequate to support the intended use CMC Reviewer Archana Devi Siddam (CBER/OTAT/DCGT/CTB)
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	No issues identified CMC Reviewer: Guo-Chiuan Hung (CBER/OTAT/DCGT/GTB1)
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	No issues identified CMC Reviewer: Mercy Quagraine (CBER/OTAT/DCGT/CTB)
MF(b) (4)	Lonza Houston Inc.	Lonza Houston Cell and Gene Therapy Manufacturing Facility	Yes	No review available. Refer to DMPQ memo for details. Facility information is included in BLA.
BB-MF (b) (4) Type V Master File	(b) (4)	Manufacturing and Laboratory Facilities and Quality Systems	Yes	No review available. Information pertinent to manufacturing facility is provided in the BLA.
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	Relevant sections in BLA reviewed by CMC reviewer Andrew Timmons (CBER/OTAT/DCGT/GTB1)
BB-MF (b) (4)	(b) (4)	(b) (4)	Yes	Relevant sections in BLA reviewed by CMC reviewer Andrew Timmons (CBER/OTAT/DCGT/GTB1)

STN (b) (4)	(b) (4)	(b) (4)	Yes	Relevant sections in BLA reviewed by CMC reviewer Andrew Timmons (CBER/OTAT/DCGT/GTB1)
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## 10. REVIEWER SUMMARY AND RECOMMENDATION

### A. EXECUTIVE SUMMARY

The CMC review team concludes that the manufacturing process, test methods and control measures for betibeglogene autotemcel (beti-cel; Zynteglo) are capable of yielding autologous products with consistent quality attributes deemed acceptable for commercial manufacturing under the BLA.

beti-cel is an autologous gene therapy product intended to treat patients with  $\beta$ -thalassemia who require regular red blood cell (RBC) transfusions. beti-cel can increase the amount of total hemoglobin (Hb) to normal or near-normal levels to eliminate the need for chronic RBC transfusions and iron management therapies. The clinical benefits of this one-time beti-cel treatment are expected to last for the patient's lifetime.


beti-cel consists of a CD34+ cell enriched population that contains hematopoietic stem and progenitor cells (HSCs) transduced with a lentiviral vector (LVV), referred to as BB305 LVV, containing the  $\beta^{A-T87Q}$ -globin transgene sequence. The sequence of the  $\beta^{A-T87Q}$ -globin protein encoded by BB305 LVV is identical to that of endogenous adult  $\beta$ -globin with the exception of a glutamine substitution for threonine at position 87 (T87Q), which enables tracking of the  $\beta$ -globin encoded by the transgene sequence via reverse phase high performance liquid chromatography (RP-HPLC) in patients' peripheral blood. The adult Hb containing  $\beta^{A-T87Q}$ -globin chains (HbA<sup>T87Q</sup>) has comparable oxygen kinetics to adult Hb containing wild-type  $\beta$ -globin chains (HbA). As  $\beta^{A-T87Q}$ -globin transgene expression is regulated by the  $\beta$ -globin locus control region and erythroid-specific promoter, it is expressed only in erythroid cells and not in other hematopoietic cell types. The  $\beta^{A-T87Q}$ -globin protein combines with  $\alpha$ -globin to correct the  $\alpha/\beta$ -globin imbalance in erythroid cells, producing functional HbA<sup>T87Q</sup>.

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 beti-cel. After myeloablative conditioning and beti-cel infusion, transduced HSCs engraft in the bone marrow and differentiate to reconstitute the hematopoietic system, including RBCs that contain HbA<sup>T87Q</sup> to treat the patient's  $\beta$ -thalassemia.


To manufacture beti-cel, autologous hematopoietic progenitor cells obtained by apheresis (HPC-A) are collected from each patient at a Qualified Treatment Center



(QTC) for use in the manufacture of drug product. The apheresis material is then shipped to the Lonza-Houston, Inc. drug substance/drug product manufacturing facility where CD34+ cells are selected and then transduced with BB305 LVV to manufacture beti-cel drug product. To do this, the HPC-A are enriched for cells expressing CD34 by (b) (4)



At the end of drug substance manufacture, the transduced cells, which are suspended in (b) (4)



beti-cel drug product is supplied frozen as a suspension in cryopreservation solution for intravenous infusion in 20 mL fluoro-ethylene-propylene bags. Each bag contains  $2.0 - 20 \times 10^6$  cells/mL, frozen in approximately 20 mL of solution. The minimum dose is  $5.0 \times 10^6$  CD34+ cells/kg patient weight.

More than one drug product lot may be required to achieve the minimum beti-cel dose. As such, a patient may undergo additional mobilization cycles to provide HPC-A for use in the manufacture of an additional drug product lot to provide a total CD34+ cell count that meets or exceeds the target dose. Shipment of drug product lot(s) to the QTC for infusion occurs once the dose requirement has been met and all product lot(s) have been released.

The BB305 LVV is derived from HIV-1 and is replication-incompetent and self-inactivating (SIN). It 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. The packaged genomic viral RNA encodes no viral genes and contains less than (b) (4) of the HIV-1 genome. Essential proteins are encoded by three packaging plasmids used in the transfection of HEK293T cells during BB305 LVV production.

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.

**B. RECOMMENDATION****I. APPROVAL**

This biological license application (BLA) provides an adequate description of the manufacturing process and characterization of betibeglogene autotemcel (beti-cel; Zynteglo). The CMC review team has concluded that the manufacturing process, along with associated test methods and control measures, is capable of yielding a product with consistent quality characteristics. This information satisfies the CMC requirements for biological product licensure per the provisions of section 351(a) of the Public Health Service (PHS) Act controlling the manufacture and sale of biological products. Based on the information provided in the BLA submission and the information gathered during the pre-license inspection of the Lonza-Houston, Inc. facility and the FDA/ORA inspection of the (b) (4) facility in December 2021, the CMC review team recommends regular approval of this BLA.

*Post-Marketing Commitments (PMCs):*Robustness PMC

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

Sampling PMC

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

(b) (4) PMC

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

(b) (4) PMC

bluebird bio, Inc., commits to adding an additional (b) (4) test using (b) (4) of the (b) (4) volume (i.e., 20 mL) that is removed prior to DP formulation (referred to as the (b) (4)), while continuing to perform the DP (b) (4) test using (b) (4) volume, and passing results from both these (b) (4) tests will be required for DP release. The qualification results for this additional (b) (4) test on the (b) (4) sample matrix will be submitted to the BLA for CBER review on or before March 31, 2023, and these qualifications will include (b) (4) from the (b) (4) DP manufacturing facility.

(b) (4) Leachable Study PMC

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 Feasibility Assessment Report Submission: February 28, 2023.

(b) (4) Testing PMC

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. Complete test reports for this (b) (4) testing on the (b) (4) bag will be submitted as a final study report by December 31, 2022.

(b) (4) Study PMC

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

Complete test reports for this testing will be submitted as a final study report by December 31, 2022.

*Postmarketing Requirement (PMR):*

Leachables and Extractable Studies and related Toxicological Risk Assessment

Section 505(o) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute (section 505(o)(3)(A), 21 U.S.C. 355(o)(3)(A)).

We have determined that an analysis of spontaneous postmarketing adverse events reported under section 505(k)(1) of the FDCA will not be sufficient to assess a known serious risk of patient exposure to any unknown extractables and leachables, at this time, from the (b) (4) bag, in association with the use of betibeglogene autotemcel.

Furthermore, the pharmacovigilance system that FDA is required to maintain under section 505(k)(3) of the FDCA is not sufficient to assess this serious risk.

Therefore, based on appropriate scientific data, we have determined that you are required to conduct the following studies:

A study to justify the sample processing steps in the concentration and liquid-liquid extraction 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.

We acknowledge the timetable you submitted on August 10, 2022 which states that you will conduct this study according to the following schedule:

Draft Protocols Submission: August 31, 2022

Final Protocols Submission: November 30, 2022

Extractable Study Completion Date: February 28, 2023

Leachable Study Completion Date: January 30, 2024

Interim Study Report Submission: April 30, 2023 (Extractable)

Final Report Submission: March 30, 2024 (Leachable and Toxicological Risk Assessment).

Please submit the protocols to your IND 15324, with a cross-reference letter to this BLA, STN BL 125717/0 explaining that these protocols were submitted to the IND. Please refer to the sequential number for each study/clinical trial and the submission number as shown in this letter.

## II. COMPLETE RESPONSE (CR)

Not applicable

## III. SIGNATURE BLOCK

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Jakob Reiser Review Committee Chair Research Biologist OTAT/DCGT/GTIB	Concur	
Tal Salz Biologist OTAT/DCGT/GTB1	Concur	
Andrew Timmons Biologist OTAT/DCGT/GTB1	Concur	
Brian Stultz Research Biologist OTAT/DCGT/CTTB	Concur	

Anna Kwilas Lead Biologist OTAT/DCGT/GTB2	Concur	
Kimberly Schultz Branch Chief OTAT/DCGT/GTB2	Concur	
Denise Gavin. Branch Chief OTAT/DCGT/GTB1	Concur	
Steven Oh Acting Director OTAT/DCGT	Concur	

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### **Module 3**

#### **3.2.S BB305 LVV DRUG SUBSTANCE**

##### **3.2.S.1.1 - 1.3 Nomenclature, Structure and General Properties**

*Reviewed by JR*

#### **Nomenclature**

The company code for the Lentiviral Vector (LVV) used in the manufacture of betibeglogene autotemcel (beti-cel) is BB305 LVV. A USAN/INN name will not be assigned for the BB305 LVV.

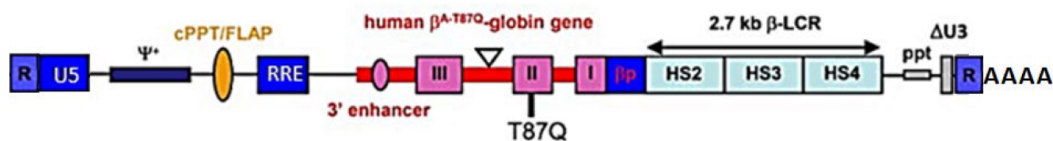
#### **Structure and General Properties**

The BB305 LVV particle (Figure 1) (b) (4)



(b) (4)

(b) (4)

**Figure 2: Diagram of BB305 LVV RNA**

From left to right: R and U5 segments; R = repeat; U5 = unique 5' Long Terminal Repeat (LTR);  $\Psi^+$  = Psi packaging signal; cPPT/FLAP = central polypurine tract/DNA flap; RRE = Rev responsive element; 3' enhancer = 3'  $\beta$ -globin enhancer; III, II, I = exons III, II, I;  $\nabla$  = 372-bp IVS2 deletion in intron 2; T87Q =  $\beta_{A-T87Q}$  mutation (ACA [Thr] to CAG [Gln]);  $\beta p$  = human  $\beta$ -globin promoter; HS2, HS3, HS4 = DNase I hypersensitive sites (HS) HS2, HS3, and HS4;  $\beta$ -LCR = human  $\beta$ -globin locus control region; ppt = polypurine tract;  $\Delta U3$  = unique 3' region of the LTR with 400-bp deletion of the promoter, resulting in the self-inactivating property. AAAA denotes the polyadenylated tail to the viral genomic RNA. (b) (4)

(b) (4)

BB305 LVV is manufactured using a four-plasmid system consisting of one transfer plasmid encoding the genome of the LVV and three packaging plasmids that encode the proteins needed for packaging the LVV particles, reverse transcription of the LVV RNA into a complementary DNA, and integration into the genome of transduced cells.

### 3.2.S.2 Manufacture

#### 3.2.S.2.1 Manufacturer(s)

*Reviewed by JR*

BB305 LVV used to manufacture beti-cel for commercial use will be manufactured, tested, and stored at the manufacturing sites listed in Table 1. The sites responsible for

manufacture, qualification, and storage of the HEK293T master cell bank (MCB) and working cell banks (WCBs) are listed in Table 2.

**Table 1 - BB305 LVV Manufacturing, Testing, and Storage Sites**

Facility Name and Address	Identification Numbers	Responsibility
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	BB305 LVV manufacturing and in-process testing
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	BB305 LVV in-process, release, and stability testing
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	BB305 LVV in-process, release, and stability testing
bluebird bio Research Triangle 1733 T.W. Alexander Drive Durham, North Carolina 27703 USA	FEI: 3015451326 DUNS: 117192706	BB305 LVV release and stability testing
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	BB305 LVV storage

DUNS = Data Universal Numbering System; FEI = FDA Establishment Identifier; LVV = lentiviral vector

**Table 2 - HEK293T Master Cell Bank and Working Cell Bank Manufacturer and Testing Site Information**

Facility Name and Address	Identification Numbers	Responsibility
<b>Historical</b>		
(b) (4)	FEI: Not Available DUNS: Not Available	MCB Manufacturing and Testing
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	MCB Testing

(b) (4)	FEI: Not Available DUNS: Not Available	MCB Testing
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	WCB Manufacturing
<b>Current</b>		
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	WCB Manufacturing MCB and WCB Storage WCB Testing
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	MCB and WCB Storage

DUNS = Data Universal Numbering System; FEI = FDA Establishment Identifier; MCB = Master Cell Bank; WCB = Working Cell Bank.

**Reviewer Comment:** In response to CMC IR#1, sent 11/19/2021, bluebird bio indicated in Amendment 8 received 11/19/2021 that the (b) (4) site was included as a long-term storage location for BB305 LVV intended for use in beti-cel manufacturing at (b) (4), the European drug product manufacturing site for the EU Marketing Authorization Application, and if the BB305 LVV needed to be returned to the United States for manufacturing drug product at Lonza. Bluebird bio proposed to remove (b) (4) from BB305 LVV Manufacturing, Testing, and Storage Sites. In addition, this section of the BLA was updated to reflect the transfer in ownership of the QC testing laboratory located in Durham, North Carolina from bluebird bio to (b) (4) which occurred in September 2021, as noted during the 12 November 2021 beti-cel application orientation meeting with FDA. This is acceptable.

### 3.2.S.2.2 Description of Manufacturing Process

*Reviewed by AET*

BB305 LVV is produced by the transient transfection of human embryonic kidney (HEK) 293T cells using the transfer plasmid (pBB305) and three packaging plasmids ((b) (4) (Table 3). See **Control of Raw Materials NOT of Biological Origin** for more information about the plasmids.

(b) (4)

(b) (4)

130 pages determined to be not releleasable: (b)(4)



(b) (4)

beti-cel DS is manufactured and processed into DP (b) (4). Stability testing of DS is not applicable.

### 3.2.P DRUG PRODUCT (BETI-CEL)

#### 3.2.P.1 Description and Composition of the Drug Product

*Reviewed by TS*

beti-cel consists of an autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with BB305 LVV encoding the  $\beta^{A-T87Q}$ -globin gene, suspended in (b) (4) cryopreservation solution containing 5% dimethylsulfoxide (DMSO).

#### 3.2.P.2 Pharmaceutical Development

*Reviewed by TS*

##### 3.2.P.2.1 Components of the Drug Product

**3.2.P.2.1.1 Drug Substance:** transduced, washed cells suspended in (b) (4) and consists of an autologous CD34+ cell enriched population that contains HSCs transduced with BB305 LVV encoding the  $\beta^{A-T87Q}$ -globin gene.

**3.2.P.2.1.2 Excipients:** (b) (4)

##### 3.2.P.2.2 Drug Product

###### 3.2.P.2.2.1 Formulation Development

The composition of (b) (4) is described in section 3.2.P.4.1. (b) (4) was selected for its cryopreservative qualities, absence of protein, and for its suitability for infusion into humans. Studies supporting cell concentration for cryopreservation in (b) (4) are described in 3.2.P.2 Pharmaceutical Development. (b) (4) viability and (b) (4) recovery were acceptable between (b) (4) but the total cell concentration release specification is  $2.0 \times 10^6$ -  $20 \times 10^6$  cells/mL based on clinical manufacturing experience.

Post-thaw viability and recovery at room temperature were evaluated using (b) (4) and demonstrated that the DP remains viable in (b) (4) for at least 4 hours at room temperature after the DP bag has been thawed (b) (4) viability and (b) (4) recovery). Data provided in 3.2.P.8.3 support the stability of the DP in the (b) (4) throughout the DP shelf-life (12 months).

### 3.2.P.2.2.2 Overages

There are no overages in the beti-cel DP.

### 3.2.P.2.2.3 Physicochemical and Biological Properties

Physicochemical and biological properties are described in Sections **3.2.S.1.2 Structure** and **3.2.S.1.3 General Properties**. There are no differences between the properties of the DS and the DP.

### 3.2.P.2.3 Manufacturing Process Development

Process development and comparability studies performed during the product lifecycle are described in **3.2.S.2.6.2 Studies Supporting Manufacturing Changes**. The studies described apply to the DP as well as DS. Information unique to the DP is described here.

#### 3.2.P.2.3.1 Manufacturing Development History

Table 79 summarizes changes introduced to the formulation and cryopreservation of the DP during product development. There were no changes in the DP manufacturing process (i.e., formulation and cryopreservation) through HGB-204, HGB-207, HGB-212. The DP manufacturing process used during those clinical studies is the same as the commercial process. During the first clinical study (HGB-205; Phase 1/2; SCD and  $\beta$ -thal), several changes were made. In the original manufacturing process, (b) (4)

The clinical site where the DP manufacturing occurred was the (b) (4) manufacturing site.

**Table 79 - Comparative Summary of DP Manufacturing Process Development**

Clinical Study	HGB-205	HGB-204	HGB-207, HGB-212
Manufacturing Site	(b) (4)	(b) (4)	LHI- (b) (4), LHI- (b) (4)
Trial Initiation Date	2013	2014	2016
Cryopreservation	(b) (4)	Drug Product	
Formulation	(b) (4)	(b) (4)	(5% DMSO), (b) (4) FEP bags
Storage	-140°C	-140°C	
Infusion	(b) (4)	(b) (4)	

#### 3.2.P.2.3.2 Process Characterization Studies

This section describes the process characterization studies and results to support the proposed in-process control limits, the NOR and PARs of process parameters, and

classification of process parameters in the formulation and cryopreservation unit operation as summarized in section **3.2.P.3.4 Controls of Critical Steps and Intermediates**. Process parameters were classified based on severity, potential for occurrence, and capability to detect, and each parameter was assigned with a risk priority number (RPN), as was described in **3.2.S.2.6.3 Process Characterization studies**. Parameters with RPN (b) (4) were characterized.

(b) (4) DP large-scale characterization study reports were provided. Different container closure systems were used in the characterization studies including (b) (4) FEP cryobag (b) (4) and (b) (4) FEP cryobag.

#### **Characterization of Scale-down models (RPT-0437)**

The commercial beti-cel manufacturing platform utilizes (b) (4) FEP bags as the DP container closure system. The (b) (4) vials are used for sample retains and the (b) (4) FEP Cryobag are used for long-term stability and were used for HD PPQ runs. To facilitate process DP characterization activities, the scale-down container models were characterized. (b) (4) lots of CD34+ cells from (b) (4) unique HDs were transduced with LVV BB305 and cryopreserved at (b) (4) cells/mL (DP commercial acceptance criteria: 2 to 20 x 10<sup>6</sup> cells/mL) in different containers. There was (b) (4) in cryopreservation media before freeze (NOR: (b) (4)). Post-thaw TNC viability was (b) (4) and TNC recovery was (b) (4) for all conditions (i.e., containers) and met the study specifications (TNC viability (b) (4) and recovery (b) (4)).

(b) (4)

(b) (4)

(b) (4)



(b) (4)

#### **Overall Reviewer's Assessment of Section 3.2.P.2.3.2 DP Process**

##### **Characterization Studies:**

The characterization studies described support the DP process control strategy as described in Section 3.2.P.3.4 Controls of Critical Steps and Intermediates.

#### **3.2.P.2.4 Container Closure System**

Beti-cel container closure system is described in **3.2.P.7 Container Closure System**.

#### **3.2.P.2.5 Microbiological Attributes**

*Reviewed by TS*

Beti-cel is comprised of living cells and is manufactured under (b) (4)

. DP lot release testing includes (b) (4) sterility and endotoxin testing with samples that are aseptically obtained from the final container immediately prior to freezing of the DP bag. The DS is tested for (b) (4). Each DP bag is (b) (4)

Each product lot is evaluated for sterility and endotoxin. Container closure integrity testing, including (b) (4), demonstrated that the DP bag remains integral following representative filling, freezing, shipping, and thawing conditions.

#### **3.2.P.2.6 Compatibility**

In-use Stability

*Reviewed by AK*

(b) (4)

(b) (4)

(b) (4)

*Reviewer Comment: In response to CMC IR#1 dated 11/19/2021, bluebird bio clarified that the cell suspension was transferred using a (b) (4) from the DP bag into a (b) (4)*

*for this study. RPT-0437 was provided to support the use of these surrogate container closures.*

(b) (4)

### 3.2.P.3 Manufacture

#### 3.2.P.3.1 Manufacturer(s)

*Reviewed by TS*

**Table 84 - Manufacturing and Testing Facilities for beti-cel Drug Product**

Facility	FDA Identification No.	Responsibility
Lonza Houston, Inc	FEI: 3013629214	Drug product manufacturing,

14905 Kirby Dr., Houston, TX 77047, USA	DUNS: 832903004	packaging, labeling; and release testing (%CD34+ cells, (b) (4), cell concentration, viability, appearance)
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)

### 3.2.P.3.2 Batch Formula

*Reviewed by TS*

Formulation and cryopreservation operations are detailed in **3.2.P.3.3 Description of Manufacturing Process**. A batch (i.e., lot) of DP may be packaged in either one or two 20-mL bags, depending on the total number of cells present. Up to 2 DP lots may be administered to the patient to meet the dose (Table 85).

**Table 85 - Drug Product Batch Formula**

Component	Amount per Batch
Autologous CD34+ cell enriched population transduced with lentiviral vector encoding the $\beta^{A-T87Q}$ -globin gene	(b) (4) $\times 10^6$ - $20 \times 10^6$ cells/mL
(b) (4)	20 mL per bag, up to two bags per lot

### 3.2.P.3.3 Description of Manufacturing Process

*Reviewed by TS*

#### Overview of the DS Manufacturing Process

The beti-cel manufacturing process is (b) (4) (Figure 27). The DS is processed into the DP (b) (4). The DP manufacturing process consists of the Formulation and Cryopreservation step, which includes (b) (4)

As described in **3.2.P.3.3 Chain of Identity**, each individual patient is infused with DP manufactured with their own hematopoietic progenitor cells.

(b) (4)

### Step-by-step Description of the DP Manufacturing Process

#### Formulation and Cryopreservation

After completion of (b) (4)

cells are cryopreserved in one or two (b) (4) 20 mL (b) (4) Fluoro-Ethylene-Propylene (FEP) bags filled to 20 mL final volume each.

If a beti-cel lot does not contain enough CD34+ cells to achieve the necessary dose for the patient ( $\geq 5 \times 10^6$  CD34+ cells/kg), then a second mobilization cycle is initiated and a second lot of beti-cel is manufactured. The dose is calculated by (b) (4)

All available cells are formulated. Samples for release testing are taken from each bag via (b) (4). The filled cryopreservation bag(s) is transferred to the cryopreservation room and visually inspected (IPC) for defects such as bag integrity, visible particulates, and cell clumps. Following visual inspection, a product label is applied to the flap of the bag detailing lot and patient specific information (refer to the Inspection Report (EIR) for more details). Each bag is then separately placed in a transparent overwrap bag (secondary



container) and inserted into a metal cassette that has been labeled with a label that contains both product and patient information.

Each product-containing cassette is frozen to (b) (4) using a (b) (4). Freezing is performed using a (b) (4) by the manufacturer and is selected by the operator as defined in the batch record. The NORs and PARs for each step of the (b) (4) were established. The manufacturing batch record includes controls to ensure that the correct (b) (4) is chosen by the operators. (b) (4)

When the (b) (4) is complete (hold at (b) (4)), the DP is transferred to LN<sub>2</sub> within (b) (4) using LN<sub>2</sub> in a cryogenic (b) (4) system. The cassette is stored in the vapor phase of liquid nitrogen at ≤ 140°C. DP process parameters are listed in section **3.2.P.3.4 Controls of Critical Steps and Intermediates**.

***Reviewer Comment:** According to the information that was provided in Amendment 35 in response to the 02/03/2022 CMC IR, during the PPQ campaign, (b) (4) were used for the HD PPQ lots to allow for retain of more samples, but the commercial manufacturing process will only use (b) (4) to freeze the final DP bags and samples.*

#### Product Packout and Shipment

The beti-cel lot(s) remain in storage at the DP manufacturer site until bluebird bio confirms the lot(s) for shipment. The Lot Information Sheet (LIS) is required to authorize/initiate the shipment and accompanies the DP shipment. The LIS documents the DP lots required to be shipped and administered. Additional information about the LIS is discussed in section **3.2.P.3.3.2 Chain of Identity**. Please see Figure 33: Zynteglo – US – Lot information sheet for a representative LIS. beti-cel in the metal cassette is placed in a cassette holder that is subsequently placed into vapor phase of liquid nitrogen of the qualified (b) (4) cryoshipper. All bags of DP manufactured for the patient to achieve the required cell dose are packed out in the same cryoshipper. As discussed in **3.2.S.2.3 Control of Materials - Hematopoietic Progenitor Cells - Apheresis (HPC-A)**, (b) (4) DP lots may be manufactured and required to achieve the minimum required dose ( $\geq 5 \times 10^6$  CD34+ cells/kg). Therefore, up to (b) (4) product bags each containing up to 20 mL of cells will be shipped per patient. The cryoshipper is qualified to maintain a temperature of ≤ -140°C throughout the duration of shipment and up to (b) (4). Upon receipt at the clinical site, beti-cel is required to be stored at ≤ -140°C.

***Reviewer Comment:** In amendment 67 in response to CMC IR dated 5/13/2022, the applicant indicated that bluebird bio is responsible for courier selection and shipping of DP and HPC-A as defined in Quality Agreements. A single courier, (b) (4) has been qualified and approved for shipping DP and HPC-A per bluebird bio vendor qualification program. Qualification and approval of couriers includes quality assessment and execution of a Quality Agreement and listing on bluebird bio's approved vendor list.*

**Overall Reviewer's Assessment of Section 3.2.P.3.3 Description of Manufacturing Process:**

The description of the DP manufacturing process is appropriate and the controls and control limits are acceptable.

**3.2.P.3.3 Chain of Identity**

*Reviewed by TS*

To ensure that the correct cells are tracked from apheresis through the manufacturing process to infusion into the same patient, a chain of identity (COI)/chain of custody (COC) system has been developed which includes human-readable text (labels) and optical machine-readable linear and two-dimensional barcodes. Traceability is maintained by a computerized system (b) (4) together with paper-based manufacturing batch records (BRs). The COI is maintained across operations from patient enrollment to DP delivery to the qualified treatment center (QTC) for administration. There are distinct phases, each with defined COI data elements:

- **Phase 1: Enrollment, HPC-A collection and shipment**
- **Phase 2: HPC- A receipt and DP manufacturing and labeling**
- **Phase 3: DP disposition and shipping**
- **Phase 4: DP receipt at QTC and patient administration**

There are three COI unique traceability identifiers that are used for licensure and are linked throughout bluebird bio's COI process via combination of paper and electronic system verifications that occur by and between bluebird bio, the QTC, and the DP Manufacturer. Throughout the COI Process, successful verifications are required before proceeding with the next step. The COI identifiers are:

- **Patient Identifier(s)** include patient name, Date of Birth (DOB), patient ID, and COI ID. They are assigned by the QTC and/or bluebird bio and remain unchanged from enrollment to delivery of the DP
- **HPC-A Product Identifier** is also known as the Donation Identification Number (DIN) and assigned by the QTC responsible for collecting the HPC-A
- **Drug Product (DP) Manufacturing/Product Identifier(s)** are assigned by the manufacturer and include the DP lot number and bag number which are specific to each DP lot.

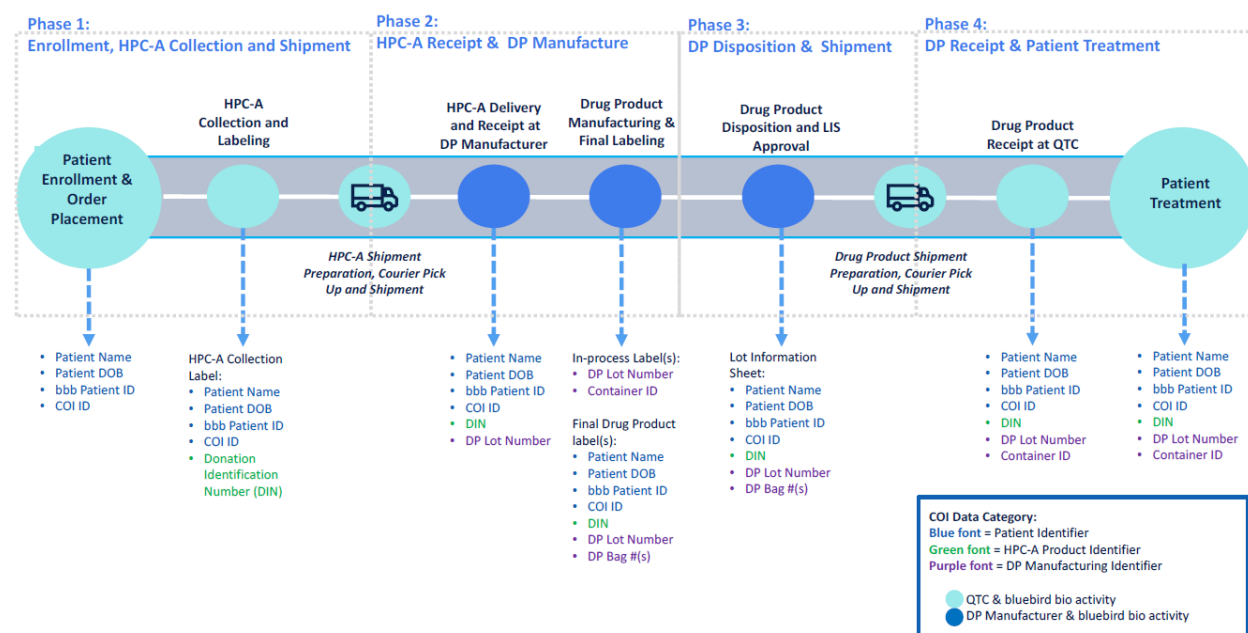
For clinical development, the Patient Identifier(s) consisted solely of the clinical trial Subject ID(s).

Bluebird bio uses a Lot Information Sheet (LIS) to support commercial manufacturing and to enhance the COI. The LIS is generated for each patient and contains all COI identifiers. Additionally, it includes the number of DP lots and infusion bags provided for the patient, as well as the patient-specific dose encompassing the total number of CD34+ cells (from all bags and lots) divided by the patient's weight. The LIS is included with the shipment of DP(s).

*Reviewer Comment: the link between two DP lots intended for a single patient is established in the LIS and (b) (4) system through the patient number. Therefore, the lots are linked. All in process and final labels are generated through (b) (4)*

The HPC-A labels, in-process DS Labels (Pre-Stimulation and transduction), DP labels (infusion bag and cassette), and LIS, require specific COI unique identifiers. The bluebird bio COI system activates during patient enrollment and order placement. A QTC may consist of an apheresis collection center, cell therapy laboratory, and/or the infusion center and is qualified by bluebird bio's Quality Assurance Unit prior to use in the treatment process. Figure 28 illustrates each phase and the COI identifiers which will be printed on labels. Table 86 provides an example of COI for a patient/lot. Table 87 provides description of the activities supporting COI at each COI phase.

**Figure 28: Chain of Identity - Flow Diagram**



**Table 86 - Chain of Identity – Example of Identifiers and Labels**

COI Unique Identifier Category	Example COI Data Element	Example Format COI Data Element	Process		
			QTC	DP Manufacturer	Bluebird bio
Patient Identifier(s)	Patient Name <sup>a</sup>	Doe, Charlie Alex	(b)	(4)	
	Date of Birth (DOB) <sup>a</sup>	YYYY-MM-DD			
	Patient ID (bbb Patient ID) <sup>b</sup>	(b) (4)			

	Chain of Identity Identifier (COI ID) <sup>b</sup>	(b) (4)
HPC-A Product Identifier	Donation Identification Number (DIN) <sup>a</sup>	(b) (4)
DP Manufacturing Product Identifier(s)	DP Lot Number <sup>c</sup>	(b) (4)
	DP Bag Number <sup>c</sup>	Bag X of Y
	Container ID <sup>c</sup>	(b) (4)

(b) (4)

<sup>a</sup> QTC communicated/assigned COI Data Element

<sup>b</sup> bluebird bio assigned COI Data Element

<sup>c</sup> DP Manufacturer assigned COI Data Element

(b) (4)

(b) (4)

(b) (4)

### **Suitability for Use**

During the execution of the PPQ campaign, bluebird bio's COI system successfully showed all applicable COI unique identifiers traceable throughout the COI Process in executed batch documentation. No deviation reported during the PPQ series impacted the bluebird bio COI system ability to ensure HPC-A donor-to-recipient, (b) (4) product tracking. Therefore, evidence of the COI system suitability for use by assuring HPC-A collected from a patient to make DPs, is returned to that same patient, is provided.

#### **Overall Reviewer's Assessment of Section 3.2.P.3.3 Chain of Identity:**

COI has been reviewed during inspection of the Lonza Houston (b) (4) facility inspection. CBER inspectors audited the COI and had extensive discussions with the LHI SMEs to ensure that the hybrid system (of (b) (4) and paper-based BR) is able to maintain the integrity of production data and the COI of each autologous DP lot. This is further discussed in the EIR. The COI system is acceptable.

### **3.2.P.3.4 Controls of Critical Steps and Intermediates**

#### *Reviewed by TS*

The identification, characterization, and classification of DP process parameters and the establishment of acceptable ranges are discussed in section **3.2.P.2.3 Manufacturing**

**Process Development** and support the defined control strategy for Formulation and Cryopreservation as listed in Table 88.

**Table 88 - DP Process Parameters**

Unit Operation	Process Parameter	NOR	PAR	Classification
Formulation and Cryopreservation	Cryopreservation Volume (mL)	(b) (4)	(4)	
	(b) (4) concentration at freeze ( $\times 10^6$ cells/mL)			
	Time in cryopreservation solution before freeze (b) (4)			
	(b) (4) Profile			
After Cryopreservation	Time at (b) (4) in (b) (4) (after freezing, (b) (4) LN <sub>2</sub> ) (hours)	(b) (4)	(4)	
	Transfer time from (b) (4) to LN <sub>2</sub> storage (b) (4)			

<sup>a</sup> The total amount of time controlled by this non-CPP includes the amount of time for the performance of the visual inspection process.

**Reviewer Comment:** Table 88 was updated according to the agreement reached in amendments 35 and 49 in responses to the CMC IRs dated 02/03/2022 and 03/25/2022 regarding the NOR and PAR for hold time in cryopreservation media. Additionally, the cryopreservation Profile is an OP and was added to the table in Amendment 35 in response to the CMC IR dated 02/03/2022.

In addition to DP process parameters, Visual Inspection IPC is performed by (b) (4)

#### **Overall Reviewer's Assessment of Section 3.2.P.3.4 DP Controls of Critical Steps and Intermediates**

The established commercial DP process parameters and in-process control are acceptable based on manufacturing experience and characterization studies.

#### **3.2.P.3.5 Process Validation and/or Evaluation**

*Reviewed by TS*

Prospectively identified parameters and attributes were assigned Process Validation Acceptance Criteria (PVAC) based on process characterization studies and manufacturing experience as described in sections **3.2.S.2.6** and **3.2.P.2**.



(b) (4)

**PPQ Acceptance Criteria**

For process validation, final product release acceptance criteria must be met, IPCs must be within the established action limits, process parameters are set to the NORs, and acceptance criteria for process-related CQAs, such as impurities, must meet the established acceptance criteria. The prospectively identified parameters and attributes were assigned Process Validation Acceptance Criteria (PVAC) as listed in Table 90, based on process characterization studies and manufacturing experience as described in Sections **3.2.S.2.6** and **3.2.P.2**. The PVAC were determined based on statistical analysis of historical process data, scientific knowledge, and technical expertise. Any excursions from the PVAC observed during the execution of the validation runs was investigated for impact to the PPQ and documented in the PPQ report.

(b) (4)

(b) (4)





(b) (4)

**Overall Reviewer's Assessment of Section 3.2.S.2.5 Process Validation:**

Some modifications to DP release methods were implemented post validation and therefore, some release tests, including CFC,  $\beta^{\text{A-T87Q}}$ -globin Quantitative Protein Expression, and in (b) (4) were performed on PPQ retains. The use of (b) (4) material in (b) (4) PPQ runs precluded evaluation of Potency in the (b) (4) lots as well as demonstration of consistent  $\beta^{\text{A-T87Q}}$ -globin expression. Moreover, shipping validation was limited to one lot which was not manufactured and tested using the commercial process and facility. While considering these limitations, the totality of data and information provided supports successful validation of the beti-cel manufacturing process.

**3.2.P.4 Control of Excipients**

*Reviewed by BS*

The sole excipient in the DP is (b) (4), a commercially available cryopreservation medium that includes 5% dimethyl sulfoxide (DMSO). (b) (4) is obtained from (b) (4) and is manufactured under cGMP. A Letter of Authorization to Master File (DMF) BB-MF (b) (4) is provided. Quality Agreements are in place to ensure bluebird bio will be notified of any manufacturing changes. (b) (4) is manufactured with components that meet compendial requirements except for (b) (4). The (b) (4) used in manufacturing has a requirement for purity of (b) (4).

Upon receipt at the drug product manufacturing site, (b) (4) is visually inspected for package integrity. (b) (4) is released for production based on inspection of the supplier CoA and identity testing by (b) (4). Acceptance criteria for identity testing are DMSO content of (b) (4).

**3.2.P.4.1 Specifications**

This information is covered in the cross-referenced master file. Specifications in the cross-referenced master file are acceptable.

**3.2.P.4.2 and 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures**

This information is covered in the cross-referenced master file. Analytical methods in the cross-referenced master file are acceptable.

**3.2.P.4.4 Justification of Specifications:**

This information is covered in the cross-referenced master file. Justifications of specifications in the cross-referenced master file are acceptable.

**3.2.P.4.5 Excipients of Human or Animal Origin:**

Not applicable

**3.2.P.4.6 Novel Excipient:**

Not applicable

**3.2.P.5 Control of Drug Product***Reviewed by AK, TS and Brad Strader***3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)**

The final agreed upon beti-cel lot release acceptance criteria are summarized in Table 96.

**Table 96 - Release Specifications for beti-cel**

Quality Attribute	Test	Method	Acceptance Criteria	Justification
Potency and Strength	Vector Copy Number (VCN)	(b) (4) qPCR	(b) (4)	Highest and lowest values that resulted in a patient achieving transfusion independence (TI)
	% LVV+ Cells	(b) (4) qPCR	(b) (4)	Greater than the highest value observed for any patient who did not achieve TI
	(b) (4)	(b) (4)	(b) (4)	Lower bound: manufacturing/clinical experience; Upper bound: mean (b) (4) of lots manufactured within commercial PARs
	(b) (4)	(b) (4)	(b) (4)	Mean – (b) (4) of lots manufactured within commercial PARs
	Colony Forming Cells (CFC)	(b) (4)	(b) (4)	Lowest value observed that resulted in a patient achieving TI
			(b) (4)	Necessity for recapitulation of stem cell niche
Identity	$\beta^{\text{A-T87Q}}$ -globin Quantitative Protein Expression	(b) (4)	(b) (4) $\beta^{\text{A-T87Q}}$ -globin (relative to (b) (4))	Lower bound: lowest value observed that resulted in a patient achieving TI; Upper bound: mean (b) (4) of lots manufactured within commercial PARs
	(b) (4)	(b) (4)	(b) (4)	N/A
	(b) (4)	(b) (4)	(b) (4)	N/A
Purity and Content	(b) (4)	(b) (4)	(b) (4)	Lowest value observed that resulted in a patient achieving TI

	(b) (4)	(b) (4)	(b) (4)	Lower bound: calculated based on lower bound of (b) (4); Upper bound: highest value observed that resulted in a patient achieving TI
	Total Cell Concentration		2.0E+06 to 20E+06 total cells/mL	Highest and lowest values that resulted in a patient achieving TI
	(b) (4)		(b) (4)	Manufacturing/ clinical experience
Safety	Sterility	(b) (4)	No Growth	Requirement
	Endotoxin	(b) (4)	(b) (4)	Based on the compendial requirement of (b) (4) with a (b) (4) safety factor
	Mycoplasma	(b) (4)	None Detected	Requirement
Quality	Appearance	Visual assessment	Colorless to white to red, including shades of white or pink, light yellow, and orange.	Based on observation of DP prior to freezing

**Reviewer Comment:** In CMC IR #6, dated 6/10/2022, bluebird bio was asked to tighten the VCN, (b) (4), total cell concentration, (b) (4),  $\beta^A$ -T87Q-globin expression and in (b) (4) acceptance criteria compared to the initially proposed criteria to better reflect clinical study and manufacturing experience. bluebird bio provided updated acceptance criteria and justifications in Amendment 75 which we agreed with. All updates are reflected in Table 96.

### Sampling time points

Beti-cel release tests are performed on samples taken at various steps in the (b) (4) manufacturing process. An outline of the sampling time points for release testing is provided in Table 97 - Sampling Points, Matrices and Justifications for beti-cel Release Testing.

**Table 97 - Sampling Points, Matrices and Justifications for beti-cel Release Testing**

Release Test	Sampling Point	Matrix	Justification
Mycoplasma	(b) (4)		
(b) (4)			
(b) (4)			
(b) (4)			
Total Cell Concentration			

(b) (4)			DP bags (1 or 2).
Vector Copy Number	(b) (4)		
(b) (4)			
%LVV+ Cells			
$\beta^A$ -T87Q-globin Quantitative Protein Expression			
Forming Cells (CFC)			
Appearance			
Sterility			
Endotoxin			

**Reviewer Comment:** In CMC IR #6 and #7, dated 6/10/2022 and 6/28/2022, respectively, bluebird bio was asked to include sampling of the DP for (b) (4) Total Cell Concentration, (b) (4) to confirm on the final formulated DP. In amendments 74 and 82, bluebird bio agreed to add the requested testing on cryopreserved DP samples but indicated that additional assay validation would be needed to implement the requested testing. In amendment 89, bluebird bio agreed to submit a post approval supplement (PAS) to implement the additional testing as part of a PMC. Note, bluebird bio still intends to determine beti-cel dose based on the DS test results to remain consistent with dosing in the clinical trials. Whether this continues to be acceptable will be determined upon review of the correlation data provided in the PAS.

#### Release of out of specification (OOS) commercial product

The need to have a mechanism for releasing OOS commercial product was discussed with bluebird during the LHI-(b) (4) inspection. A follow-up IR was sent on 7/25/2022. Bluebird responded in Amendment 94 that a mechanism to release OOS commercial beti-cel was still in development and until the pathway was implemented, no OOS commercial beti-cel lots would be released.

### **3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures**

**Reviewer Comment:** Sterility (b) (4), Endotoxin (b) (4), Mycoplasma (b) (4), and Appearance (visual inspection) were reviewed by DBSQC. DBSQC determined that the assays are performed and were validated adequately with the exception of the sterility assay. A PMC was agreed upon to further validate the sterility assay and add additional sample volume from the (b) (4) to the sterility test sample to further confirm the absence of contamination. Please see DBSQC review for additional details.

#### **Cell Count and Viability**

4 pages determined to be not releleasable: (b)(4)

(b) (4)

(b) (4)


**Vector Copy Number (VCN)**

To assess VCN, (b) (4)

2 pages determined to be not releleasable: (b)(4)

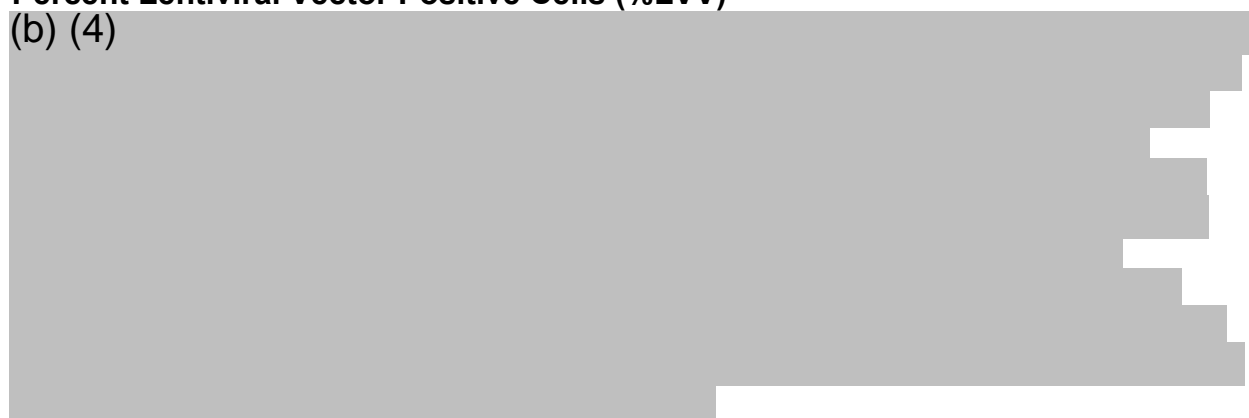


(b) (4)

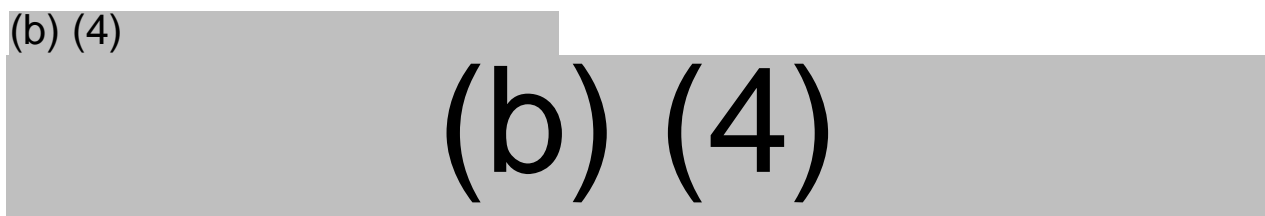
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**Percent Lentiviral Vector Positive Cells (%LVV)**

(b) (4)

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(b) (4)

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(b) (4)



(b) (4)

(b) (4)

**Colony Forming Cells (CFC)**

DP and an assay control are (b) (4)

3 pages determined to be not releleasable: (b)(4)

(b) (4)

*Reviewer Comment: Based on the information/data provided, this assay has been adequately validated.*

**HbA<sup>T87Q</sup> Quantitative protein expression**

$\beta^{T87Q}$  globin transgene product identity and quantified expression in beti-cel is determined using (b) (4)

[Redacted]

(b) (4)

[Redacted]

3 pages determined to be not releleasable: (b)(4)

(b) (4)

### 3.2.P.5.4 Batch Analyses

A total of (b) (4) beti-cel lots were manufactured for use in HGB-207 (n=(b) (4)) and HGB-212 (n=(b) (4)). A total of (b) (4) subjects were treated in HGB-207 (n=(b) (4)) and HGB-212 (n=(b) (4)). Of those (b) (4) subjects, (b) (4) subjects were treated with (b) (4) DP lots. (b) (4) DP lots were manufactured for (b) (4) subjects who were subsequently not treated as they withdrew from the study before drug administration. The Phase (b) (4) lots were manufactured at (b) (4) clinical manufacturing facilities, including the commercial facility LHI- (b) (4) as described in section **3.2.S.2.6.1 Manufacturing Development History**. Product data analysis was performed to determine the DP release specification as described in **3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)**. Of the (b) (4) infused subjects, (b) (4) were transfusion independent (TI), (b) (4) were not TI, and (b) (4) were not yet evaluable at the time of clinical review.

*Reviewer Comment: based on the information provided in amendment 84 in response to CMC IR dated 07/15/2022 the (b) (4) subjects that were not evaluable at the time of clinical review are now passed the 12 months follow up time and are all TI.*

Commercial specifications were established using available data from (b) (4) beti-cel lots manufactured for the HGB-207 and HGB-212 clinical trials with cell concentrations at transduction and (b) (4) that fell within the proposed commercial PARs for these parameters.

### 3.2.P.5.5 Characterization of Impurities

beti-cel is manufactured from beti-cel drug substance (b) (4). Characterization of potential impurities in beti-cel drug substance is discussed in Section **3.2.S.3.2 Impurities**.

### 3.2.P.6 Reference Standards or Materials

Testing of beti-cel does not require a reference standard for control and monitoring. Descriptions of assay controls and system suitability controls used in individual test methods are included in section **3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures**.

### 3.2.P.7 Container Closure System

*Reviewed by Carolina Panico (TEB); Summarized by TS.*

The container closure system consists of a primary package container, the (b) (4) Cryopreservation bag, a secondary package container (b) (4) bag), and a tertiary package container (cryocassette). The primary container closure is a 20-mL fluorinated ethylene propylene (FEP) cryopreservation bag with maximum fill volume of (b) (4). The (b) (4) is manufactured by (b) (4). Please see Table 106 and Figure 29 for the specifications and representative drawings of the (b) (4) respectively.

(b) (4)

(b) (4)

**Reviewer Comment:** The DP container closure is not FDA cleared (at the time of the review of the original BLA) and has been reviewed by DCGT/TEB as a consultation. Please refer to the full TEB review memo for details and information about the container



*closure system. The applicant did not conduct (b) (4) testing on the (b) (4) bag to demonstrate the bag can withstand drop events and extreme temperature conditions. This testing is being requested in a PMC. The Applicant agreed to these PMC requests in amendment 91 (August 1, 2022). In addition, the applicant provided insufficient information on the extractables and leachables chemical profile of the (b) (4) bag (consult review conducted by CDRH). This testing is being requested in a PMR and was discussed and concurred by the CBER Safety Working Group. The Applicant agreed to these PMR requests in amendment 98 (August 10, 2022).*

### 3.2.P.8 Stability

*Reviewed by AK*

#### 3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data

The long-term stability of beti-cel has been evaluated in a variety of studies. These include:

- Long-term studies of (b) (4) healthy donor DP lots (including (b) (4) PPQ lots) extending through 12 months at the recommended long-term storage condition ( $\leq -140^{\circ}\text{C}$ ).
  - (b) (4) DP lots manufactured at LHI-(b) (4)  
(b) (4) DP lots manufactured at (b) (4)  
(b) (4) manufactured at LHI-(b) (4)
  - LHI-(b) (4) lots were tested for VCN,  $\beta^{\text{A-187Q}}$ -globin expression (+/-), cell concentration, (b) (4), sterility, endotoxin
  - (b) (4) lots were tested for VCN, cell concentration, (b) (4), sterility, endotoxin; (b) (4) also tested for (b) (4)  
(b) (4) also tested for  $\beta^{\text{A-T87Q}}$ -globin expression (quantitative) and CFC
  - LHI-(b) (4) PPQ lots were tested for VCN, (b) (4) cell concentration, (b) (4), sterility endotoxin
  - Samples of all long-term stability lots were stored in (b) (4) Fluoro-Ethylene-Propylene (FEP) bags.
  - With the exception of single results for (b) (4) in (b) (4) lots (b) (4) at different time points, all results met the proposed commercial acceptance criteria, and no trends were observed. An investigation into the (b) (4) OOS results determined that these were due to inconsistent handling of the stability samples during the (b) (4) procedure. A CAPA was implemented to provide sufficient detail on how to conduct the (b) (4) procedure at the testing site.
- Historical lot testing of retains from (b) (4) beti-cel clinical lots for  $\beta^{\text{A-T87Q}}$ -globin expression and CFC
  - Testing was only performed at one time point but results were consistent with the results obtained from lots tested at the time of release
- Comparison of test results of a clinical beti-cel lot (b) (4) at the time of manufacture and following storage for (b) (4) at  $\leq -140^{\circ}\text{C}$ .

- This data was obtained for the in-use stability study (see 3.2.P.2.6 Compatibility for results)
- Accelerated (b) (4) and stress (b) (4) studies of (b) (4) DP PPQ lots (b) (4) ) stored as (b) (4)

**Reviewer Comment:** Note, (b) (4) testing cannot be performed on healthy donor beti-cel lots. While these data do support a beti-cel shelf life of 12 months when stored at the intended long-term storage condition of  $\leq -140^{\circ}\text{C}$ , there were no assessments of (b) (4) cells other than on beti-cel lot (b) (4) cell data were requested in CMC IR#7 dated 6/28/2022. In Amendment 82, bbb submitted (b) (4) cell data following cryopreservation for lovotibeglogene autotemcel (lovo-cel), a LVV-transduced CD34+ cell product related to beti-cel being evaluated for the treatment of sickle cell disease. Data from (b) (4) lots indicated that there was a difference of (b) (4) cells between the (b) (4) and the DP sampled following cryopreservation. bluebird bio has agreed to gather additional (b) (4) cell stability data in their post approval stability protocol.

### 3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment

Post-approval, during (b) (4), one beti-cel lot manufactured at the commercial manufacturing site, using the commercial manufacturing process with CD34+cells from healthy donors, will be placed on stability at the long-term storage condition of  $\leq 140^{\circ}\text{C}$ . These lots will be tested as indicated in Table 107.

**Table 107 - Annual Post-Approval Stability Testing at Long-term Storage Conditions ( $\leq -140^{\circ}\text{C}$ )**

Method	(b) (4)	Time Point (Months)				
		0	3	6	9	12
Vector Copy Number (VCN)	(b) (4)	P	P	P	P	P
%LVV+ Cells		P	NR	P	NR	P
(b) (4)		P	NR	P	NR	P
Colony Forming Cells (CFC)		P	P	P	P	P
		P	P	P	P	P
$\beta^{\text{A-T87Q}}$ -globin		P	P	P	P	P
Quantitative Protein Expression		P	P	P	P	P
(b) (4)		P	NR	P	NR	P
Total Cell Concentration		P	P	P	P	P
		(b) (4) E+06 to 20E+06 cells/mL				

Cell Viability	(b) (4)	P	P	P	P	P
Sterility	No Growth	P	NR	NR	NR	P
Endotoxin	(b) (4)	P	NR	NR	NR	P

Additionally, stability may be evaluated in support of significant beti-cel manufacturing changes.

**Reviewer Comment:** In CMC IR #6, dated 6/10/2022, bluebird bio was asked to i) include %LVV+ cells, (b) (4) cells testing to the protocol; ii) update the stability acceptance criteria based on the updates to the lot release specifications; iii) include an acceptance criterion for  $\beta^{A-T87Q}$ -globin expression; and iv) include in (b) (4) testing of any beti-cel lots manufactured from patient material. In amendment 75, bluebird bio updated the VCN and CFC stability acceptance criteria and agreed to include in (b) (4) testing of any beti-cel lots manufactured from patient material that may be placed on stability. Note, the  $\beta^{A-T87Q}$ -globin expression acceptance criterion is lower than that necessary for beti-cel release because the expression level in healthy donor lots is lower than that in patient lots. Also, a lower cell viability acceptance criterion is being allowed because lot release cell viability is assessed on a fresh sample and some degree of viability loss is expected upon cryopreservation. In amendment 82, bluebird bio agreed to include %LVV+ cells, (b) (4) cells testing to the protocol with appropriate acceptance criteria. This addition is reflected in Table 107.

### 3.2.A APPENDICES

#### 3.2.A.1 Facilities and Equipment

*Reviewed by JR*

A pre-license inspection (PLI) of the Lonza Houston, Inc. (abbreviated as LHI) facility located at 14905 Kirby Drive, Houston, TX 77047 in support of approval of BLA 125717 was conducted between 2/14/2022 and 2/18/2022

No FDA Form 483 was issued at the end of this PLI. However, a number of discussion items were conveyed to the LHI management during daily wrap-up sessions and during the final close out meeting. Details are provided in the EIR.

The BB305 LVV is manufactured by (b) (4), Inc. (a contract manufacturer for bluebird bio) in (b) (4). DMPQ recommended to waive PLIs for the (b) (4) manufacturing facility and the other facilities involved in BB305 LVV testing.

The (b) (4) manufacturing facility was inspected in (b) (4) by the FDA/ORA Team Biologics. The inspection of the (b) (4) facility was classified as Voluntary Action Indicated (VAI).

### 3.2.A.2 Adventitious Agents Safety Evaluation

Information in this section is integrated in the section 3.2.S.2.3 Control of Materials BB305 LVV Drug Substance and section 3.2.S.2.3 Control of Materials beti-cel Drug Substance.

### Viral Clearance Studies

Viral clearance studies were not performed on BB305 LVV or the beti-cel DP. However, studies on residual BB305 LVV impurity clearance during the beti-cel manufacturing process was conducted and found acceptable (sections 3.2.S.2.6 Manufacturing Process Development and 3.2.S.3.2 Impurities).

### 3.2.A.3 Novel Excipients

Not applicable – no new excipients are used.

## 3.2.R Regional Information (USA)

### Executed Batch Records

*Reviewed by TS*

Unexecuted and one executed batch record is provided for the beti-cel PPQ lot (b) (4) which was manufactured in 2019. The unexecuted batch record was provided in amendment 74 in response to IR #6 dated June 10, 2022 and incorporated changes according to various FDA comments provided to the applicant throughout the BLA review period, including a change from using up to (b) (4) LVV lots to using up to (b) (4) LVV lots per transduction.

*Reviewer Comment: Batch records were further reviewed during inspection of the LHI-PI facility for comparison with deviations. See EIR report for additional details.*

### Covid Risk Assessment Drug Product Manufacturing

*Reviewed by TS*

Beti-cel is an autologous product and does not require donor testing for SARS-CoV-2. The Applicant assessed the potential risk to safety, integrity, strength, purity, and quality (SISPQ) of an unintended expansion of the SARS-CoV-2 virus in the beti-cel manufacturing process potentially resulting in the final product with increased SARS-CoV-2 viral load. Based on the assessments performed, there is a low risk of transmission of the COVID-19 virus into patient apheresis material, LVV or in DP. Since ACE-2, the receptor used by SARS-CoV-2, is lacking in target cells, the cells within the apheresis product are an unlikely target for SARS-CoV-2 infection. Additionally, for a SARS-CoV-2 positive subject to be infused, the subjects will likely have developed specific antibodies able to counteract the virus, further reducing the risk of auto-transmission of SARS-CoV-2. The manufacturing process is controlled for adventitious agents and performed using closed systems whenever possible and using aseptic processing, including appropriate levels of gowning, the use of sterile sleeves, coveralls, masks, hair nets, beard covers etc.

*Reviewer Comment: The manufacturing process is appropriately controlled to reduce the risk of amplified SARS-CoV-2 viral load.*

## **Method Validation Package**

*Reviewed by JR*

Full method validation reports are provided. Analytical Procedures and Validation of Analytical Procedures are described in sections 3.2.S.4.2 (Analytical Procedures BB305 LVV) and 3.2.S.4.3 (Validation of Analytical Procedures BB305 LVV) and in sections 3.2.P.4.2 (Analytical Procedures beti-cel) and 3.2.P.4.3 (Validation of Analytical Procedures beti-cel).

## **Module 1**

### **A. Environmental Assessment or Claim of Categorical Exclusion**

*Reviewed by JR*

bluebird bio, Inc. is claiming a categorical exclusion under 21 CFR 25.31(c) from the need to prepare an environmental assessment. The agency action bluebird bio is requesting complies with the categorical exclusion criteria. Furthermore, bluebird bio is not aware of any extraordinary circumstances that would require the preparation of an environmental assessment.

beti-cel is composed of genetically modified human cells. FDA generally considers products that consist of genetically modified human cells to be eligible for the naturally occurring categorical exclusion [21 CFR 25.31(c)] because these cells have stringent nutritional requirements for survival and therefore are not viable in the environment as described in the “Guidance for Industry: Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vectored Vaccines and Related Recombinant Viral or Microbial Products (2015)”.

Furthermore, bluebird bio is not aware of any extraordinary circumstances that would require the preparation of an environmental assessment. BB305 LVV used in the manufacturing of beti-cel is created using a third-generation, split HIV-1 genome, plasmid system generating a replication defective, self-inactivating vector; BB305 LVV lots are fully tested during manufacturing; and clinical trial subjects are monitored after administration according to current FDA guidelines. bluebird bio has found no evidence of replication competent lentivirus in over 62 BB305 LVV lots or 63 subjects administered beti-cel during investigational studies.

*Reviewer Comment: The applicant's Claim of Categorical Exclusion is acceptable.*

### **B. Reference Product Designation Request**

bluebird bio claims a reference product exclusivity period of 12 years from the date of approval of this BLA. According to bluebird bio, approval of this BLA will constitute “first licensure” for Zynteglo and there are no licensed biological products that are structurally

related to Zynteglo for which bluebird bio, Inc., one of its affiliates, licensors, predecessors in interest, or related entities are the current or previous license holders.

**Reviewer Comment:** *The Reference Product Exclusivity Board Meeting to discuss Zynteglo took place on August 8, 2022. The Product Exclusivity Board supported the applicant's Claim of Categorical Exclusion.*

### C. Labeling Review

*Reviewed by JR*

#### Full Prescribing Information (PI):

The following sections of the PI were reviewed: Section 2 (Dose and Administration), Section 3 (Dosage Forms and Strengths), Section 11 (Description), Section 12 (Clinical Pharmacology – Mechanism of Action) and Section 16 (How supplied / storage and handling). Description of beti-cel dosage form and mechanism of action is consistent with other sections in the BLA. Procedures for receipt and preparation of beti-cel at clinical sites are described in sufficient details and are acceptable.

#### Carton and Container Label:

Figure 30 shows the Zynteglo - US - Cassette Label. The Zynteglo - US - Infusion Bag Label and the Zynteglo - US - Infusion Bag Label - PT Identifiers are shown in Figures 31 and 32, respectively. Figure 33 shows the Zynteglo - US - Lot information Sheet.

**Figure 30: Zynteglo - US - Cassette Label**




betibeglogene autotemcel zynteglo™			
Suspension for IV Infusion 20 mL containing 2.0 to 20 x 10 <sup>6</sup> cells/mL (1.7 to 20 x 10 <sup>6</sup> CD34 <sup>+</sup> cells/mL)		3 NDC 73554-3111-1 4	
<b>For autologous use only. For intravenous use only. Rx only.</b>			
Contains genetically modified autologous hematopoietic stem cells suspended in cryopreservation solution containing 5% DMSO.			
Keep infusion bag(s) in the metal cassette(s). Store in the vapor phase of liquid nitrogen at ≤ -140°C until ready for thaw and administration. Once thawed do not re-freeze.			
See full prescribing information for dosage and administration.			
<b>Do not irradiate. Do not use an in-line blood filter or infusion pump.</b>			
Not evaluated for infectious substances. No preservatives.			
See Lot Information Sheet for number of infusion bags and CD34 <sup>+</sup> cells per kg for this patient.			
<b>Confirm Patient Identifiers</b>			
Last Name: \$LastName\$		LOT: \$LOT\$	
First Name: \$FirstName\$		EXP: \$Expiry\$	
Date of Birth: \$DOB\$		Bag X of X	
bbb Patient ID: \$bbb_PatientID\$			
COI ID: \$bbb_COI_ID\$			
DIN \$DIN1_DIN2\$		U.S.Lic. # 2160	
		Manufactured for: bluebird bio, Inc. Somerville, MA 02145 1-833-999-6378 ZYNTGLO.com	
P/N: XXXXXXXX Label P/N: XXXXXXXX			

Figure 31: Zynteglo - US - Infusion Bag Label

betibeglogene autotemcel  
zynteglo™

Suspension for IV Infusion  
20 mL containing 20 to 20 x 10<sup>6</sup> cells/mL  
(1.7 to 20 x 10<sup>6</sup> CD34+ cells/mL)

  
3 NDC 73554-3111-1 4

---

**For autologous use only. For intravenous use only. Rx only.**  
Contains genetically modified autologous hematopoietic stem cells  
suspended in cryopreservation solution containing 5% DMSO.  
Not evaluated for infectious substances.  
Do not irradiate. Do not use an in-line blood filter or infusion pump.  
See full prescribing information for dosage and administration.  
See Lot Information Sheet for number of infusion bags and CD34+  
cells per kg for this patient.

P/N: XXXXXXXX  
Label P/N: XXXXXXXX

Manufactured for: bluebird bio, Inc.  
Somerville, MA 02145

Figure 32: Zynteglo - US - Infusion Bag Label - Patient Identifiers

betibeglogene autotemcel  
zynteglo™

Suspension for IV infusion  
20 mL containing 2.0 to 20 x 10<sup>6</sup> cells/mL  
(1.7 to 20 x 10<sup>6</sup> CD34+ cells/mL)

**Confirm Patient Identifiers**

Last Name: \$LastName\$  
First Name: \$First Name\$  
Date of Birth: \$DOB\$  
bbb Patient ID: \$bbb\_PatientID\$  
COI ID: \$bbb\_COI\_ID\$  
LOT: \$LOT\$  
EXP: \$Expiry\$  
Bag X of X  
DIN \$DIN1\_DIN2\$



 U.S. Lic. # 2160  
Label P/N: XXXXXXXX



Figure 33: Zynteglo - US - Lot information Sheet

betibeglogene autotemcel  
zynteglo™  
Suspension for IV infusion  
20 mL containing 2.0 to 20 x 10<sup>6</sup> cells/mL  
(1.7 to 20 x 10<sup>6</sup> CD34+ cells/mL)

3  4  
NDC 73554-3111-1

**LOT INFORMATION SHEET**

**SAVE THIS DOCUMENT AND HAVE IT AVAILABLE AT THE TIME OF ZYNTEGLO INFUSION**

**PATIENT INFORMATION**

Name (Last, First):  
Date of Birth (DD-MMM-YYYY):  
bluebird bio Patient ID:  
COI ID:  
Weight at First Collection (kg):

**INFORMATION ON SUPPLIED LOT(S)**

For autologous use only. For intravenous use only.  
Confirm patient identifiers. Read the prescribing information before use.  
The following lot(s) was manufactured and included in the shipment for this patient:

Lot Number	Manufacture Date (DD-MMM-YYYY)	DIN (List all collections)	Number of Infusion Bags	CD34+ Cells (x 10 <sup>6</sup> CD34+ cells)	Expiry Date (DD-MMM-YYYY)


Total Number of Infusion Bags:  Total Dose Volume: 20 mL x total # of infusion bags Total Dose: {N.N} x 10<sup>6</sup> CD34+ cells/kg

The minimum recommended dose of ZYNTEGLO is 5.0 x 10<sup>6</sup> CD34+ cells/kg.

**INSTRUCTIONS FOR STORAGE AND DISPOSAL**

Keep infusion bag(s) in the metal cassette(s). Store in the vapor phase of liquid nitrogen at ≤ -140°C until ready for thaw and administration. Use immediately after thawing; shelf life after thawing maximum of 4 hours at room temperature (20°C - 25°C). Once thawed do not re-freeze.

This medicine contains genetically modified cells.

 Manufactured for: bluebird bio, Inc.  
Somerville, MA 02145  
Manufactured by: U.S. Lic. # 2160  
Lonza, Inc. 1-833-999-6378  
Houston, TX 77047 ZYNTEGLO.COM

ZYNTEGLO and the bluebird bio logo are trademarks of bluebird bio, Inc.

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**Reviewer Comment:**

Updated versions of the cassette and infusion bag labels and the Lot Information Sheet were provided in amendment 84 in response to CMC IR #8 dated July 14, 2022, in amendment 92 in response to CMC IR #9 dated July 29, 2022 and in amendment 98 in response to CMC #10 dated August 9, 2022. For the infusion bag label, the following information was added: "Not evaluated for infectious substances", "Do not irradiate", "Do not use an in-line blood filter".

**Modules 4 and 5****Analytical Procedures and Validation of Analytical Procedures for Assessment of Clinical and Animal Study Endpoints***Reviewed by AK*




**Vector copy number (VCN)**


BB305 VCN in patient samples was determined by a qPCR assay performed and qualified by the (b) (4)




BB305 VCN in patient samples was also determined by a qPCR assay performed and qualified by (b) (4) This assay detects (b) (4)



**Reviewer Comment:** These assays are considered suitable for the analysis of study/subject samples. In Amendment 83, bluebird bio indicated that for the Phase 1/2 and long-term follow-up studies (HGB-204, HGB-205, and LTF-303), samples were evaluated at the laboratory at (b) (4). For the Phase 3 studies (HGB-207 and HGB-212), samples were evaluated at (b) (4). A bridging study conducted between (b) (4) and (b) (4) for the VCN test method. (b) (4) sets of peripheral blood samples from (b) (4) healthy donors were (b) (4)




. The percent difference between calculated VCNs from (b) (4) ranged from (b) (4) for samples with (b) (4). The % difference for the (b) (4) was (b) (4), however, the absolute difference was low (b) (4)



(b) (4) *Additionally, these VCN results are lower than any VCN values observed in any beti-cel study clinical samples. Therefore, this variability was considered acceptable.*

**Insertion site analysis**


S-EPTS/LM-PCR (shearing extension primer tag selection ligation-mediated PCR) is used to monitor BB305 insertion sites in patient samples. The method is performed and was qualified (b) (4)




***Reviewer Comment:** bluebird bio should perform additional studies to potentially lower the LOQ of this assay but this assay is considered to be suitable for the analysis of study/subject samples.*

**RP-HPLC  $\beta^{\text{A-T87Q}}$ -globin expression assay (b) (4) evaluation)**

(b) (4)




(b) (4)



**RP-HPLC  $\beta^{\text{A-T87Q}}$ -globin expression assay (b) (4) evaluation)**

(b) (4)



**Reviewer Comment:** These assays are considered suitable for the analysis of study/subject samples. In Amendment 83, bluebird bio indicated that for the Phase 1/2 and long-term follow-up studies (HGB-204, HGB-205, and LTF-303), samples were evaluated at the laboratory at (b) (4). For the Phase 3 studies (HGB-207 and HGB-212), samples were evaluated at (b) (4). The reference standard (Lot No. (b) (4)) was used to demonstrate concordance between assay results. Representative chromatograms, relative peak area, percent of total  $\beta$ -like globin chains, and hypothetical amount of each globin chain (assuming (b) (4) total Hb) data were provided supporting assay concordance.