

CBER CMC BLA Review Memorandum

BLA STN 125788

Product Name

LYFGENIA
lovotibeglogene autotemcel

Reviewers

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BLA#: STN 125788

APPLICANT NAME AND LICENSE NUMBER

Name: bluebird bio, Inc.

License Number: 2160

PRODUCT NAME/PRODUCT TYPE

Non-proprietary/Proper Name/USAN: lovotibeglogene autotemcel

Proprietary Name: LYFEGNIA

Company Codename(s): lovo-cel; bb1111; LentiGlobin BB305 Drug Product for Sickle Cell Disease

UNII Code: LOVOTIBEGLOGENE AUTOTEMCEL: 2C6A9NH2Z8

(b) (4) : (b) (4)

NDC Code: NDC73554-1111-1

GENERAL DESCRIPTION OF THE FINAL PRODUCT

- Pharmacological category: Autologous Hematopoietic Stem-Cell-Based Gene Therapy
- Dosage form: Suspension for infusion
- Strength/Potency: Between 1.7×10^6 and 20×10^6 cells/mL (b) (4) $\times 10^6$ CD34+ cells/mL)
- Route of administration: Intravenous infusion
- Indication: Treatment of patients 12 years and older with sickle cell disease and a history of vaso occlusive events (VOEs)

MAJOR MILESTONES

Pre-IND meeting	August 30, 2013
IND submission (BB-IND-15905)	March 14, 2014
IND allowed to proceed	April 10, 2014
Fast Track designation granted	May 8, 2014
Orphan Drug designation granted	February 26, 2014
Regenerative Medicine Advanced Therapy granted	October 26, 2017
Rare Pediatric Disease designation granted	May 14, 2020
Pre-BLA meeting	February 13, 2023
BLA 125788/0 submission	April 21, 2023
BLA filed	June 20, 2023
Mid-Cycle communication	August 15, 2023
Late-Cycle meeting	October 6, 2023
BLA 125788 approval date	December 8, 2023
PDUFA Action Due Date	December 20, 2023

CMC/QUALITY REVIEW TEAM

Reviewer/Affiliation	Section/Subject Matter
Takele Argaw (TA) CBER/OTP/OGT/DGT2/GTIB	BB305 LVV: 3.2.S.1 General information, 3.2.S.2 Manufacture, 3.2.S.3 Characterization, 3.2.S.4 Control of DS, 3.2.S.5 Reference standards or materials, 3.2.S.6 Container closure system
Alan Baer (AGB) CBER/OTP/OGT/DGT2/GTIB	BB305 LVV: 3.2.S.4 Control of DS; lovo-cel DP: 3.2.P.5 Control of DP

Graeme Price (GEP) CBER/OTP/OGT/DGT2/GTB5	Module 1; BB305 LVV: 3.2.S.3.2 Impurities, 3.2.S.5 Reference standards or materials; Iovo-cel DS: 3.2.S.1 General information, 3.2.S.2 Manufacture, 3.2.S.3 Characterization, 3.2.S.6 Container closure system; 3.2.P Iovo-cel DP: 3.2.P.1 Description and composition of DP, 3.2.P.2 Pharmaceutical development, 3.2.P.3 Manufacture, 3.2.P.7 Container closure system; 3.2.A Appendices; 3.2.R Regional information (USA); Other eCTD modules: Environmental assessment/claim of categorical exclusion, Reference product designation request, Labeling, Procedures and validation of analytical procedures for assessment of clinical and animal study endpoints
Brian Stultz (BS) CBER/OTP/OGT/DGT1/GTB3	BB305 LVV: 3.2.S.2.3 Control of materials; Iovo-cel DS: 3.2.S.2.3 Control of materials; Iovo-cel DP: 3.2.P.4 Control of excipients, 3.2.P.8 Stability
Andrew Timmons (AET) CBER/OTP/OGT/DGT2/GTB5	BB305 LVV 3.2.S.2.6 Manufacturing process development, 3.2.S.7 Stability; Iovo-cel DS: 3.2.S.2.6 Manufacturing process development

INTER-CENTER CONSULTS REQUESTED

Not applicable

SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/ Status
21APR2023	STN 125788/0.0	Original BLA submission
30MAY2023	STN 125788/0.1	Response to DMPQ IR#1 (Testing facility information)
01AUG2023	STN 125788/0.5	Response to CMC IR#1 (sent 21JUL2023)
14AUG2023	STN 125788/0.8	Response to CMC IR#2 (sent 07AUG2023)
01SEP2023	STN 125788/0.10	Response to DBSQC IR#1 (microbiological methods)
11SEP2023	STN 125788/0.12	Response to CMC IR#3 (sent 01SEP2023)
18SEP2023	STN 125788/0.14	Response to DBSQC IR#2 (UPLC assay validation)
20OCT2023	STN 125788/0.17	Response to late cycle meeting (stability update)
07NOV2023	STN 125788/0.22	Response to Clinical IR#10 (also included corrected 3.2.P.5.6 reflecting that all HGB-206 subjects received Iovo-cel manufactured using Process 2a)
08NOV2023	STN 125788/0.23	Response to PMRs
20NOV2023	STN 125788/0.25	Response to CMC IR#4 (sent 13NOV2023)
20NOV2023	STN 125788/0.26	Response to CMC IR#5 (sent 17NOV2023)
21NOV2023	STN 125788/0.27	Response to PMCs
29NOV2023	STN 125788/0.30	Draft container and carton labels
29NOV2023	STN 125788/0.31	Response to CMC IR#6 – Final DP specifications (sent 28NOV2023)
01DEC2023	STN 125788/0.32	Response to DBSQC IR#3 (HCP and (b) (4) assay validations)
01DEC2023	STN 125788/0.35	Revised container and carton labels
04DEC2023	STN 125788/0.37	Response to CMC IR#6 (sent 30NOV2023)

REFERENCED REGULATORY SUBMISSIONS (e.g., IND BLA, 510K, Master File, etc.)

Submission Type and number	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
BB-MF-(b) (4)	(b) (4)	(b) (4)	Yes	No issues identified CMC Reviewer: Guo-Chiuan Hung (CBER/OTP/OGT/DGT1/GTB3)
BB-MF-(b) (4)	(b) (4)	(b) (4)	Yes	No issues identified CMC Reviewer: Archana Siddam (CBER/OTP/OCTHT/DCT1/CTB1)

Submission Type and number	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
BB-MF-(b) (4)	(b) (4)	(b) (4)	Yes	No issues identified CMC Reviewer: Iain Farrance (CBER/OTP/OCTHT/DCT1/CTB1)
BB-MF-(b) (4)	(b) (4)	(b) (4)	Yes	No issues identified CMC Reviewer: Elena Gubina (CBER/OTP/OGT/DGT1/GTB3)
BB-MF-(b) (4)	(b) (4)	(b) (4)	Yes	No issues identified CMC Reviewer: Mercy Quagraine (CBER/OTP/OCTHT/DCT1/CTB1)
BB-MF-(b) (4)	(b) (4)	(b) (4)	Yes	Relevant sections in BLA reviewed by CMC reviewer Takele Argaw (CBER/OTP/OGT/DGT2/GTIB)
BB-MF-(b) (4)	(b) (4)	(b) (4)	Yes	Relevant sections in BLA reviewed by CMC reviewer Takele Argaw (CBER/OTP/OGT/DGT2/GTIB)
DMF-(b) (4)	(b) (4)	(b) (4)	Yes	Relevant sections in BLA reviewed by CMC reviewer Brian Stultz (CBER/OTP/OGT/DGT1/GTB3)
BB-MF-(b) (4)	(b) (4)	Facility Information	Yes	MF voided: No review is available. Facility was subject to pre-license inspection. Information regarding manufacturing facility is provided in the BLA

REVIEWER SUMMARY AND RECOMMENDATION

EXECUTIVE SUMMARY

The CMC review teams concludes that the manufacturing process, process controls, and test methods for lovotibeglogene autotemcel (lovo-cel; LYFGENIA) are capable of producing autologous products with consistent quality attributes that are acceptable for commercial distribution under this BLA.

lovo-cel is an autologous gene therapy product intended for the treatment of patients with sickle cell disease (SCD) and a history of vaso-occlusive events (VOEs). lovo-cel consists of an autologous CD34+ cell enriched population containing hematopoietic stem and progenitor cells (HSCs) transduced with a non-replicating lentiviral vector (LVV) referred to as BB305, containing the human β^{A-T87Q} -globin transgene sequence. The human β^{A-T87Q} -globin transgene encodes a protein identical to that of the endogenous adult β -globin protein with the exception of a threonine to glutamine substitution at residue 87. Transgene expression is regulated by the erythroid lineage-specific β -globin promoter and locus control region, so it is only expressed in erythroid cells and not in other hematopoietic cell types. Progeny erythrocytes derived from a modified HSC incorporate the β^{A-T87Q} -globin into adult hemoglobin (HbA) to form HbA^{T87Q}; this HbA^{T87Q} inhibits polymerization of sickle Hb under hypoxic conditions leading to reduced erythrocyte sickling, preventing VOEs, and reducing hemolytic anemia that are the hallmarks of SCD. Because the therapeutic β^{A-T87Q} -globin transgene is integrated into the chromosomal DNA of modified HSCs, which are a self-renewing population, a single lovo-cel treatment is expected to provide lifelong clinical benefit to the patient. HbA^{T87Q} has comparable oxygen binding and transfer characteristics to HbA containing the wild-type β -

globin chains and can be tracked by reverse phase high-performance liquid chromatography in the peripheral blood of treated patients.

Starting material for lovo-cel manufacture consists of autologous hematopoietic progenitor cells obtained by apheresis (HPC-A) collected from SCD patients following HSC mobilization with plerixafor. HPC-A is collected at a Qualified Treatment Center (QTC) and shipped to the drug substance (DS)/drug product (DP) manufacturing facility (b) (4)

[REDACTED]

The lovo-cel DS is immediately forward processed by (b) (4) the DP which is filled into one or two fluoro-ethylene-propylene bags, depending on the number of cells available. The bags containing DP are cryopreserved in a controlled rate freezer and stored in vapor phase liquid nitrogen ($\leq -140^{\circ}\text{C}$) until thawed for use. (b) (4)

[REDACTED] DP manufacture.

The lovo-cel DP is supplied frozen in 20 mL fluoro-ethylene-propylene bags as a suspension in cryopreservation media, for intravenous infusion after thawing. Each bag contains ~20 mL of a suspension of $1.7 - 20 \times 10^6$ cells/mL. The minimum dose is 3.0×10^6 CD34+ cells/kg of patient weight. More than one DP lot may be needed to achieve the minimum lovo-cel dose; in this instance the patient may undergo an additional mobilization and apheresis cycle to provide HPC-A for manufacturing an additional DP lot. The two DP lots will be infused back-to-back to meet or exceed the total CD34+ cell dose. Shipment of DP from the manufacturing site to the QTC for infusion occurs once sufficient cells to meet the minimum dose are available and the product lot(s) have been released. lovo-cel is administered as an intravenous infusion following myeloablative chemotherapy. The infused, transduced CD34+ cells engraft into the bone marrow and differentiate to reconstitute the patient's hematopoietic system, including production of erythrocytes that contain HbA^{T87Q} to prevent sickling and thus treat the patient's SCD.

The BB305 LVV is derived from a third-generation human immunodeficiency virus (HIV)-1 backbone, which has been modified to render it replication-incompetent and self-inactivating (SIN). To minimize the potential for generating replication-competent virus, viral proteins necessary for LVV production are expressed from three separate packaging plasmids used to transfect HEK293T cells grown in suspension during BB305 LVV manufacture. (b) (4)

[REDACTED]

Manufacturing process consistency is assured by qualification and tracking of raw materials and reagents, in-process monitoring and testing, manufacturing process validation and continuous process verification, and lot release and stability testing programs. Autologous product traceability is assured using a validated chain-of-identity system.

RECOMMENDATION:

APPROVAL

This Biological License Application (BLA) adequately describes the manufacturing process and characterization of lovotibeglogene autotemcel (lovo-cel; LYFGENIA). The CMC review team has concluded that the manufacturing process, along with associated test methods and control measures, are capable of producing a product with consistent quality characteristics. This information satisfies the CMC requirements for biological product licensure per the provisions of Section 315(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, associated amendments, and gathered during the pre-license inspections of the (b) (4), respectively, the CMC team recommends regular approval of this BLA.

Post-marketing commitment (PMC):

bluebird bio, Inc., commits to perform additional robustness assessments of the (b) (4) assay. The final report will be submitted as a “Postmarketing Commitment – Final Study Reports” by December 31, 2024.

Post-marketing requirement (PMR):

As authorized under Section 505(o)(3) of the Federal Food, Drug, and Cosmetic Act (FDCA), we have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to assess a serious risk of patient exposure to any unknown, at this time, extractables and leachables from the (b) (4) bag used to store and administer lovo-cel. In addition, 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, bluebird bio, Inc. will be required to conduct the following studies:

A study to evaluate leachables of the (b) (4) bag over the duration of the shelf life of lovo-cel. This evaluation will also include a full toxicological risk assessment for the identified leachables and extractables.

The ongoing leachable compound evaluation (a PMR for BLA 125755 and BLA 125717) will be extended to (b) (4) in support of lovo-cel to align with the duration of the long-term stability protocol establishing the intended shelf-life, according to the following schedule submitted on November 8, 2023:

Milestone	Timeline
Amend Leachable Testing Protocol (to add (b) (4) aged sample)	Final study protocol by January 26, 2024
Study completion	Study completion by January 30, 2025
Submit Leachable Testing Results and Toxicological Assessment	Final Study Report by March 30, 2025

COMPLETE RESPONSE (CR)

Not applicable

SIGNATURE BLOCK

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Graeme Price Review Committee Chair Supervisory Biologist CBER/OTP/OGT/DGT2/GTB5	Concur	
Takele Argaw Research Biologist CBER/OTP/OGT/DGT2/GTIB	Concur	
Alan Baer Staff Fellow CBER/OTP/OGT/DGT2/GTIB	Concur	
Brian Stultz Supervisory Biologist CBER/OTP/OGT/DGT1/GTB2	Concur	
Andrew Timmons Biologist CBER/OTP/OGT/DGT2/GTB5	Concur	
Kimberly Schultz Division Director CBER/OTP/OGT/DGT2	Concur	
Denise Gavin Director CBER/OTP/OGT	Concur	

Review of CTD

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3.2.S DRUG SUBSTANCE [BB305 Lentiviral Vector, All]

(b) (4)

[REDACTED]

[REDACTED]

110 pages determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

3.2.P DRUG PRODUCT [lovo-cel suspension for infusion, All]

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

Reviewed by GEP

The lovotibeglogene autotemcel (lovo-cel) DP consists of autologous CD34+ enriched hematopoietic stem cells obtained from patients with SCD and transduced with the BB305 LVV encoding the β^{A-T87Q} -globin gene, suspended in (b) (4) cryopreservation solution. lovo-cel is supplied as a suspension for intravenous infusion in (b) (4) fluorinated ethylene propylene bags. Components of lovo-cel are shown in Table 78.

Table 78. Components of lovo-cel

Component	Function	Quality Standard	Amount per batch
Autologous CD34+ cell-enriched HSC obtained from patients with SCD and transduced with lentiviral vector encoding the β^{A-T87Q} -globin gene (lovo-cel drug substance)	Drug substance	Specified in Section 3.2.P.5.1	1.7×10^6 to 20×10^6 cells/mL
(b) (4)	Excipient for suspension and preservation of cells in ultralow temperature environments	Specified in Section 3.2.P.4.1	20 mL per bag, up to 2 bags per lot

lovo-cel is administered as a single dose by intravenous infusion, with a single lot of DP consisting of 1 or 2 bags. Multiple DP lots may be required to meet the minimum lovo-cel dose of 3.0×10^6 CD34+ cells/kg patient weight. Thus, a patient may undergo an additional mobilization with apheresis collection cycles to obtain sufficient cells for DP manufacture to meet or exceed the minimum CD34+ cell dose. Shipment of DP lot(s) to the Qualified Treatment Center (QTC) for infusion occurs once the dose requirement has been met and all product lot(s) have been released. The multiple DP lots comprising a single dose are documented in the Lot Information Sheet accompanying the final product shipment.

3.2.P.2 Pharmaceutical Development

Reviewed by GEP

3.2.P.2.1 Components of the Drug Product

3.2.P.2.1.1 Drug Substance

Autologous CD34-enriched cell suspension transduced with the BB305 LVV encoding the β^{A-T87Q} -globin gene, washed, and suspended in (b) (4)

3.2.P.2.1.2 Excipients

The sole excipient in lovo-cel is (b) (4) a commercial cryopreservation solution containing 5% dimethyl sulfoxide (DMSO).

3.2.P.2.2 Drug Product

3.2.P.2.2.1 Formulation Development

Lovo-cel is formulated in commercially available cryopreservation media, (b) (4) (containing 5% DMSO, described in 3.2.P.4 Control of Excipients). This cryomedia was used for all batches of lovo-cel described in sections 3.2.P.3.5 Process Validation and/or Evaluation and. (b) (4) was selected based on its cryoprotectant properties, absence of protein, and ability to be infused without further manipulation at the treatment site. Suitability of this formulation was examined by assessing (b) (4) and recovery data at (b) (4) post thaw from process development and engineering DP runs manufactured at commercial scale from mobilized healthy donor HPC-A, as summarized in Table 79.

(b) (4)

In this study, DP bags were removed from (b) (4)

Reviewer comment: Note that these post-thaw stability studies were performed using BB02 process development and engineering lots (representing beti-cel, a similar product containing the same structural elements, CD34+ cells transduced with the same BB305 vector but intended to treat β -thalassemia). Use of these lots to assess post-thaw stability is acceptable as the cells were derived from healthy donors, manufactured

using a similar process, and formulated and filled in an identical way to lovo-cel. The assessment that DP is stable at room temperature for 4 hours post-thaw is acceptable. Although both recovery and viability were slightly decreased at this time relative to immediately post-thaw, effect on delivered dose is expected to be minimal.

3.2.P.2.2.2 Overages

There are no overages.

3.2.P.2.2.3 Physicochemical and Biological Properties

Lovo-cel is intended to treat patients with SCD, a hereditary blood disorder caused by a point mutation in codon 6 of the β -globin gene that results in formation of an abnormal β^{E6V} -globin (β^{S} -globin) that is incorporated into hemoglobin to form sickle hemoglobin (HbS). SCD occurs in those who are homozygous for β^{S} -globin. In these individuals hypoxia causes HbS to form rigid polymers within erythrocytes, resulting in erythrocytes assuming a sickle shape and hemolytic anemia, life-threatening infections, and multiple organ damage characteristic of VOE's.

Lovo-cel is intended to deliver autologous CD34+ enriched hematopoietic stem cells transduced with BB305 LVV to contain functional $\beta^{\text{A-T87Q}}$ -globin under control of an erythroid lineage-specific human β A-globin promoter and enhancer elements from the β -globin locus control region. This will drive transgene expression during erythroid differentiation, resulting in erythrocytes that possess the $\beta^{\text{A-T87Q}}$ -globin combined with endogenous α -globin to form HbA^{T87Q}. $\beta^{\text{A-T87Q}}$ -globin has anti-sickling properties as the side chain of glutamine at position 87 sterically inhibits a hydrophobic pocket, blocking a valine residue on the adjacent Hb molecule from binding and therefore inhibiting polymerization. Key properties relating to lovo-cel DP performance and biological activity are shown in Table 80.

(b) (4)

3.2.P.2.3 Manufacturing Process Development

Processes for lovo-cel DP formulation and cryopreservation changed over the HGB-205, HGB-206 and HGB-210 studies, as outlined in Table 81.

Table 81. lovo-cel DP formulation and cryopreservation process development

Parameter	HGB-205	HGB-206	HGB-210
Manufacturing site	(b) (4)	(4)	
First manufacture date			
Cryopreservation			
Formulation			
CCS			
Storage			
Infusion			

(b) (4)

Reviewer comment: The original HGB-205 process involved significant (b) (4) manipulation to formulate product for infusion. This process was discontinued in 2015 and was not used in the HGB-206 or -210 studies nor proposed for commercial use.

The applicant conducted a risk assessment of formulation and cryopreservation processes using a FMEA model to identify high, medium, and low risk parameters that could affect viability, post-thaw recovery, and/or colony formation ability. The FMEA also leveraged knowledge from the beti-cel process as outlined in Table 82.

Reviewer comment: beti-cel and lovo-cel are both autologous CD34+ enriched cell products transduced with the BB305 LVV, but are manufactured by different CMOs for different indications (treatment of transfusion-dependent β -thalassemia, in the case of beti-cel). DP manufacture is largely identical for these two products, making it appropriate to apply process knowledge from beti-cel to assess the lovo-cel process.

(b) (4)

(b) (4)

1 page determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

Reviewer assessment: There are no concerns with characterization of the DP formulation and freeze process, or the process parameter criticality classifications and operating ranges established as a result.

3.2.P.2.4 Container Closure System

The Iovo-cel container closure system (CCS) is reviewed in Section 3.2.P.7 Container Closure System.

3.2.P.2.5 Microbiological Attributes

Iovo-cel is manufactured under aseptic conditions and final DP samples drawn from the CCS are tested for sterility and endotoxin for lot release. DP is stored frozen and contains no preservatives. Container closure integrity testing (CCIT) was performed to confirm that the CCS maintains a sterile barrier. Bags were filled with (b) (4)

Reviewer assessment: Results from the CCIT are acceptable to demonstrate microbiological suitability of the CCS.

3.2.P.2.6 Compatibility

In-use studies were performed to show DP stability and compatibility with infusion sets. These studies were conducted using (b) (4) healthy donor-derived lots manufactured at the bluebird bio process-development laboratory and (b) (4) lot manufactured at (b) (4); all lots were formulated in (b) (4) and cryopreserved in (b) (4) bags. The study design used (b) (4)

1 page determined to be not releasable: (b)(4)

Overall Reviewer's Assessment of Section 3.2.P.2:

The information provided in this section is acceptable. Formulation and cryopreservation development studies were adequately performed, and container closure system microbiological attributes and product compatibility have been demonstrated using appropriate test methods and in-use stability studies.

3.2.P.3 Manufacture

Reviewed by GEP

3.2.P.3.1 Manufacturer(s)

The manufacturing and testing sites for lovo-cel DP are listed in Table 85.

Table 85. Manufacturing and testing facilities for lovo-cel DP

Facility	FDA identification numbers	Responsibilities	Release tests performed
(b) (4)			

Reviewer comment: Information regarding testing performed at each facility was provided in Amendment #1 (response to DMPQ IR of 23MAY2023, received 30MAY2023). This information is acceptable.

3.2.P.3.2 Batch Formula

The batch formula for lovo-cel is summarized in Table 85 (see Section 3.2.P.1 Description and Composition of the Drug Product).

3.2.P.3.3 Description of Manufacturing Process

Manufacture of lovo-cel DP continues from the manufacture of lovo-cel DS (described in 3.2.S.2 Manufacture) with (b) (4). DP manufacturing consists of the formulation and cryopreservation step, involving (b) (4)

(b) (4)

Chain-of-identity (COI) is maintained by a combination of physical labeling and procedural controls, with the same batch number used throughout the manufacturing process. End-to-end traceability of cells from collection, through manufacturing, receipt at the QTC, and administration to the patient is described in Chain-of-Identity (COI).

The DP manufacturing process flow is shown in Figure 32.

(b) (4)

Formulation, filling, and cryopreservation

(b) (4)

(b) (4)

Once freezing is complete the cassette(s) is transferred to vapor phase LN₂ for storage.

Product packout and shipment

When released product is to be shipped to the QTC, the metal cassette(s) containing lovo-cel are placed in a cassette holder that is then loaded into vapor phase LN₂ of a cryoshipper qualified to maintain a temperature of $\leq -140^{\circ}\text{C}$ throughout the duration of shipment. Up to 4 bags of DP manufactured for the patient to achieve the required dose are packed into the same cryoshipper. (b) (4)

. A Lot Information Sheet (LIS; described below) providing QTC staff with complete information regarding the DP for a given product is included in each shipment. Once packout is complete the product is shipped. On receipt at the QTC, lovo-cel is required to be stored at $\leq -140^{\circ}\text{C}$.

Chain-of-Identity (COI)

The applicant has established a COI strategy based on current Good Tissue Practices, as defined in 21 CFR 1271.290, to ensure that all operations from patient enrollment to delivery of DP to the QTC for administration are controlled to assure that each individual patient is infused with DP manufactured from their own hematopoietic progenitor cells.

The COI system is maintained by a combination of physical labeling and procedural controls based on three unique traceability identifiers:

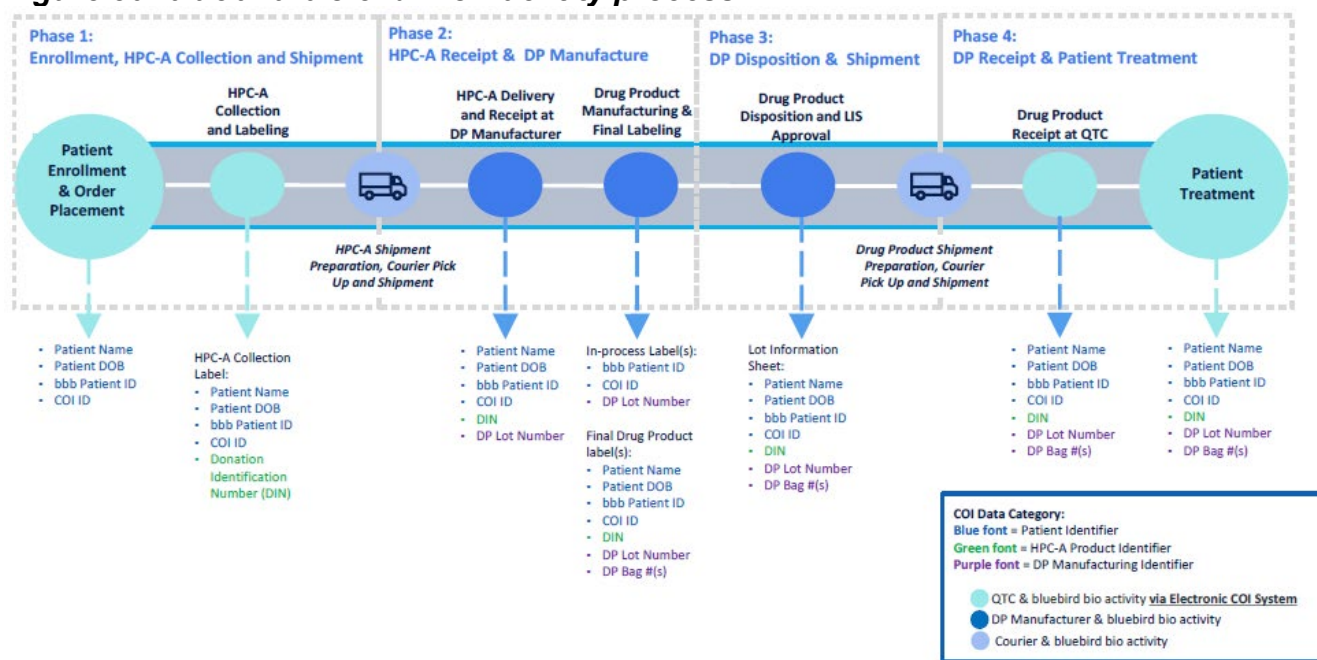
- *Patient Identifiers:* These are unique and specific to the donor/patient. Patient identifiers are assigned early in the treatment process and remain unchanged from enrollment to delivery of the product. Patient identifiers are specifically assigned by the QTC and/or bluebird bio (bbb) and include Patient Name, date of birth (DOB) bbb Patient ID, and bbb COI ID.
- *HPC-A identifier:* This is unique and specific to each HPC-A collection and is used to identify the autologous HPC-A material. The HPC-A identifier follows the International Society of Blood Transfusion (ISBT) global standard 128 for human tissue and cell labeling requirements and is also known as the Donation Identification Number (DIN). This is assigned by the QTC responsible for collecting the HPC-A.
- *Drug Product (DP) Manufacturing/Product Identifiers:* These are unique and specific to each DS and DP batch and are used to identify the manufactured drug product. DP Manufacturing/Product identifiers are assigned by the DP manufacturer and include DP lot number and bag number.

For clinical development the sole bbb patient identifier was the clinical trial Subject ID. Commercial patient identifiers are Patient Name, DOB, bbb Patient ID, and bbb COI ID. The commercial HPC-A identifier and DP Manufacturing/Product ID are the same as for clinical trial manufacture.

A Lot Information Sheet (LIS) is generated for each patient during commercial manufacturing. The LIS contains the Patient Identifiers, HPC-A Identifier, and DP Manufacturing/Product ID in addition to specific information related to the DP lot(s) included in the shipment. This includes the number of DP lots and infusion bags provided for the patient, and the patient-specific dose (CD34+ cells/kg) defined as the total number of CD34+ cells from all bags and lots divided by the patient's weight. The LIS is included in each DP shipment and provides a complete set of unique traceability identifiers specific for that patient. This is intended to increase transparency and provide additional detail about the patient and their autologous DP to further assure that each patient receives the correct DP.

The unique identifiers are linked via a combination of paper and electronic system verifications that occur between bluebird bio, the QTC, and the manufacturer, starting at patient enrollment. Successful COI verifications are required before proceeding with the next step, as outlined in Figure 33.

Figure 30. bluebird bio chain-of-identity process



There are 4 COI phases, each containing defined COI data elements tracked by bluebird bio, the QTC, and the DP manufacturer, as follows:

Phase 1: Enrollment, HPC-A collection and shipment. COI transactions occur between bluebird bio and QTC.

- Patient enrollment and order placement: QTC provides patient identifiers (Patient Name, DOB) to bbb who assign unique bbb Patient ID and unique bbb COI
- HPC-A collection: QTC assigns HPC-A identifier (DIN); COI traceability is assured via verification of COI unique identifiers on HPC-A label(s)
- HPC-A shipment from QTC to DP manufacturer: Verification of COI unique identifiers on HPC-A label(s) and shipment documentation

Phase 2: HPC-A receipt and DP manufacture. COI transactions occur between bbb and the DP Manufacturer.

- HPC-A delivery and receipt at DP Manufacturer: Verification of COI unique identifiers on HPC-A label(s) with both site records and shipment documentation
- DP manufacturing and labeling: DP Manufacturer verification of COI identifiers in DS in-process labels, batch documentation or associated records with the product

Phase 3: DP disposition and shipment. COI transactions occur between bbb and the DP Manufacturer.

- DP disposition: bbb verification of COI identifiers in executed batch record during disposition and LIS approval.
- DP shipment from DP manufacturer to QTC: DP Manufacturer verification of COI identifiers on DP cassette(s) with shipment documentation and LIS

Phase 4: DP receipt and patient treatment. COI transactions occur between bluebird bio and QTC.

- DP receipt at QTC: COI traceability is assured by QTC verification of COI unique identifiers on shipment documentation, LIS and DP labels.
- DP administration: QTC verification of COI unique identifiers present on LIS and DP labels

Labeling of HPC-A, in-process DS, and DP is a key component of the traceability system. Labels contain a combination of human-readable text and optical machine-readable linear and 2-dimensional barcodes. Information included on each label, and example data elements and formats, are shown in Table 87.

Table 87. Chain-of-Identity (COI) identifiers for labels

COI Unique identifier Category	Example COI data element	Example format COI data element	Process	
			(b)	(4)
Patient identifiers	Patient Name ^A	Doe, Alex Charlie		
	Date of Birth (DOB) ^A	YYYY-MM-DD		
	bbb Patient ID ^B	(b) (4)		
	bbb COI ID ^B	(b) (4)		
HPC-A identifier	DIN ^B	(b) (4)		
DP Manufacturing/Product identifiers	DP lot number ^C	(b) (4)		
	DP bag number ^C	Bag X of Y		

DIN = Donation Identification Number

^A QTC communicated/assigned COI data element

^B bluebird bio assigned COI data element

^C DP Manufacturer assigned data element

Validation of the COI system is described in Section 3.2.P.3.5 Process Validation and/or Evaluation, Chain-of-identity (COI) validation.

Overall Reviewer's Assessment of Section 3.2.P.3.3:

The description of the DP manufacturing process, including cryopreservation, packout, shipment, and the COI system, is acceptable.

3.2.P.3.4 Controls of Critical Steps and Intermediates

Process parameters used during DP manufacture were established as described in 3.2.P.2.3 Manufacturing Process Development and are shown in Table 88. No performance attributes are defined for these steps.

Table 88. Process parameters for DP formulation and cryopreservation

Parameter	Classification	Set point	Normal operating range	Proven acceptable range		
lovo-cel formulation process step						
Cell concentration at freeze (cells/mL)	CPP		(b) (4)			
Formulation media temperature (°C)	CPP					
Time in cryoprotectant before freeze (min)	Non-CPP					
Number of DP bags	OP	20 mL – 1 bag 40 mL – 2 bags				
lovo-cel cryopreservation proces						
CRF profile	Non-CPP	See Table 93				
Time in CRF at (b) (4)	OP					
Time on (b) (4)	OP					

CPP = Critical Process Parameter, OP = Operating Parameter, min = minutes, CRF = Controlled-Rate Freezer, LN₂ = liquid nitrogen

Cell concentration at freeze is controlled within the NOR via the total cell count at the end of DS post-transduction wash step. Formulation media temperature is controlled by storage of formulation media under refrigeration per the batch record instructions. The volume of cryopreservation media used for resuspension is based on the total number of cells, yielding either 20 mL (one bag) or 40 mL (two bags) of final product once all sampling is complete.

Overall Reviewer's Assessment of Section 3.2.P.3.4:

Critical steps during the formulation and cryopreservation processes are adequately controlled. There are no concerns.

3.2.P.3.5 Process Validation and/or Evaluation

(b) (4)

(b) (4)

Overall Reviewer's Assessment of Section 3.2.P.3.5:

Validation of the lovo-cel manufacturing process and associated activities (shipping, COI, and CPV) is acceptable. While deviations occurred during the PPQ campaign, resulting in invalidation and replacement of (b) (4) PPQ runs due to extrinsic root causes, these deviations were appropriately investigated with implementation of CAPAs initiated to prevent recurrence of these events. There are no outstanding concerns regarding validation of the lovo-cel DS/DP manufacturing process.

3.2.P.4 Control of Excipients

Reviewed by BS

The sole excipient in 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 Excipients

Not applicable

Overall Reviewer's Assessment of Section 3.2.P.4:

(b) (4) is the sole excipient for lovo-cel. There are no concerns regarding this cryopreservation medium.

3.2.P.5 Control of Drug Product

Reviewed by AGB

3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)

Table 98. Release and post-approval stability specifications for lovo-cel

Quality Attribute	Test	Method	Acceptance Criteria	
			Release	Justification
Potency and Strength	Vector Copy Number (VCN)	(b) (4)	(b) (4)	LL aligns lowest observed VCN in clinical lot achieving VOE-CR in study HGB-206. UL aligns beti-cel upper limit
	(b) (4)	(b) (4)	(b) (4)	Limit aligns lowest observed clinical lot achieving VOE-CR
	(b) (4)	(b) (4)	(b) (4)	LL aligns to minimum observed value in studies HGB-206 and HGB-210
	(b) (4)	(b) (4)	(b) (4)	Mean (b) (4)
	(b) (4)	(b) (4)	(b) (4)	Limit aligns lowest observed clinical lot achieving VOE-CR
			(b) (4)	(b) (4)

Quality Attribute	Test	Method	Acceptance Criteria	
			Release	Justification
	β^A -T87Q-globin Quantitative Protein Expression	(b) (4)	(b) (4) β^A -T87Q-globin (relative to (b) (4))	LL aligns lowest observed clinical lot achieving VOE-CR. UL mean (b) (4)
Identity	β^A -T87Q-globin quantitative protein expression		(b) (4)	Not applicable
	(b) (4)	(b) (4)	(b) (4)	Meets set markers
Purity and Content	(b) (4)		(b) (4)	Mean (b) (4) manufacturing experience
	(b) (4)	(b) (4)	(b) (4)	Based on calculations and clinical experience
	Total cell concentration ^C		(b) (4)	Based on calculations and clinical experience
	(b) (4)		(b) (4)	DS: aligns to beti-cel experience and mean - (b) (4) of HGB-206/210 lots DP: aligns to DS AC
Safety	Sterility ^D	(b) (4)	No growth	Compendial method
	Endotoxin	(b) (4)	(b) (4)	Compendial method
	Mycoplasma	(b) (4)	None detected	Compendial method
Quality	Appearance	Visual assessment	Colorless to white to red, including shades of white or pink, light yellow, and orange	Clinical experience

qPCR = Quantitative Polymerase Chain Reaction; %LVV+ = Percent lentiviral vector-positive; vc = vector copy; tc = transduced cell; dg = diploid genome; UPLC = Ultra-performance Liquid Chromatography; DS = Drug Substance; DP = Drug Product; EU= Endotoxin Units; LL = lower limit; UL = upper limit

^A (b) (4) assessment only applicable to patient lot material and availability; cannot be performed on Healthy Donor (HD) stability lots

^B Performed on cryopreserved Drug Substance and cryopreserved Drug Product

^C Performed on fresh Drug Substance and cryopreserved Drug Product

^D Performed on In-Process Drug Substance Supernatant and Final Drug Product

^E Test on final Drug Product performed with modified (b) (4) method using reduced sample volume (b) (4) of total drug product volume)

Reviewer comment: DP commercial lot release specifications and justification of specifications were reviewed and revised in Amendment #25 (response to CMC IR of 03NOV2023, received 20NOV2023) and Amendment #31 (response to CMC IR of 28NOV2023 received 31 29NOV2023). The (b) (4) specification was revised to align with the minimum observed value in studies HGB-206 and HGB-210. The (b) (4) cells specification was revised from the originally proposed (b) (4) (based on mean (b) (4) (mean (b) (4) of the (b) (4) manufacturing experience. (b) (4) for DS was revised from the originally proposed (b) (4) based on the lowest observed value from clinical experience with beti-cel; the (b) (4) specification also aligns closely with the mean (b) (4) of all Process 2A lots used in studies HGB-206 and HGB-210. The DP (b) (4) specification of (b) (4) was revised from the originally proposed (b) (4) to align with the revised DS (b) (4) specification.

These final commercial specifications were confirmed in Amendment #31 which also included a revised justification of specifications and confirmation that the commercial specifications would also be applied to the stability testing program. Final commercial specifications are acceptable.

Drug product release tests are performed on samples taken at various steps during the (b) (4) Iovo-cel DS/DP manufacturing process. Information on the sampling points for tests that are performed on process intermediates, DS/DP, including justifications for testing at process stages other than DP are provided.

Table 99. Sampling points, matrices, and justifications for Iovo-cel release testing

Release Test	Sampling Point	Testing Matrix	Justification
Mycoplasma	(b) (4)		
Sterility			
(b) (4)			
Total cell concentration			
(b) (4)			
Vector copy number (VCN)			
(b) (4)			
(b) (4)			
β A-T87Q-globin quantitative protein expression and identity			
(b) (4)			
(b) (4)			
(b) (4)			
(b) (4)			
(b) (4)			
Total cell concentration			
(b) (4)			
Appearance			
Sterility			
Endotoxin			

^A Matched untransduced (UT) patient CD34+ cells for (b) (4) test are obtained at day (b) (4) culture washes.

Reviewer comment: In Amendment #26 (response to CMC IR of 17NOV2023, received 20NOV2023) the applicant confirmed that the (b) (4) identity test sample is pulled from formulated DP (b) (4). The sample for β^{A-T87Q} -globin QPE, which serves as a transgene-specific identity test, is pulled (b) (4) to conserve cells for dosing. The manufacturing process and COI procedures do not allow for mix-ups between sampling and DP fill. These procedures are in alignment with the commercial beti-cel release testing strategy.

Overall Reviewer's Assessment of Sections 3.2.P.5.1 and 3.2.P.5.6:

DP specifications were revised in Amendments #25 and #31 following communications with the applicant, as described above. The final commercial lot release specifications for lovo-cel are acceptable and adequately justified based on clinical and manufacturing experience. There are no outstanding concerns with lovo-cel DP specification or justification of these specifications.

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

Reviewed by AGB

(b) (4)

25 pages determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

Sterility

Verification of Sterility method for lovo-cel DP was performed using healthy donor CD34+ cells transduced with BB305 LVV in accordance with (b) (4) using (b) (4)

sample volume of (b) (4). Additionally, the verification of the sterility method for the lovo-cel (b) (4) was performed in accordance with (b) (4) of the (b) (4) volume.

Reviewer Comment: Fully reviewed by DBSQC, assay determined to be appropriate for its intended use.

Endotoxin

Verification of the (b) (4) method performed using healthy donor (b) (4) with BB305 LVV (Mock DP). Specifically, the matrix used in method verification consisted of Mock DP at (b) (4)

Reviewer Comment: Fully reviewed by DBSQC, assay determined to be appropriate for its intended use.

Mycoplasma

(b) (4) mycoplasma method was verified per (b) (4). Healthy donor (b) (4) with BB305 LVV (Mock DP) was used for verification. The verification was evaluated for the detection of mycoplasma challenge organisms at (b) (4) forming units (CFU) in the Mock DP. (b) (4) were used to demonstrate the presence and absence of Mycoplasma, respectively. *Reviewer Comment: Fully reviewed by DBSQC, assay determined to be appropriate for its intended use.*

Appearance

Validation of the appearance method was performed using samples that represent colors within and outside of the appearance color specification acceptance criteria for lovo-cel when compared to the color standard. The samples used for validation were prepared using commercially available reagents, including (b) (4) standards.

(b) (4)

(b) (4)

Reviewer Comments: Assay determined to be appropriate for its intended use.

Overall Reviewer's Assessment of Sections 3.2.P.5.2 and 3.2.P.5.3:

Additional information regarding robustness testing of the VCN assay was provided in Amendment #26 following an information request and was found acceptable. This amendment also contained information regarding robustness testing for the (b) (4) assay, which was not sufficient. The applicant proposed additional robustness testing for this assay as a post-marketing commitment, with a final study report to be submitted by 31JUL2024. This timeline is acceptable. The analytical methods used for lot release testing of lovo-cel are otherwise appropriate for their intended use and have been adequately validated.

3.2.P.5.4 Batch Analyses

Reviewed by AGB

The batch analysis test results are presented for lovo-cel batches manufactured for subjects with SCD treated in clinical studies HGB-206 and HGB-210. All lovo-cel batches were manufactured at (b) (4) according to Process 1, Process 2, and Process 2a. For HGB-206, Group A subjects were treated with lovo-cel manufactured with Process 1, Group B subjects were treated with lovo-cel manufactured with Process 2, and Group C subjects were treated with lovo-cel manufactured with Process 2a. All subjects in HGB-210 to date have been treated with lovo-cel manufactured with Process 2a.

For the HGB-206 clinical study this includes (b) (4) batches manufactured using BB305 with adherent produced LVV. These lovo-cel batches were used to treat (b) (4) subjects in clinical study HGB-206. (b) (4) lovo-cel batches were manufactured using BB305 suspension LVV. These lovo-cel batches were used to treat (b) (4) subjects in clinical study HGB-206. Of these (b) (4) subjects, (b) (4) subjects were treated with (b) (4) batches of lovo-cel where (b) (4) batch was manufactured with aLVV and (b) (4) batch was manufactured with sLVV. The HGB-210 clinical study treated patients with batches of lovo-cel solely manufactured with sLVV. Over the course of HGB-210, the manufacture of sLVV was transferred from (b) (4) (3.2.S.2.6 Manufacturing Process History). To date, (b) (4) lovo-cel batches have been manufactured for the HGB-210 clinical study, from which (b) (4) subjects have been treated.

In addition, the cells for all Process 1 and Process 2 lovo-cel batches were obtained from bone marrow, the cells for all Process 2a lovo-cel batches were obtained by apheresis. (b) (4) subjects with SCD received lovo-cel in clinical study HGB-205 (b) (4) batches). Drug product for this study was manufactured at the treatment location, (b) (4) according to Process 0.

Manufacturing history(s) and a comparison(s) of Process for the cellular DS and LVV BB305 DS are respectively provided in 3.2.S.2.6 Manufacturing Process Development (Cell DS) and Manufacturing Process History (BB305 Lentiviral Vector).

Product data analysis performed to determine lovo-cel DP release specification are described in Specification section 3.2.P.5.1 and Justification of Specification section 3.2.P.5.6.

Reviewer comments: Sponsor also notes that as of August 2022, (b) (4) lots of betibeglogene autotemcel, for Transfusion Dependent Thalassemia, under clinical studies HGB- 204, HGB-207 and HGB-212, are also manufactured by (b) (4) using similar manufacturing and testing strategies, helping support the manufacture and safety evaluation of lovo-cel DP.

Deviations: Under process 2a Clinical lots, (b) (4) lot was rejected due to a shipment problem (Lot #BB03(b) (4)), another Clinical lot was rejected due to an OOS on VCN (Lot # BB03-(b) (4)). For Lot # BB03-(b) (4) , DP lot release criteria 'Sickled Cells' result not generated for this lot due to a contamination of the un-transduced control. Request to release this material was discussed with and agreed to by FDA.

3.2.P.5.5 Characterization of Impurities

Reviewed by AGB

Lovo-cel is manufactured from lovo-cel cellular material and LVV BB305 drug substance with (b) (4) . Characterization of potential impurities in lovo-cel and LVV BB305 drug substances are discussed in respective Impurities Sections 3.2.S.3.2.

Overall Reviewer's Assessment of Sections 3.2.P.5.4 and 3.2.P.5.5:

The lovo-cel batch analysis information and impurities assessments are acceptable and were adequate to support the final commercial DP lot release specifications.

3.2.P.6 Reference Standards or Materials

Reviewed by AGB

The testing strategy (in-process, release, and stability testing) of lovo-cel does not require a reference standard for control and monitoring.

3.2.P.7 Container Closure System

Reviewed by GEP

The container closure system for lovo-cel consists of a (b) (4) 20 mL fluorinated ethylene propylene (FEP) cryopreservation bag (primary package container) enclosed within a (b) (4) (secondary package container) placed within a cryocassette (tertiary package container).

(b) (4) bags are manufactured by (b) (4) per cGMP in an (b) (4) clean room to the specifications outlined in Table 112. The maximum fill is (b) (4) but the nominal volume for lovo-cel is 20 mL. No color additives or perfluorinated compounds are used during bag manufacture. Bags are (b) (4) pressure tested with filtered (b) (4) by a validated method to achieve a sterility assurance level (SAL) of \geq (b) (4) .

Figure 33. Schematic diagram of the cryogenic storage cassette (tertiary packaging)

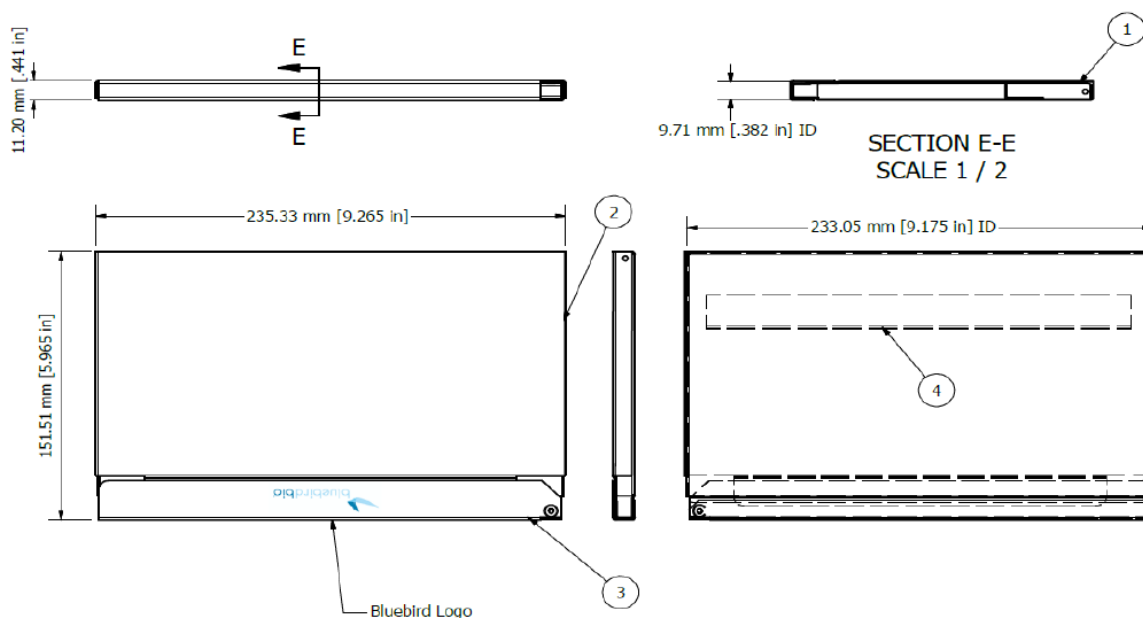


Figure 36. Cryocassette parts: 1. Base, 2. Cover, 3. Latch, 4. Flange. The latch is blue to differentiate it from similar products.

3.2.P.8 Stability

Reviewed by BS

3.2.P.8.1 Stability Summary and Conclusion

Lovo-cel is a patient specific DP and is manufactured with a limited number of CD34+ cells obtained from the patient, therefore, lovo-cel cannot ethically be used for stability testing. For this reason, stability studies are performed on lots of drug product manufactured from CD34+ cells obtained from healthy donors. Data supports the suitability of healthy donor CD34+ cells as surrogates for SCD patient CD34+ cells (provided in 3.2.S.2.6 - DS Process Design and Characterization). Additionally, the patient container/closure for lovo-cel is a 20 mL (b) (4) Fluoro-Ethylene-Propylene (FEP) cryopreservation bag (b) (4) with a routine 20 mL fill volume. A similar reduced size container closure is used for stability samples, including the same material of construction. The container closure for stability samples is a (b) (4) FEP cryopreservation bag (b) (4) with a routine (b) (4) fill volume. Samples for accelerated and stress studies, as well as the (b) (4) assay, are aliquoted prior to cryopreservation at a target concentration of (b) (4) $\times 10^6$ viable cells/mL into (b) (4) cryogenic vials. The stability of lovo-cel is supported by DP lots on long term stability at $\leq -140^\circ\text{C}$ as well as one accelerated study (b) (4) study, and one (b) (4) study performed on PPQ lots. The test methods used for stability are a subset of the lovo-cel release test methods.

Stability is demonstrated by VCN, (b) (4), and Total Cell Concentration and confirmed by results of (b) (4), and the $\beta^{\text{A-T87Q}}$ globin Quantitative Protein Expression assay.

Bluebird requests a shelf life of (b) (4) months for lovo-cel stored at the long-term storage condition of $\leq -140^{\circ}\text{C}$.

Reviewer Comment: Stability data supports a shelf life of (b) (4) months (see Table 131). However, the (b) (4) cryopreservation bag leachables study was conducted for only 12 months. The shelf-life for lovo-cel cannot exceed that supported by the leachables study for the container closure system and should therefore be set at 12 months until the final leachables study has been submitted and reviewed.

Conclusions from Stability Studies

At the recommended long-term storage condition ($\leq -140^{\circ}\text{C}$), all stability attributes except (b) (4) and Total Cell Concentration remain within acceptance criteria for at least 6 months for (b) (4), 9 months for (b) (4) for (b) (4) lots of representative drug product, and no trends were observed. (b) (4) and Total Cell Concentration results were compromised by assay variability due to inconsistent thawing, but no trend was observed. A CAPA was implemented to control the thaw procedure at the testing site.

Accelerated stability studies at (b) (4) for one PPQ lot are complete with no apparent trends observed. Data supports temperature excursions outside the long-term storage and shipping conditions of $\leq -140^{\circ}\text{C}$. Stress stability studies (b) (4) for (b) (4) PPQ lots are complete.

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

Post-approval, one drug product lot will be manufactured at the commercial manufacturing site during each calendar year, using the commercial manufacturing process with CD34+ cells from healthy donors. The drug product will be placed on stability at the long-term storage condition of $\leq -140^{\circ}\text{C}$ and evaluated according to the stability protocol in Table 113. Additionally, drug product stability may be evaluated in support of significant drug product manufacturing changes.

Table 113. Post-approval stability testing protocol

Test	Acceptance Criteria	Time Point (Months)				
		0	3	6	9	12
Vector Copy Number	(b) (4)	P	P	P	P	P
(b) (4)		P	NR	NR	NR	P
(b) (4)		P	NR	NR	NR	P
(b) (4)		P	NR	NR	NR	NR
(b) (4)		P	P	P	P	P
		P	P	P	P	P
β A-T87Q-globin Quantitative Protein Expression		P	P	P	P	P
(b) (4)		P	NR	NR	NR	P
Total Cell Concentration		P	P	P	P	P
(b) (4)		P	P	P	P	P
Sterility	No Growth	P	NR	NR	NR	NR
Endotoxin	(b) (4)	P	NR	NR	NR	NR

Test	Acceptance Criteria	Time Point (Months)					(b) (4)
		0	3	6	9	12	

vc = vector copies; dg = diploid genome; tc = transduced cell

P: Testing is planned at this timepoint.

NR: Testing is not required at this timepoint.

^A The In (b) (4) assessment only applicable to patient lot material and availability; cannot be performed on Healthy Donor lots.

Reviewer Comment: The β^{A-T87Q} -globin expression stability acceptance criterion is lower than that necessary for lovo-cel release because the expression level in healthy donor lots is lower than that in patient lots. The (b) (4) assay is included in the long term stability protocol in the event that SCD patient-derived lots become available for testing and are not required for clinical use (such as if the patient withdraws from treatment after product has been manufactured); this is expected to be an uncommon situation.

3.2.P.8.3 Stability Data

Results of stability studies performed at long-term ($\leq -140^{\circ}\text{C}$, vapor phase liquid nitrogen), accelerated (b) (4) and stressed (b) (4) conditions, as well as a (b) (4) cycle are provided in this section. The available stability data for lovo-cel drug product lots are summarized in Table 114.

(b) (4)

Reviewer Comment: Actual stability data is not provided here for space considerations. The totality of stability data supports an (b) (4) shelf life (although shelf-life is limited to 12 months due to the duration of the CCS leachables study). The last stability update submitted in Amendment #17 (received 20OCT2023) is suitable, with no trends observed.

3.2.A APPENDICES

Reviewed by GEP

3.2.A.1 Facilities and Equipment

A pre-license inspection (PLI) of the (b) (4) facility located at (b) (4) was conducted by CBER/DMPQ and CBER/OGT inspectors between (b) (4) to support approval of BLA 125788. A Form FDA 483 was issued at the end of the inspection. The firm adequately responded to the observation. All inspectional issues were resolved, and the inspection was classified as voluntary action indicated (VAI). Details are provided in the Establishment Inspection Report (EIR)

A PLI of the (b) (4) facility located at (b) (4) was conducted for the BB305 lentiviral vector manufacturing and fill/finish activities by CBER/DMPQ and CBER/OGT inspectors between (b) (4). No objectionable issues were identified, no Form FDA 483 was issued, and the inspection was classified no action indicated (NAI). Details are provided in the EIR.

Overall Reviewer's Assessment of Section 3.2.A.1:

Manufacturing facilities for the BB305 LVV and lovo-cel were inspected and fully reviewed by CBER/DMPQ. Please see that review for details. Facilities were deemed suitable to support the BLA.

3.2.A.2 Adventitious Agents Safety Evaluation

Control of adventitious agents in lovo-cel and the BB305 LVV is achieved via a three-tiered approach summarized below:

- (b) (4)

***Reviewer comment:** Descriptions of adventitious agents control are integrated into sections 3.2.S.2.3 Control of Materials, 3.2.S.2.3 Control of Materials, 3.2.P.2.5 Microbiological Attributes, 3.2.P.4 Control of Excipients, 3.2.P.5 Control of Drug Product, and 3.2.S.4 Control of Drug Substance.*

Viral Clearance Studies

Viral clearance studies were not performed on the BB305 LVV or lovo-cel DP. However, clearance of BB305 LVV during lovo-cel manufacture was assessed in section 3.2.S.3.2 Impurities and found acceptable.

Overall Reviewer's Assessment of Section 3.2.A.2:

Adventitious agents and microbiological contamination are adequately controlled during BB305 LVV and lovo-cel manufacture. There are no outstanding concerns regarding adventitious agent safety.

3.2.A.3 Novel Excipients

Not applicable – no new excipients are used.

3.2.R Regional Information (USA)**Executed Batch Records***Reviewed by GEP*

Executed batch records for (b) (4) lot of BB305 LVV (Fill/Finish lot (b) (4) ; PPQ run (b) (4) manufactured at (b) (4) , and (b) (4) lot of lovo-cel (SCD lot # BB03-(b) (4) PPQ run (b) (4) manufactured at (b) (4) are provided. An unexecuted master batch record (MBR) for the lovo-cel DS/DP (MBR-7476 Revision 5, effective 11APR2022) is also provided.

Reviewer comment: Batch records (including associated deviations) were further reviewed during inspection of the (b) (4) facilities. The master batch records are consistent with the procedures described in the BLA and are followed during BB305 LVV and lovo-cel DS/DP manufacture. See EIRs for additional details.

COVID Risk Assessment During Product Manufacture*Reviewed by GEP*

A risk assessment to evaluate potential transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) to bluebird bio products (bei-cel, eli-cel, and lovo-cel) was performed per the recommendations in FDA guidance documents “Manufacturing Considerations for Licensed and Investigational Cellular and Gene Therapy Products During COVID-19 Public Health Emergency” (January 2021) and “Good Manufacturing Practice Considerations for Responding to COVID-19 Infection in Employees in Drug and Biological Product Manufacturing (June 2020)”. This assessment considered collection and processing of the apheresis material, manufacture of the lentiviral vector, and manufacture of the autologous CD34+ enriched cell DP. No medium or high risks were identified, and no risk control plan is necessary. lovo-cel is derived from autologous HPC-A, so donor screening for infectious diseases is not required and if the patient were infected at the time of apheresis collection they would likely have developed SARS-CoV-2-specific antibodies by the time of lovo-cel infusion. BB305 LVV is tested for adventitious viruses using cell lines permissive for SARS-CoV-2, which would enable detection of contamination during vector production cultures. The risk assessment concluded that there was a low risk of SARS-CoV-2 expansion during the lovo-cel manufacturing process. Procedures followed during CGMP manufacturing are assessed as sufficient to control any potential SARS-CoV-2 contamination during the manufacturing process.

Reviewer assessment: The manufacturing process is appropriately controlled to reduce the risk of SARS-CoV-2 transmission to patients.

Method Validation Package

Reviewed by AGB

Full method validation reports, along with specifications and analytical method descriptions, for BB305 Lentiviral Vector (3.2.S.4.2 Analytical Procedures and 3.2.S.4.3 Validation of Analytical Procedures) and lovo-cel product (3.2.P.5.2 Analytical Procedures and 3.2.P.5.3 Validation of Analytical Procedures) are provided. Information on the BB305 LVV reference standard is also provided. There are no specifications or reference standards for the lovo-cel drug substance as it is directly processed to drug product without a hold step. The testing strategy for drug product release encompasses tests performed on representative samples taken at various steps during the manufacturing process including drug substance. The testing strategy (in-process, release, and stability testing) of lovo-cel DP does not require a reference standard for control and monitoring.

Combination Products

Not applicable – lovo-cel is not regulated as a combination product.

Comparability Protocols

Not applicable – no comparability protocol is provided.

Other eCTD Modules

Module 1

A. Environmental Assessment or Claim of Categorical Exclusion

Reviewed by GEP

bluebird bio, inc. claims a categorical exclusion from the need to prepare an environmental assessment (EA) pursuant to 21 CFR 35.31(c) and is not aware of any extraordinary circumstances that would require preparation of an EA. The rationale for categorical exclusion is that:

- The lovotibeglogene autotemcel (lovo-cel) DP is composed of genetically modified human cells that have stringent nutritional requirements for survival and are not viable in the environment (as outlined in the 2015 FDA Guidance for Industry: Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vectored Vaccines and Related Recombinant Viral or Microbial Products).
- The BB305 LVV used in lovo-cel manufacture uses a 3rd-generation, split HIV-1 genome design requiring transfection of multiple plasmids to generate a replication-deficient, self-inactivating vector. BB305 is fully tested during manufacture and clinical study subjects have been monitored for, and shown no evidence of, replication-competent lentivirus. BB305 disposition is controlled at the manufacturing site and there is limited risk of BB305 LVV released to the environment.

Reviewer assessment: The applicant's claim for categorical exclusion from EA is acceptable.

B. Reference Product Designation Request

The applicant claims reference product exclusivity for 12 years from the date of approval of lovo-cel for treatment of patients with sickle cell disease and a history of vaso-occlusive events, with orphan drug exclusivity for 7 years from approval for this

indication per 21 CFR 316.31 based on orphan drug designation #13-4204 granted February 26, 2014.

Reviewer comment: This is under review.

C. Labeling Review

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). The description of lovo-cel dosage form and mechanism of action is consistent with other sections in the BLA. Procedures for receipt and preparation of lovo-cel at clinical sites are described in sufficient details and are acceptable.


Carton and Container Label:

Container labels (infusion bag patient identification label and product label, and cryocassette label) are shown in Figure 37. The lot information sheet accompanying the product shipment of the QTC is shown in Figure 38.

Reviewer comment: The final container and cassette labels and lot information sheet were submitted in Amendment #35 (received 01DEC2023) and are acceptable.

Figure 34. Container and cassette labels

A.



**lovotibeglogene autotemcel
lyfgenia™**

Suspension for IV infusion
20 mL containing 1.7 to 20 x 10⁶ cells/mL
(1.4 to 20 x 10⁶ CD34+ cells/mL)

Confirm Patient Identifiers

Last Name:

First Name:

Date of Birth:

bbb Patient ID:


COI ID:

LOT:

EXP:


Bag of

DIN




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

B.



**lovotibeglogene autotemcel
lyfgenia™**

Suspension for IV infusion
20 mL containing 1.7 to 20 x 10⁶ cells/mL
(1.4 to 20 x 10⁶ CD34+ cells/mL)




³ NDC 73554-1111-1 ⁰

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. **Dispense with Medication Guide.**


P/N: XXXXXXXX Manufactured for: bluebird bio, Inc.
 Label P/N: XXXXXXXX Somerville, MA 02145

C.



**lovotibeglogene autotemcel
lyfgenia™**

Suspension for IV infusion
20 mL containing 1.7 to 20 x 10⁶ cells/mL
(1.4 to 20 x 10⁶ CD34+ cells/mL)




³ NDC 73554-1111-1 ⁰

For autologous use only. For intravenous use only. Rx only.
 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.
Do not irradiate. Do not use an in-line blood filter or infusion pump.
 Not evaluated for infectious substances. No preservatives.
 See Lot Information Sheet for number of infusion bags and CD34+ cells
 per kg for this patient. **Dispense with Medication Guide.**

Confirm Patient Identifiers

Last Name: <input type="text" value="\$LastName\$"/>	LOT: <input type="text" value="\$LOT\$"/>
First Name: <input type="text" value="\$FirstName\$"/>	EXP: <input type="text" value="\$Expiry\$"/>
Date of Birth: <input type="text" value="\$DOB\$"/>	Bag <input type="text" value="X"/> of <input type="text" value="X"/>
bbb Patient ID: <input type="text" value="\$bbb_PatientID\$"/>	
COI ID: <input type="text" value="\$bbb_COI_ID\$"/>	
DIN <input type="text" value="\$DIN1_DIN2\$"/>	




U.S. Lic. # 2160

Manufactured for: bluebird bio, Inc.
 Somerville, MA 02145
 1-833-999-6378
 LYFGENIA.com


Container labels: A. Infusion bag patient identifier label; B. Infusion bag product label; C. Cryocassette label.

Figure 35. Lot information sheet



lovotibeglogene autotemcel
lyfgenia™

Suspension for IV infusion
20 mL containing 1.7 to 20 x 10⁶ cells/mL
(1.4 to 20 x 10⁶ CD34+ cells/mL)



3 NDC 73554-1111-1 0

LOT INFORMATION SHEET

SAVE THIS DOCUMENT AND HAVE IT AVAILABLE AT THE TIME OF LYFGENIA 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 (× 10 ⁶ CD34+ cells)	Expiry Date (DD-MMM-YYYY)

**Total Number of
Infusion Bags:**

**Total Dose
Volume:**

20 mL x total #
of infusion bags

**Total
Dose:**


× 10⁶ CD34+ cells/kg

The minimum recommended dose of LYFGENIA is 3.0 × 10⁶ 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.



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

Manufactured by:

(b) (4)

Manufactured for: bluebird bio, Inc.
Somerville, MA 02145
U.S. Lic. # 2160
1-833-999-6378
LYFGENIA.com

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Modules 4 and 5

Analytical Procedures and Validation of Analytical Procedures for Assessment of Clinical and Animal Study Endpoints

Reviewed by GEP

(b) (4)

