

**CBER CMC BLA Review Memorandum**

**BLA STN 125755**

**Skysona**  
**elivaldogene autotemcel**

**Reviewers**

**Anna Kwilas, CMC Reviewer/Chair OTAT/DCGT/GTB2**  
**Tal Salz, CMC Reviewer OTAT/DCGT/GTB1**  
**Brian Stultz, CMC Reviewer OTAT/DCGT/CTTB**  
**Andrew Timmons, CMC Reviewer OTAT/DCGT/GTB1**  
**Massoud Motamed, CMC Reviewer OTAT/DCGT/GTB2**

1. **BLA#:** STN 125755

2. **APPLICANT NAME AND LICENSE NUMBER**

bluebird bio, Inc.; License # 2160

3. **PRODUCT NAME/PRODUCT TYPE**

Non-Proprietary/Proper/USAN: elivaldogene autotemcel (eli-cel)

Proprietary Name: Skysona

Company codename: Lenti-D Drug Product

UNII Code: KUM75TD6SG

NDC Codes: NDC73554- 2111 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: 4 to 30 x 10<sup>6</sup> cells/mL (3.6 to 30 × 10<sup>6</sup> CD34+ cells/mL)

d. Route of administration: Intravenous infusion

e. Indication: To slow the progression of neurologic dysfunction in boys 4-17 years of age with early, active cerebral adrenoleukodystrophy (CALD)

5. **MAJOR MILESTONES**

Initial IND Submission (BB-IND 15433)	March 27, 2013
IND allowed to proceed	April 26, 2013
Orphan Drug Designation granted	April 19, 2012
Breakthrough Therapy Designation granted	May 21, 2018
Rare Pediatric Disease designation granted	August 9, 2017
Pre-BLA Meeting	June 21, 2021
BLA Submission	October 18, 2021
First Committee Meeting	November 12, 2021
Filing Meeting	December 9, 2021
Mid-Cycle Meeting	February 24, 2022
External Late-Cycle Meeting	May 31, 2022
PDUFA action due date (original)	June 17, 2022
Major Amendment Acknowledgement	January 13, 2022
Advisory Committee Meeting	June 9-10, 2022
PDUFA action due date:	September 16, 2022

6. **CMC/QUALITY REVIEW TEAM**

Reviewer/Affiliation	Section/Subject Matter
Anna Kwilas, CMC Reviewer/Chair OTAT/DCGT/GTB	eli-cel DP: Specifications, Analytical methods; Stability
Andrew Timmons, CMC Reviewer OTAT/DCGT/GTB	Lenti-D lentiviral vector (LVV)

Brian Stultz, CMC Reviewer OTAT/DCGT/CTTB	Lenti-D LVV and eli-cel DS: Control of Materials, Control of excipients
Tal Salz, CMC Reviewer OTAT/DCGT/CTTB	eli-cel DS/DP: Manufacturing Process, Process Characterization and Validation, Comparability, Chain of Identity, DP Characterization, Impurities, Container Closure System
Massoud Motamed, CMC Reviewer OTAT/DCGT/CTTB	(b) (4) eli-cel DP Potency assays
Steven Bauer, Ph.D. OTAT/DCGT/CTTB	Consult review for (b) (4)
Elena Gubina, Ph.D. OTAT/DCGT/CTTB	Consult review for (b) (4)
Archana Devi Siddam, Ph.D. OTAT/DCGT/CTTB	Consult review for (b) (4)
Guo-Chiuan Hung, Ph.D. OTAT/DCGT/CTTB	Consult review for (b) (4)
Mercy Quagraine, Ph.D. OTAT/DCGT/CTTB	Consult review for (b) (4)
Carolina Panico, M.D. Ph.D. OTAT/DCGT/TEB	Consult review for (b) (4) Cryobag

## 7. INTER-CENTER CONSULTS REQUESTED

Reviewer/Affiliation	Section/Topic	In agreement with consult recommendations (Yes/No <sup>1</sup> )
Zhaobo Fan, Ph.D. CDRH/OSEL/DBCMS Caroline Pinto, Ph.D. CDRH/OSEL/DBCMS	(b) (4) bag	

## 8. SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/ Status
9/23/2021	125755/1	Initial submission of CMC Module 3
10/18/2021	125755/2	Initial submission of Clinical Module 5
1/7/2022	125755/8	Response to IR #1 (CMC IR #1) dated 12/28/2021
2/4/2022	125755/16	Response to IR #1 (CMC IR #1) dated 12/28/2021
2/11/2022	125755/17	Response to IR #2 (CMC IR #2) dated 2/3/2022
2/28/2022	125755/21	Response to IR #2 (CMC IR #2) dated 2/3/2022
4/12/2022	125755/38	Response to IR #11 (CMC IR #3) dated 3/28/2022

4/19/2022	125755/41	Response to IR #15 (CMC IR #4) dated 4/7/2022
4/19/2022	125755/42	Response to IR #11 (CMC IR #3) dated 3/28/2022
4/29/2022	125755/48	Response to IR #17 (CMC IR #5) dated 4/15/2022
4/29/2022	125755/49	Response to IR #15 (CMC IR #4) dated 4/7/2022
5/20/2022	125755/56	Response to IR #23 (CMC IR #6) dated 5/13/2022
5/25/2022	125755/59	Response to IR #11 (CMC IR #3) dated 3/28/2022
5/27/2022	125755/60	Summary of tcon held to discuss sampling for sterility testing
6/8/2022	125755/64	Module 3 update based on agreements reached during BLA review
6/17/2022	125755/66	Response to IR #26 (CMC IR #7) dated 6/10/2022
6/21/2022	125755/68	Response to IR #28 (Clinical IR) dated 6/15/2022
6/24/2022	125755/70	Response to IR #26 (CMC IR #7) dated 6/10/2022
6/30/2022	125755/70	Agreement to DBSQC PMC
7/15/2022	125755/79	Response to IR #37 (Clinical IR) dated 7/11/2022
7/15/2022	125755/80	Response to IR #34 (CMC IR #8) dated 7/1/2022
7/15/2022	125755/81	Response to IR #11 (CMC IR #3) dated 3/28/2021
7/28/2022	125755/84	Response to IR #38 (CMC IR #9) dated 7/25/2022
7/29/2022	125755/85	Response to IR #39 dated 7/25/2022 (Module 3 update)
8/4/2022	125755/88	Response to IR #34 (CMC IR #8) dated 7/1/2022
8/17/2022	125755/92	Response to IR #42 (CMC IR #10) dated 8/12/2022
8/17/2022	125755/93	Response to PMCs/PMR dated 8/12/2022
9/8/2022	125755/112	Response to IR #45 (CMC IR #11) dated 8/18/2022

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

Submission Type & #	Holder	Referenced Item	LoA	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/CTTB)

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/CTB1)
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	Facility information is included in BLA.
BB-MF (b) (4) Type V Master File	(b) (4)	Manufacturing and Laboratory Facilities and Quality Systems	Yes	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/CTB1)
BB-MF (b) (4)	(b) (4)	Pharmaceutical Closure (Stopper) (b) (4)	Yes	Relevant sections in BLA reviewed by CMC reviewer Andrew Timmons (CBER/OTAT/DCGT/CTB1)
STN (b) (4)	(b) (4)	Pharmaceutical Closure (Stopper) Elastomeric Formulations, Coatings and Films	Yes	Relevant sections in BLA reviewed by CMC reviewer Andrew Timmons (CBER/OTAT/DCGT/CTB1)

## 10. REVIEWER SUMMARY AND RECOMMENDATION

### I. EXECUTIVE SUMMARY

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

eli-cel is an autologous gene therapy product intended to treat patients with childhood cerebral adrenoleukodystrophy (CCALD). eli-cel consists of a CD34+ cell enriched population, that contains hematopoietic stem cells (HSCs), transduced with a lentiviral vector (LVV), referred to as Lenti-D. Lenti-D contains the coding sequence for the ATP-binding cassette, sub-family D, member 1 (ABCD1) gene, which encodes the human adrenoleukodystrophy protein (ALDP). The proposed eli-cel mechanism of action is that following engraftment, these HSCs differentiate into various cell types, including monocytes that migrate to the brain where they further differentiate into macrophages and cerebral microglia that can produce functional ALDP. The functional ALDP can then enable the local degradation of very long chain fatty acids (VLCFAs) in the brain, which in turn can stabilize the disease by preventing further inflammation and demyelination. The clinical benefits of one-time eli-cel treatment are expected to last for the patient's lifetime.

The Lenti-D LVV is a nonreplicating, self-inactivated lentivirus, based on HIV-1, pseudotyped with the Vesicular Stomatitis Virus glycoprotein (VSV-G). The LVV is manufactured at a contract manufacturing facility (b) (4) via transient transfection of HEK293T cells with five plasmids expressing (1) the vector genome, (2) Gag-Pol, (3) Rev (4) tat, and (5) VSV-G. The packaged genomic viral RNA encodes no viral genes and contains less than 25% of the HIV-1 genome. The HEK293T cells are expanded then transfected. At the completion of the culture period, (b) (4) harvested, (b) (4), purified (b) (4) chromatography, (b) (4), formulated by (b) (4). The LVV is then filled into (b) (4) vials and stored at  $\leq 65^{\circ}\text{C}$ . Lenti-D stability at  $\leq 65^{\circ}\text{C}$  was supported up to (b) (4).

To manufacture eli-cel, autologous hematopoietic progenitor cells obtained by apheresis (HPC-A) are collected from each patient at a Qualified Treatment Center (QTC) following HSC mobilization with granulocyte-colony stimulating factor (G-CSF) and plerixafor. The apheresis material is then shipped to the Lonza-Houston, Inc. drug substance (DS)/drug product (DP) manufacturing facility (Houston, TX). The HPC-A is enriched for cells expressing CD34 by (b) (4)

(b) (4). The enriched CD34+ cells are then (b) (4) /growth factors (b) (4), enriched CD34+ cells are (b) (4) in the presence of Lenti-D LVV, (b) (4) to transduce the cells. After transduction, the cells are washed (b) (4). The washed transduced cells, (b) (4)

(b) (4). To produce the DP, the DS (b) (4), formulated (b) (4) 4 to  $30 \times 10^6$  cells/mL ( $3.6$  to  $30 \times 10^6$  CD34+ cells/mL) and filled into one or two 20 mL fluoro-ethylene-propylene bags, depending on the number of cells produced. Filled bags are examined for appearance, placed in individual metal cassettes, then cryopreserved using a (b) (4) and stored at  $\leq -140^\circ\text{C}$  in vapor phase liquid nitrogen until lot release testing is complete. There are (b) (4) steps between DS and DP manufacture, which takes place over a (b) (4) period. eli-cel stability in vapor phase liquid nitrogen ( $\leq -140^\circ\text{C}$ ) was supported up to 9 months.

eli-cel DP is supplied as a frozen suspension of cells for intravenous infusion. The minimum dose is  $5.0 \times 10^6$  CD34+ cells/kg patient weight. However, other than the samples taken for lot release testing and retain samples, each patient receives the entire DP manufactured. eli-cel is shipped frozen in a vapor phase liquid nitrogen shipper to the administration site once patient administration has been scheduled. The DP bag(s), contained within individual cassettes, are secured in a metal rack within the shipper. Following receipt at the administration site, eli-cel is stored in vapor phase liquid nitrogen ( $\leq -140^\circ\text{C}$ ) until the scheduled treatment time, when it is thawed and infused within 4 hours. Patients receive eli-cel after myeloablative conditioning.

Manufacturing process consistency is assured through 1) raw material and reagent qualification programs, 2) in-process monitoring, 3) in-process control testing, and 4) lot release and stability testing. Raw materials derived from animals and humans are appropriately controlled to ensure the absence of microbial contaminants and adventitious agents. Lot release test methods are suitably validated or verified and product specifications are adequate to ensure product quality and consistency with DP used in the clinical study. The manufacturing process has been adequately validated and continuous process verification is in place. Because of the autologous nature of the product, Chain of Identity/Chain of Custody (COI/COC) is established at the collection site and maintained through the manufacturing process and administration by conducting label checks at specified times throughout the process.

## **J. RECOMMENDATION**

### **K. APPROVAL**

This biological license application (BLA) provides an adequate description of the manufacturing process and characterization of elivaldogene autotemcel (eli-cel; Skysona). 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 along with post-marketing commitments and requirements listed below satisfy 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 (b) (4), the CMC review team recommends approval of this BLA.

CBER Lot release:

eli-cel has been deemed exempt from CBER lot release testing or protocol review.

Post-Marketing Commitments (PMCs):

*Robustness*

bluebird bio, Inc., commits to perform the additional robustness assessments of the (b) (4) assays as described in BLA 125755.

Final Report Submission: June 30, 2023

*Sampling*

bluebird bio, Inc., commits to add testing of eli-cel cryopreserved drug product (DP) for (b) (4) as described in BLA 125755.

Final Report Submission: February 28, 2023

*In-use stability*

bluebird bio, Inc., commits to perform a supplemental in-use stability study of eli-cel assessing the stability of (b) (4) under the intended conditions as described in BLA 125755.

Final Report Submission: March 31, 2023

(b) (4) *leachable study*

bluebird bio, Inc., commits to assess the feasibility of detecting

(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) *bag (b) (4) Testing*

bluebird bio 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) *bag (b) (4) Study*

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



(b) (4) (e.g., (b) (4)  
. The testing will include (b) (4)  
. Complete test reports for this testing will be submitted as  
a final study report by December 31, 2022.

Postmarketing Requirements (PMRs):

(b) (4) bag

1. A study to support the extractable data provided for the (b) (4) bag, including the sample processing steps in the (b) (4) and an appropriate identification process used for the extractables. The milestones and timelines for this study are as follows:  
Final Protocol Submission: November 30, 2022  
Study Completion: February 28, 2023  
Final Study Report Submission: April 30, 2023
2. A study to evaluate leachables of the (b) (4) bag over the duration of the shelf life of elivaldogene autotemcel. This evaluation will also include a full toxicological risk assessment for the identified leachables and extractables. The milestones and timelines for this study are as follows:  
Final Protocol Submission: November 30, 2022  
Study Completion: January 30, 2024  
Final Study Report Submission: March 30, 2024

**II. COMPLETE RESPONSE (CR)**

Not applicable

**III. SIGNATURE BLOCK**

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Anna Kwilas Lead Biologist OTAT/DCGT/GTB2	Concur	
Andrew Timmons Biologist OTAT/DCGT/GTB1	Concur	
Tal Salz Biologist OTAT/DCGT/GTB1	Concur	
Brian Stultz Research Biologist OTAT/DCGT/CTTB	Concur	

Massoud Motamed 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	

## Table of Contents

<b>Module 3</b>	<b>18</b>
<b>3.2.S DRUG SUBSTANCE - Lenti-D Lentiviral Vector (LVV)</b>	<b>18</b>
3.2.S.1.1 - 1.3 Nomenclature, Structure and General Properties	18
3.2.S.2 Manufacture	19
3.2.S.2.1 Manufacturer(s)	19
3.2.S.2.2 Description of Manufacturing Process	19
Manufacturing process steps	20
Batch Numbering, (b) (4) and Scale Definition	21
Storage and Shipping	22
3.2.S.2.3 Control of Materials	22
Control of Raw Materials NOT of Biological Origin	25
Control of Raw Materials of Biological Origin	27
Control of Starting (i.e., Source) Material(s)	29
Generation of the Seed Stock and Expression Construct (e.g., vector and plasmid)	29
Cell Banking System – Generation, Characterization, and Testing	33
3.2.S.2.4 Controls of Critical Steps and Intermediates	37
Critical Process Parameters (CPPs)	37
In Process Controls (IPCs)	38
Analytical procedures for in-process tests	38
3.2.S.2.5 Process Validation and/or Evaluation	39
Process performance qualification (PPQ) studies	39
Commercial Manufacturing Control + Continued Process Verification	42
(b) (4) Qualification	43
(b) (4) Studies	43
(b) (4) (b) (4) Simulation	44
(b) (4) Validation	44
Shipping Validation	44
3.2.S.2.6 Manufacturing Process Development	45
Commercial Manufacturing Process Development and Characterization	45
Lenti-D LVV Quality Attribute Identification and Classification	45
Lenti-D LVV Process Parameter Identification and Risk Assessment	46
Lenti-D LVV Process Parameter Characterization with (b) (4)	46
Manufacturing Process History	47
Manufacturing changes implemented after PPQ runs	48
3.2.S.3 Characterization	49
3.2.S.3.1 Elucidation of Structure and Other Characteristics	49
Characterization of (b) (4)	50
3.2.S.3.2 Impurities	50
Product Related Impurities	51
Process Related Impurities	51
3.2.S.4.1 Specification(s) and 3.2.4.1.5 Justification of Specifications	52
3.2.S.4.2 Analytical Procedures and 3.2.S.4.3 Validation of Analytical Procedures	53

3.2.S.4.4 Batch Analyses.....	69
3.2.S.5 Reference Standards or Materials .....	71
3.2.S.5.1 Overview of Reference Standard Program.....	71
3.2.S.5.2 Establishment and Qualification of (b) (4) .....	71
3.2.S.5.3 Stability of (b) (4) .....	73
3.2.S.5.4 Strategy for Establishing a New PRS .....	73
3.2.S.6 Container Closure System.....	73
Container Closure Overview .....	73
3.2.S.6.2 – Stopper.....	74
3.2.S.6.3 – Seal .....	74
3.2.S.6.5 – Container Closure Suitability .....	74
Container Closure Integrity.....	74
Extractable Compound Study.....	74
3.2.S.7 Stability.....	75
3.2.S.7.1 Stability Summary and Conclusions .....	75
Long-Term Stability Study Design .....	75
3.2.S.7.3 Stability Data .....	76
Long-Term Stability Study Data.....	76
Accelerated Stability Study Data .....	78
3.2.S.7.2 Post-Approval Stability Protocol and Stability Commitment .....	79
<b>3.2.S DRUG SUBSTANCE - elivaldogene autotemcel (eli-cel).....</b>	<b>80</b>
3.2.S.1 General Information.....	80
3.2.S.1.1 Nomenclature .....	80
3.2.S.1.2 Structure.....	80
3.2.S.1.3 General Properties .....	80
3.2.S.2 Manufacture .....	80
3.2.S.2.1 Manufacturer(s).....	80
3.2.S.2.2 Description of Manufacturing Process.....	80
3.2.S.2.3 Control of Materials .....	87
Control of Materials– Raw Materials.....	87
Control of Raw Materials NOT of Biological Origin.....	93
Control of Raw Materials of Biological Origin .....	93
Control of Materials– Hematopoietic Progenitor Cells - Apheresis (HPC-A).....	96
3.2.S.2.4 Controls of Critical Steps and Intermediates .....	102
3.2.S.2.5 Process Validation and/or Evaluation.....	104
3.2.S.2.6 Manufacturing Process Development.....	104
3.2.S.2.6.1 Manufacturing Development History .....	104
3.2.S.2.6.2 Studies supporting manufacturing changes .....	106
3.2.S.2.6.3 Process Characterization Studies .....	117
3.2.S.3 Characterization .....	125
3.2.S.3.1 Elucidation of Structure and Other Characteristics.....	125
3.2.S.3.2 Impurities.....	135
3.2.S.3.2.1 Process-Related Impurities .....	135
3.2.S.3.2.2 Cellular impurities.....	139
3.2.S.4 Control of Drug Substance .....	139
3.2.S.4.1 Specification(s) and 3.2.S.4.5 Justification of Specification(s) .....	139

3.2.S.4.2 Analytical Procedures and 3.2.S.4.3 Validation of Analytical Procedures	139
3.2.S.4.4 Batch Analyses	139
3.2.S.5 Reference Standards or Materials	139
3.2.S.6 Container Closure System	139
3.2.S.7 Stability	140
3.2.S.7.1 Stability Summary and Conclusion and 3.2.S.7.3 Stability Data	140
<b>3.2.P DRUG PRODUCT elivaldogene autotemcel (eli-cel)</b>	<b>140</b>
3.2.P.1 Description and Composition of the Drug Product	140
3.2.P.2 Pharmaceutical Development	140
3.2.P.2.1 Components of the Drug Product	141
3.2.P.2.1.1 Drug Substance	141
3.2.P.2.1.2 Excipients	141
3.2.P.2.2 Drug Product	141
3.2.P.2.2.1 Formulation Development	141
3.2.P.2.2.2 Overages	141
3.2.P.2.2.3 Physicochemical and Biological Properties	141
3.2.P.2.3 Manufacturing Process Development	142
3.2.P.2.3.1 Manufacturing Process History	142
3.2.P.2.3.2 Manufacturing Process Characterization Studies	142
3.2.P.2.4 Container Closure System	147
3.2.P.2.5 Microbiological Attributes	147
3.2.P.2.6 Compatibility	147
3.2.P.3 Manufacture	148
3.2.P.3.1 Manufacturer(s)	148
3.2.P.3.2 Batch Formula	149
3.2.P.3.3 Description of Manufacturing Process	149
3.2.P.3.3 Chain of Identity	151
3.2.P.3.4 Controls of Critical Steps and Intermediates	155
3.2.P.3.5 Process Validation and/or Evaluation	156
3.2.P.4 Control of Excipients	165
3.2.P.4.1 Specifications	165
3.2.P.4.2 and 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures	165
3.2.P.4.4 Justification of Specifications	165
3.2.P.4.5 Excipients of Human or Animal Origin	165
3.2.P.4.6 Novel Excipient	166
3.2.P.5 Control of Drug Product	166
3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)	166
Sampling time points	167
Release of out of specification (OOS) commercial product	168
3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures	168
3.2.P.5.4 Batch Analyses	187
3.2.P.5.5 Characterization of Impurities	187
3.2.P.6 Reference Standards or Materials	187

3.2.P.7 Container Closure System.....	187
3.2.P.8 Stability.....	189
3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data .....	189
3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment .....	192
3.2.A APPENDICES .....	193
3.2.A.1 Facilities and Equipment .....	193
3.2.A.2 Adventitious Agents Safety Evaluation.....	193
3.2.R Regional Information (USA) .....	193
Risk Assessment for (b) (4) .....	193
Executed Batch Records .....	193
Comparability Protocols.....	194
<b>Module 1</b> .....	<b>194</b>
Environmental Assessment or Claim of Categorical Exclusion.....	194
Reference Product Designation Request.....	194
Labeling Review Full Prescribing Information (PI): .....	194
Carton and Container Label.....	195
<b>Modules 4 and 5</b> .....	<b>199</b>
Analytical Procedures and Validation of Analytical Procedures for Assessment of Clinical Study Endpoints .....	199

## Table of Figures

Figure 1 - General Structure and Composition of Lenti-D LVV.....	18
Figure 2 - Schematic of the Lenti-D LVV Manufacturing Process .....	20
Figure 3 - Lenti-D Representative Label.....	21
Figure 4 - Lenti-D LVV Characterization of (b) (4) .....	50
Figure 5 – Lenti-D Representative Batch Analysis Data.....	70
Figure 6 - Lenti-D Long Term Stability Study Data.....	77
Figure 7 - Drug Substance Manufacturing Process Flow Diagram.....	83
Figure 8 - HPC-A - Flow Diagram of Collection in a single Mobilization Cycle .....	99
Figure 9 - HPC-A - Bag Commercial Label .....	100
Figure 10 - Correlation between (b) (4) cells data from (b) (4) .....	111
Figure 11 - Eli-cel (b) (4) Results by Manufacturing Site .....	114
Figure 12 - Eli-cel (b) (4) Results by Manufacturing Site .....	115
Figure 13 - Process Characterization – Process Parameter Classification Approach .	119
Figure 14 - Process Characterization - comparison eli-cel and healthy donor lots .....	120
Figure 15 - Characterization – (b) (4) Time-course .....	127
Figure 16 - Characterization - Comparison of (b) (4) Assays .....	128
Figure 17 - Characterization - Comparison of (b) (4) Assays .....	128
Figure 18 - Characterization - Distribution of (b) (4) .....	129
Figure 19 - Characterization; Relationship Between (b) (4) .....	130
Figure 20 - Characterization - Relationship Between (b) (4) .....	131
Figure 21 - Characterization - (b) (4) .....	132
Figure 22 - Characterization - Relationship Between (b) (4) .....	133
Figure 23 - DS - Container Closure System .....	140
Figure 24 - Drug Product Manufacturing Process Flow Diagram .....	150
Figure 25 - Chain of Identity – Flow Diagram .....	153
Figure 26 - Container Closure System - Representative Drawing of (b) (4) Bag .....	188
Figure 27 - Eli-cel cassette label .....	195
Figure 28 - Eli-cel bag labels.....	197
Figure 29 – Lot Information Sheet.....	198

## Table of Tables

Table 1 - Manufacturers Involved in the Production, Testing, and Storage of Lenti-D LVV .....	19
Table 2 - Composition of the plasmid (b) (4) used for Lenti-D manufacture .....	19
Table 3 - Product Contact Materials .....	22
Table 4 - Compendial Raw Materials Not of Biological Origin .....	25
Table 5 - Non-Compendial Raw Materials Not of Biological Origin .....	26
Table 6 - Raw Materials of Biological Origin.....	27
Table 7 - Starting Materials .....	29
Table 8 - Plasmid Specifications .....	31
Table 9 - Qualification Testing for HEK293T Master Cell Bank Lot (b) (4) .....	33
Table 10 - Qualification Testing for HEK293T Working Cell Banks .....	35
Table 11 - Critical Process Parameters Established for Lenti-D Manufacture .....	37
Table 12 - PPQ Batch Results .....	39
Table 13 - Lenti-D PPQ Process Validation Acceptance Criteria (PVAC) .....	40
Table 14 - Lenti-D Summary of Deviations.....	41
Table 15 - Recommended hold times based on (b) (4) Hold Studies.....	43
Table 16 - Lenti-D Process Parameter Classification from (b) (4) .....	46
Table 17 - History of Lenti-D Manufacturing Processes .....	47
Table 18 - Structural Characterization of the Lenti-D LVV.....	49
Table 19 - Product and Process Related Impurities in the Lenti-D LVV .....	50
Table 20 - Lot Release Specifications for the Lenti-D LVV.....	52
Table 21 - Overview of analytical method validation .....	53
Table 22 - Lenti-D (b) (4) Method Validation Summary .....	55
Table 23 - Summary of (b) (4) Validation.....	57
Table 24 - Lenti-D (b) (4) Method Validation Summary .....	61
Table 25 - Lenti-D (b) (4) Method Validation Summary.....	62
Table 26 - Results of Lenti-D (b) (4) Study .....	64
Table 27 - Lenti-D Replication Competent Method Validation Summary.....	65
Table 28 - Lenti-D (b) (4) Method Validation Summary .....	67
Table 29 - Reference Standard (b) (4) Lot Release Testing .....	71
Table 30 - Reference Standard (b) (4) Characterization Testing .....	72
Table 31 - Lenti-D Reference Standard Stability Study Design.....	73
Table 32 - Lenti-D Samples Evaluated in the LVV Stability Study.....	75
Table 33 - Lenti-D Long Term Stability Study for PPQ Batches .....	76
Table 34 - Lenti-D Accelerated Stability Study Design .....	79
Table 35 - Lenti-D Post Approval Stability Protocol.....	79
Table 36 - Manufacturing and Testing Facilities for eli-cel Drug Substance.....	80
Table 37 - Classification Terms and Definitions for Process Controls .....	81
Table 38 - Compositions of Drug Substance Process Solutions .....	82
Table 39 - Incoming Requirements for HPC-A.....	84
Table 40 - Decision of (b) (4) of Apheresis Characteristics ..	84
Table 41 - Decision for Volume of CD34 Reagent .....	85
Table 42 - Non-Equipment Product-Contact Materials .....	89
Table 43- Extractable Compounds Detected.....	92
Table 44 – Raw Materials.....	93



Table 45 – Raw Materials of Biological Origin .....	93
Table 46 - HPC-A - Infectious Disease Serology Tests .....	97
Table 47 - HPC-A - Collection Device .....	97
Table 48 - HPC-A - Stability .....	101
Table 49 - HPC-A – (b) (4) Characterization .....	102
Table 50 - DS Process Parameters.....	102
Table 51 - DS In-Process Controls.....	103
Table 52 - Summary of eli-cel Drug Substance Manufacturing Process Development	105
Table 53 - Comparability - Attributes Assessed .....	108
Table 54 - Comparability - Quantitative CQA Sample Number by Site.....	109
Table 55 - Comparability – CQAs Results; LHI- (b) (4) .....	112
Table 56 - Comparability – CQAs Results; LHI- (b) (4) LHI-PL.....	112
Table 57 - Comparability - IPC Sample Number by Site .....	115
Table 58 - Comparability – IPCs Results.....	116
Table 59 - Process Characterization – Classification of Product Attributes.....	117
Table 60 - Process Characterization – (b) (4) .....	121
Table 61 - Process Characterization - (b) (4) .....	122
Table 62 - Process Characterization - Transduction .....	123
Table 63 - Process Characterization - In-Process Controls.....	125
Table 64 - Characterization Assays .....	125
Table 65 - Characterization - Correlations Between Product Attributes and clinical Biomarkers .....	134
Table 66 - Impurities - Clearance of process-Related Impurities.....	136
Table 67 - Comparative Summary of DP Manufacturing Process Development .....	142
Table 68 - DP Process Characterization - Cell Concentration and Hold Time (b) (4) .	143
Table 69 - DP Process Characterization - Cryopreservation Program .....	145
Table 70 - DP Process Characterization - Hold and Transfer Times (b) (4) .....	145
Table 71 - (b) (4) In-use Stability data.....	147
Table 72 - Manufacturing and Testing Facilities for eli-cel Drug Product .....	148
Table 73 - Drug Product Batch Formula.....	149
Table 74 - Chain of Identity – Example of Identifiers and Labels .....	153
Table 75 - Chain of Identity- Related Activities.....	154
Table 76 - DP Process Parameters.....	155
Table 77 - eli-cel PPQ Batches .....	157
Table 78 - Process Validation - Process Parameters .....	158
Table 79 - Process Validation - In Process Controls (IPCs) .....	159
Table 80 - Process Validation - Release Tests .....	160
Table 81 - Process Validation - Post Validation Testing.....	161
Table 82 - Process Validation - Impurities.....	161 +
Table 83 - Process Validation - Shipping Validation Study Route .....	163
Table 84 - Release Specifications for eli-cel .....	166
Table 85 - Sampling Points, Matrices and Justifications for eli-cel Release Testing ...	167
Table 86 – (b) (4) Method Validation .....	169
Table 87 - (b) (4) Method Validation .....	170
Table 88 - Cell Concentration Method Validation .....	170
Table 89 - (b) (4) Method Validation .....	171


Table 90 - VCN Method Validation .....	174
Table 91 - %LVV Method Validation .....	176
Table 92 - (b) (4) Method Validation .....	178
Table 93 - Summary of % ALDP+ Cells Method Validation .....	180
Table 94: Validation of a Quantitative (b) (4) Method for Determination of (b) (4) in Differentiated Lenti-D Drug Product .....	182
Table 95 – Robustness Study Results for Variation in Sample (b) (4) Conditions and Variation in Sample (b) (4) Conditions .....	185
Table 96 - Container Closure System - Specifications/Technical Information for (b) (4) Bag .....	188
Table 97 – Summary of Available eli-cel Stability Data .....	189
Table 98 - Annual Post-Approval Stability Testing at Long-term Storage Conditions (≤-140°C) .....	192

**Module 3**

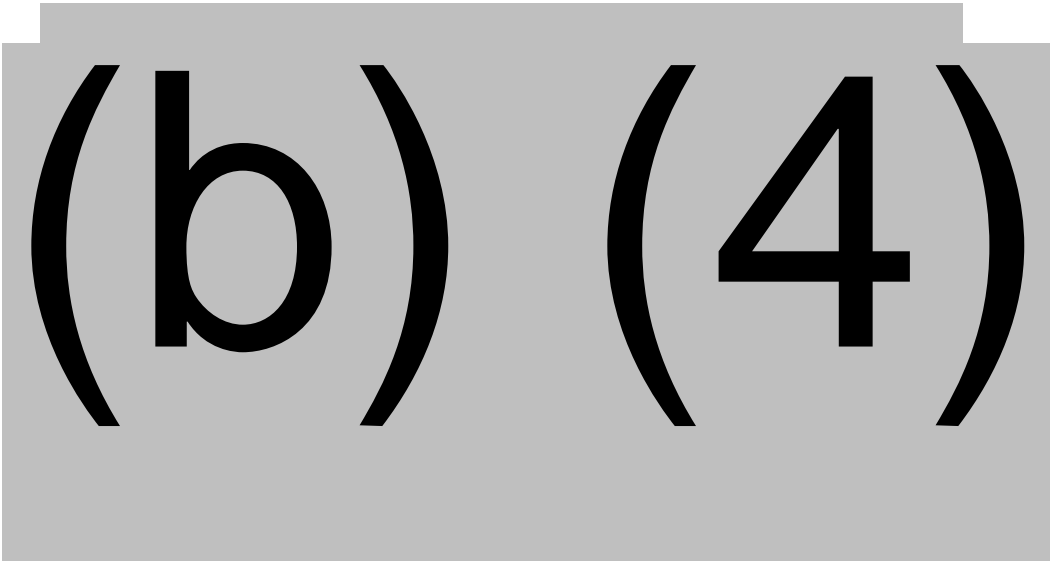
**3.2.S DRUG SUBSTANCE - Lenti-D Lentiviral Vector (LVV)**

*Reviewed by AET (unless otherwise noted)*


(b) (4)

A large rectangular area of the document is redacted with a solid gray fill.A large rectangular area of the document is redacted with a solid gray fill.

(b) (4)

A large rectangular area of the document is redacted with a solid gray fill.

(b) (4)

A large rectangular area of the document is redacted with a solid gray fill.

120 pages have been determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

(b) (4)

### **3.2.P DRUG PRODUCT elivaldogene autotemcel (eli-cel)**

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

eli-cel DP consists of an autologous CD34+ cell-enriched population containing cells transduced with the Lenti-D LVV that encodes an ABCD1 cDNA for ALDP, suspended in (b) (4) cryopreservation solution containing 5% DMSO. Eli-cel is supplied as a suspension for intravenous infusion in (b) (4) 20 mL Fluoro-Ethylene-Propylene (FEP) bags. Eli-cel is administered as a single dose by intravenous infusion. A single lot of DP may consist of 1 or 2 bags but no more than one DP lot may comprise a single dose.

#### **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

The eli-cel DS consists of (b) (4)

#### 3.2.P.2.1.2 Excipients

(b) (4)

### 3.2.P.2.2 Drug Product

#### 3.2.P.2.2.1 Formulation Development

eli-cel DP has been formulated in (b) (4) throughout development. The composition of (b) (4) is described in a BBMF (b) (4) and **3.2.P.4 Control of Excipients**. (b) (4) was selected for its cryopreservative qualities, absence of (b) (4), and for its suitability for infusion into humans. Studies supporting cell concentration for cryopreservation in (b) (4) are described in **3.2.S.2.6.3 Process Characterization Studies**. The total cell concentration release specification is  $4.0 \times 10^6$  -  $30 \times 10^6$  cells/mL based on clinical manufacturing experience. The in-use stability of eli-cel discussed in **3.2.P.2.6 Compatibility** and the log-term stability in **3.2.P.8.1 Stability Summary and Conclusion** and **3.2.P.8.3 Stability Data** support the ability of the formulation to cryoprotect cells and preserve product quality throughout the proposed DP shelf-life as well as post-thaw.

#### 3.2.P.2.2.2 Overages

There are no overages in the eli-cel DP.

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

*Reviewed by AK*

eli-cel adds functional copies of the ABCD1 gene into patients' HSCs through transduction of autologous CD34+ cells with Lenti-D LVV, thereby addressing the underlying genetic cause of the disease. After eli-cel infusion, transduced CD34+ HSCs engraft in the bone marrow. The proposed mechanism of action is that following engraftment, these HSCs differentiate into various cell types, including monocytes that migrate to the brain where they further differentiate into macrophages and cerebral microglia that can produce functional ALDP. The functional ALDP can then enable the local degradation of VLCFAs in the brain, which in turn can stabilize the disease by preventing further inflammation and demyelination.

Additional physicochemical and biological properties are described in sections **3.2.S.1 General Information**, **3.2.S.3.1 Elucidation of Structure and Other Characteristics**, and **3.2.P.1 Description and Composition of the Drug Product**. There are no differences between the properties of the DS and the DP. Attributes relevant to the performance of the DP that are monitored on each batch as part of release testing are described in **3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)**.

### 3.2.P.2.3 Manufacturing Process Development

*Reviewed by TS*

Process development and comparability studies performed during the product lifecycle are described in **3.2.S.2.6 Manufacturing Process Development**. 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 Process History

The DP manufacturing process is described in **3.2.P.3.3 Description of Manufacturing Process**. Table 67 summarizes changes introduced to the formulation and cryopreservation of the DP during product development. Formulation and Cryopreservation have remained unchanged during development and transfer to several manufacturing sites, including LHI-PL, the commercial manufacturing site. The target range for cell concentration in eli-cel was slightly revised throughout development as indicated in Table 67.

**Table 67 - Comparative Summary of DP Manufacturing Process Development**

Manufacturing Site	(b) (4)						LHI-PL
Clinical Study	ALD-102					ALD-104	
Site Initiation Date	2013	2014	2016	2018	2019		
Formulation and Cryopreservation	Manufacture and storage as described in 3.2.P.3.3 except cell concentration						
	(b) (4)	cells/mL			(b) (4)	cells/mL	

#### 3.2.P.2.3.2 Manufacturing 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 **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) (RPT-0690), 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)

(b) (4)

(b) (4)

3 pages have been determined to be not releasable: (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 support the DP process control strategy as summarized in 3.2.P.3.4 Controls of Critical Steps and Intermediates.*

### 3.2.P.2.4 Container Closure System

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

### 3.2.P.2.5 Microbiological Attributes

*Reviewed by TS*

Eli-cel is comprised of living cells and is manufactured under aseptic conditions. All container-closure components and excipients are verified to be sterile before use. DP lot release testing includes (b) (4) sterility and endotoxin testing with samples that are aseptically obtained from the final container (b) (4) of each DP bag. The DS is tested for (b) (4). Each DP bag is (b) (4) tested with (b) (4) by the manufacturer, and any (b) (4) result in the bag being rejected. Bags are (b) (4) sterilized by the vendor. 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*

Data supporting in-use stability of eli-cell was provided from a study using (b) (4) DP lot (b) (4), manufactured with (b) (4) at a cell concentration of (b) (4). After thaw, the cells were (b) (4) that were independently processed either immediately (<30 minutes timepoint), or after 1, 2, 4 and (b) (4) hours post thawing. Samples were assessed for cell concentration (b) (4), VCN, (b) (4) (b) (4) and (b) (4). The results are summarized in Table 71. Cell concentration and (b) (4), assessed by (b) (4), remains constant throughout all the timepoints. (b) (4) VCN shows a minor decrease after 60 minutes but this reduction is within the margin of error of the assay and is considered negligible. (b) (4), however the changes observed remain within the variability of the assay.

(b) (4)

*Reviewer Comment: In response to IR#2 dated 12/28/2021, bluebird bio clarified that the (b) (4)*

(b) (4) for this study. RPT-0437 was provided to support the use of these surrogate container closures

**3.2.P.2.3 Manufacturing Process Development.**

Bluebird bio also provided RPT-0243 in which cell count and (b) (4) data were obtained for (b) (4) lots held at room temperature after thaw, in the DP bags, for up to (b) (4). No loss of cell count (b) (4) was observed up to (b) (4) hours. Collectively, these data support the proposed in-use stability time of 4 hours. However, no data supporting post-thaw/in-use stability of eli-cel were provided. Any available eli-cel data were requested in IR#8 dated 7/1/2022. In response, bluebird bio performed a study within the bluebird bio analytical development lab to specifically evaluate the (b) (4) in thawed eli-cel (b) (4) DP. Eli-cel PPQ Lot (b) (4)

The data provided in Amendment 80 indicated that the (b) (4) remained stable in all samples tested. Additional in-use stability data is being requested in a PMC bluebird bio agreed to in Amendment 93.

(b) (4)

In response to CMC IR#2 dated 12/23/2021, bluebird bio further clarified that the qualified treatment center (QTC) material control and cell therapy administration procedures will govern the specific model and supplier for the infusion kits at each QTC. All intravenous administration sets that are compatible to the infusion bag ports, approved for medical use, and do not have an in-line filter would be suitable per the prescribing information. Examples of infusion kits currently used at the QTC sites that meet the proposed (b) (4) (b) (4) prescribing instructions were provided. These kits are not specifically cleared for use with hematopoietic stem cells, however they are 510(k) cleared as intravascular administration sets.

### 3.2.P.3 Manufacture

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

The eli-cel DP is manufactured and tested at the sites listed in Table 72.

**Table 72 - Manufacturing and Testing Facilities for eli-cel Drug Product**

Facility	FDA Identification No.	Responsibility
Lonza Houston, Inc 14905 Kirby Dr., Houston, TX 77047, USA	FEI: 3013629214 DUNS: 832903004	Drug product manufacturing, packaging, labeling; and release testing (b) (4) and CD34+ cell identity, (b) (4), cell concentration, appearance)
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	Stability testing and Drug product release testing (VCN, (b) (4), sterility, (b) (4)

(b) (4)	FEI: (b) (4) DUNS: (b) (4)	Stability testing and Drug product release testing (endotoxin)
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	Drug product release testing and stability testing (b) (4)
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	Stability testing and Drug product release (%LVV+ Cells)
(b) (4)	FEI: (b) (4) DUNS: (b) (4)	Stability testing and Drug product release (b) (4)

### 3.2.P.3.2 Batch Formula

*Reviewed by AK*

The eli-cel batch formula is outlined in Table 73. The (b) (4) of eli-cel DS is processed into DP, which consists of the cells (b) (4) in cryopreservation solution. A batch of DP may be packaged in either one or two 20-mL bags, depending on the total number of cells present.

**Table 73 - Drug Product Batch Formula**

Component	Amount per Batch
Autologous CD34+ cell enriched population transduced with Lenti-D LVV	4 - 30 x 10 <sup>6</sup> 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 eli-cel manufacturing process is (b) (4) (Figure 24); the DS is processed into the DP (b) (4). The DP manufacturing process consists of the Formulation and Cryopreservation step, which includes (b) (4) cryopreservation solution, filling into one or two cryopreservation bags, and freezing. Formulation activities of (b) (4), and filling are completed manually using aseptic technique. Sterile, single-use disposable product-contact consumables are used, and samples are taken through aseptic connections to the infusion bag. 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.

**Figure 24 - Drug Product Manufacturing Process Flow Diagram**

(b) (4)

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

#### **Formulation and Cryopreservation**

(b) (4)

Depending on the total cells available for cryopreservation, cells are cryopreserved in one or two (b) (4) Fluoro-Ethylene-Propylene (FEP) bags filled to 20 mL final volume each. The dose is calculated by (b) (4) by (b) (4) to obtain the number of CD34 and then dividing that number by the patient weight (in kilogram, at the time of HPC-A collection). All available cells are formulated. A label detailing lot and patient specific information is applied to the flap of the bag. The (b) (4) cells are added to the cryopreservation bag(s). Samples for release testing are taken from each bag via sampling ports, and the tubing is sealed (b) (4)

The filled cryopreservation bag(s) is transferred to (b) (4) visually inspected (b) (4) for defects such as (b) (4) (b) (4). 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 (b) (4)  
(b) (4). The program includes (b) (4)

The NORs and PARs for each step of the program were established. The manufacturing batch record includes controls to ensure that the correct program is chosen by the operators. A representative reference blank with a calibrated (b) (4) is run with every (b) (4) to record the (b) (4). When the (b) (4) program is complete (b) (4), the DP is (b) (4). The cassette is stored in the vapor phase of liquid nitrogen at  $\leq 140^{\circ}\text{C}$ . DP process parameters are listed in **3.2.P.3.4 Controls of Critical Steps and Intermediates**.

*Reviewer Comment: According to the information that was provided in Amendment 48 in response to the 4/15/2022 CMC IR, during the PPQ campaign, (b) (4) were used for the HD PPQ lots, but the commercial manufacturing process will (b) (4) to freeze the final drug product bags and samples.*

#### Product Packout and Shipment

The eli-cel lot 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 lot required to be shipped and administered. Additional information about the LIS is discussed in **3.2.P.3.3 Chain of Identity**. Please see Figure 31 for a representative LIS. Eli-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 (up to 2 bags) are packed out in the same cryoshipper. The cryoshipper is qualified to maintain a temperature of  $\leq -140^{\circ}\text{C}$  throughout the duration of shipment and up to (b) (4). Upon receipt at the clinical site, eli-cel is required to be stored at  $\leq -140^{\circ}\text{C}$ . For infusion, all bags that comprise the dose of eli-cel (as listed on the LIS) are removed from liquid nitrogen storage. Bags of eli-cel are sequentially thawed and administered immediately until the entire dose of eli-cel has been infused into the patient.

*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:

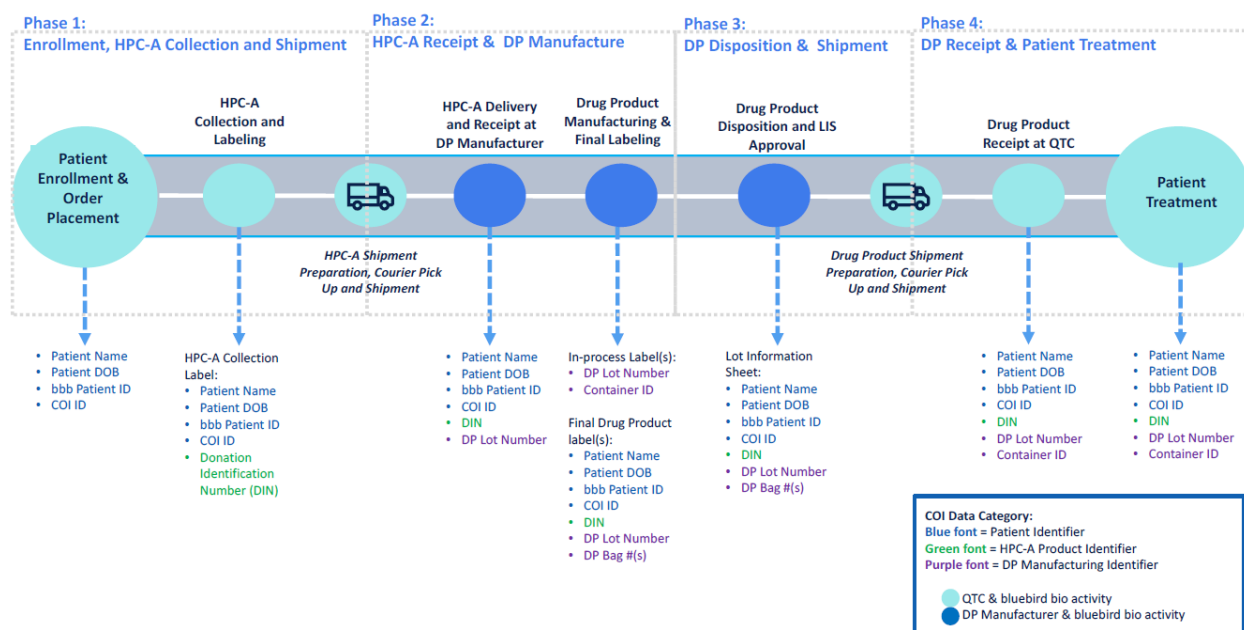
- **Patient Identifier(s)** include patient name, Date of Birth (DOB), patient ID, and COI ID. They are assigned by the QTC and/or bluebird bio and remain unchanged from enrollment to delivery of the DP
- **HPC-A Product Identifier** is also known as the Donation Identification Number (DIN) and assigned 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 infusion bags provided for the patient, as well as the patient-specific dose encompassing the total number of CD34+ cells divided by the patient's weight. The LIS is included with the shipment of DP.

The HPC-A labels, in-process DS Labels (b) (4) 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 25 illustrates each phase and the COI identifiers which will be printed on labels. Table 74 provides an example of COI for a patient/lot. Table 75 provides description of the activities supporting COI at each COI phase.

**Figure 25 - Chain of Identity – Flow Diagram**



**Table 74 - 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
			HPC-A	In-Process DS			DP		
			HPC-A Label	HPC-A Receipt	In-Process (b) (4)	In-Process (b) (4)	DP Infusion Bag Label	DP Cassette Label	Lot Information Sheet
Patient Identifier(s)	Patient Name <sup>a</sup>	Doe, Charlie Alex	(b) (4)	(b) (4)	(b) (4)	(b) (4)	✓	✓	✓
	Date of Birth (DOB) <sup>a</sup>	YYYY-MM-DD	(b) (4)	(b) (4)	(b) (4)	(b) (4)	✓	✓	✓
	Patient ID (bluebird bio Patient ID) <sup>b</sup>	bluebird bioUS0000000	(b) (4)	(b) (4)	(b) (4)	(b) (4)	✓	✓	✓
	Chain of Identity Identifier (COI ID) <sup>b</sup>	ALD22bbbUS000000	(b) (4)	(b) (4)	(b) (4)	(b) (4)	✓	✓	✓
HPC-A Product Identifier	Donation Identification Number (DIN) <sup>a</sup>	A999921120802 A888800210726	(b) (4)	(b) (4)	(b) (4)	(b) (4)	✓	✓	✓
DP Manufacturing Product Identifier(s)	DP Lot Number <sup>c</sup>	21HT702080	(b) (4)	(b) (4)	(b) (4)	(b) (4)	✓	✓	✓
	DP Bag Number <sup>c</sup>	Bag X of Y	(b) (4)	(b) (4)	(b) (4)	(b) (4)	✓	✓	✓
	Container ID <sup>c</sup>	1234567VV001	(b) (4)	(b) (4)	(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

**Reviewer comment:** Table 74 was modified to include information relevant to eli-cel in amendment 92 in response to CMC IR dated 08/12/2022.



**Table 75 - Chain of Identity- Related Activities**

<b>Phase 1: Enrollment, HPC-A Collection and Shipment</b>	
Process Step / Checkpoints	Activities Supporting COI
Patient Enrollment and Order Placement	The QTC provides Patient Identifiers (i.e., Patient Name, Patient DOB) to bluebird bio. bluebird bio assigns a unique patient ID and COI ID (per treatment).
HPC-A Collection and Labeling	DIN is assigned to each HPC-A collection and therefore, two different DINs may be included in the label of each DP as show in Table 74. COI traceability is assured via verification of COI unique identifiers present on the HPC-A label with treatment order records.
HPC-A Shipment Preparation, Courier Pickup and Shipment (from QTC to DP Manufacturer)	COI traceability is assured via verification of COI unique identifiers present on the HPC-A label with shipment request. The pickup and shipment of the HPC-A is coordinated through a bluebird bio courier and bluebird bio communicates details to the QTC. The QTC prepares the HPC-A(s) for shipment and verifies that the Patient Identifier(s) and HPC-A Identifier(s) match the shipment request.
<b>Phase 2: HPC-A Receipt and Drug Product Manufacturing COI Process</b>	
HPC-A Delivery and Receipt at DP Manufacturer	COI traceability is assured via verification of COI identifiers present on the HPC-A label with both site and incoming record(s). Prior to the receipt of the HPC-A at the DP Manufacturer, bluebird bio communicates associated Patient Identifier(s) to the DP Manufacturer with relevant manufacturing and shipment details. Upon delivery of the HPC-A, the DP Manufacturer confirms Patient Identifier(s) and HPC-A Identifier(s) with documents received in the shipment and manufacturing request. The DP Manufacturer assigns the incoming HPC-A with a DP Manufacturing/Product Identifier (i.e., DP Lot Number), and the DP Manufacturer Quality unit releases the HPC-A for DP manufacturing.
Drug Product Manufacturing and Final Labeling	COI traceability is assured via DP Manufacturing/Product Identifiers (i.e., DP lot number, container ID) and COI identifiers in the batch record. The DP Manufacturer transports the released HPC-A into the manufacturing suite to initiate DS manufacturing. The HPC-A Identifier(s) are confirmed in the batch record. Prior to final DP manufacturing, the DP Manufacturer generates final DP Infusion Bag Label(s) and Cassette Label(s) which includes all COI identifiers (except for container ID) including DP bag number. The DP Manufacturer affixes the final DP labels (Infusion Bag label(s) and Cassette label(s) to the final DP bag(s) and cassette(s), and place the cassette(s) (b) (4) into the LN2 (b) (4) for transport to the LN2 freezer (b) (4) where the DP is placed for storage.
<b>Phase 3: Drug Product Disposition and Shipping COI Process</b>	
Drug Product Disposition and Lot Information Sheet (LIS) Approval	COI traceability is assured via bluebird bio verification of COI identifiers in the executed batch record during disposition and LIS approval. The DP Manufacturer Quality unit releases the DP to bluebird bio and bluebird bio subsequently performs the final Quality unit disposition of the DP for shipment/use by verifying that all COI identifiers have been recorded and accurately traced throughout the process. Upon disposition, bluebird bio. Quality approves the LIS which contains all COI identifiers (except for container ID) and provide it to the DP Manufacturer for inclusion in the shipment of the DP to the QTC.
Drug Product Shipment Preparation, Courier Pick Up and Shipment (from DP	COI traceability is assured via DP Manufacturer verification of COI identifiers on the DP Cassette(s) with the shipping request, associated DP documentation, and LIS. The pickup and shipment of the DP is coordinated through a bluebird bio courier and bluebird bio communicates details to the

Manufacturer to QTC)	DP Manufacturer and the QTC. The DP Manufacturer prepares the DP for shipment verifying that the COI identifiers on the DP cassette label match the shipment request
<b>Phase 4: Drug Product Receipt at QTC and Patient Treatment COI Process</b>	
Drug Product Receipt at QTC	COI traceability is assured by QTC verification of COI unique identifiers present on shipping records, LIS and DP labels. The QTC provides a confirmation of receipt to bluebird bio and follows established procedures for placement into the LN2 freezer. Bluebird bio reviews shipment records and data and sends a confirmation to the QTC the DP is ready for infusion.
Patient Treatment	COI traceability is assured by QTC verification of COI unique identifiers present on LIS and final DP labels. Upon authorization from the transplant physician at the QTC, at the time of DP administration, the QTC removes the DP from the LN2 freezer and provides the LIS with the DP for infusion. The QTC verifies COI identifiers on the LIS with the DP label. The DP is thawed and infused per QTC procedures.

### 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 COI system ability the bluebird bio COI system ability to ensure HPC-A donor-to-recipient, bi-directional 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 and inspected during the Lonza facility inspection. CBER inspectors audited 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 **3.2.P.2.3.2 Manufacturing Process Characterization Studies** and support the defined control strategy for Formulation and Cryopreservation as listed in Table 76.

(b) (4)

(b) (4)

Visual inspection is performed on the final filled DP as an IPC. Trained operators assess the filled DP bag (b) (4) for defects such as (b) (4)

(b) (4) As noted in Table 76, the amount of time for visual inspection to be performed is controlled by the (b) (4)

*Overall Reviewer's Assessment of Section 3.2.P.3.4 DP Controls of Critical Steps and Intermediates: Section 3.2.P.3.4 was updated in Amendment 92 in response to CMC IR dated 08/12/2022 according to the agreements reached during the review period. 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.2.6.3 Process Characterization Studies** and **3.2.P.2.3.2 Manufacturing Process Characterization Studies**.

#### **Process Validation Overview**

The eli-cel process validation campaign was executed in 2020 according to a prospectively written protocol (VAL-VEN-PRCL-0231) and the results reported in VAL-VEN-RPT-0446. The campaign included (b) (4) PPQ runs commercial manufacturing facility, LHI-PL (Table 77). PPQ lots were also manufactured using the commercial process and control strategies (b) (4)

**3.2.S.2.6.3 Process Characterization Studies.** (b) (4) PPQ runs were validated.

Analytical methods supporting release and in-process testing of eli-cel were qualified or validated prior to testing of PPQ samples. (b) (4)

(b) (4)

**Reviewer Comment:**

(b) (4)

**Table 77 - eli-cel PPQ Batches**

(b) (4)

#### **PPQ Acceptance Criteria**

Final product release acceptance criteria must be met, IPCs must be within the established action limits, process parameters are set at to the NOR, and process-related CQAs, such as impurities, and LVV-related CQAs must meet the established acceptance criteria. The prospectively identified parameters and attributes were assigned PVAC based on process characterization studies and manufacturing experience 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 NOR or PVAC observed during the execution of the validation runs must be investigated per applicable procedure for impact to the PPQ and documented in the PPQ report.

#### **PPQ Results**

The process parameters used for each PPQ lot are detailed in Table 78. The test results from the PPQ campaign are provided in Table 79 (IPCs) Table 80 (DP release) Table 81 (DP release post-validation), and Table 82 (impurities).

4 pages have been determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

#### **Continued Process Verification (CPV)**

To ensure that process consistency will be maintained during commercial manufacturing, a CPV program was implemented as described VAL-VEN-PLAN-0038. The CPV is governed by overarching policies at bluebird bio and Lonza using SOPs. The CPV plan includes evaluation of CQAs, CPPs, selected performance attributes and non-CPPs, and IPCs. Data review and analysis are conducted every (b) (4) . Results are documented in CPV summary reports, created every (b) (4) .

(b) (4)

#### **Aseptic Process Simulation (APS)**

The APS program at LHI-PL confirms the ability to conduct aseptic processing for the manufacture of DP. Shortly, in USHT-10138: Aseptic Process Simulation Summary Report of PC702 (b) (4) Manufacturing Process, the applicant demonstrated that three APS runs were executed per the protocol (b) (4)

Testing results of three

APS runs met all specifications defined in USHT-1026. Please refer to the DMPQ review memo for additional details.

### Post-Shipment Quality Attribute Assessment

(b) (4) Liquid Nitrogen (b) (4) Vapor Shipper (b) (4) -140°C cryoshipper) (henceforth known as (b) (4) cryoshipper) is used for distribution of the elice within the United States. Validation of the (b) (4) Vapor Shipper (VAL-VEN-RPT-0337 and VAL-RPT-0003) and container closure integrity (VAL-VEN-RPT-0126) was reviewed by DMPQ and demonstrated that the shipping system operates (b) (4) for at least (b) (4) in accordance with the design specification and established performance expectation of the shipping system.

The assessment of the impact of shipping using the commercial shipper on product quality was provided in VAL-RPT-0551. These studies were performed using simulation of worse-case shipping (b) (4)

(b) (4) was used in the study. (b) (4) was manufactured from (b) (4) at the bluebird bio facility in (b) (4). The product was (b) (4). The results from the (b) (4) were compared to the results from the (b) (4).

Testing methods:

(b) (4)

Study Design

(b) (4)

**Table 83 - Process Validation - Shipping Validation Study Route**

(b) (4)

(b) (4)

[REDACTED]

[REDACTED]

(b) (4)

**Production Capacity**



LHI-PL has (b) (4) dedicated and qualified manufacturing suites (b) (4) for the production of beti-cel and eli-cel where (b) (4) beti-cel or eli-cel will be manufactured (b) (4) based on the forecasted initial demand. A Manufacturing Supervisor, Manufacturing Technicians, a Quality Control Supervisor, and Quality Control Analysts support manufacturing and QC operations to meet the indicated capacity. Additional staff are trained and capable of supporting manufacturing and quality control in the case of absences. LHI-PL has sufficient warehousing capacity to store raw materials and finished DPs. LHI-PL has demonstrated during clinical development the ability to produce bluebird bio products at this (b) (4) rate. As demand for the products increase and additional capacity is required, bluebird bio and LHI will proactively assess risks to delivery and any potential risks identified will be controlled as required prior to capacity increase.

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

*Reviewed by BS*

The sole excipient in the drug product 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) BBMF (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 (b) (4) 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

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

*Reviewed by AK*

The final agreed upon eli-cel lot release acceptance criteria are summarized in Table 84.

**Table 84 - Release Specifications for eli-cel**

Quality Attribute	Test	Method	Acceptance Criteria	Justification
Potency and Strength	Vector Copy Number (VCN)	(b) (4) qPCR	(b) (4)	(b) (4) of the clinical lots
	% LVV+ Cells	(b) (4)	(b) (4)	(b) (4) of the clinical lots
	(b) (4)	(b) (4)	(b) (4)	(b) (4) of the clinical lots
	(b) (4)	(b) (4)	(b) (4)	Manufacturing/clinical experience
	(b) (4)	(b) (4)	(b) (4)	(b) (4) of the clinical lots (b) (4)
	%ALDP+ Cells	(b) (4)	(b) (4)	(b) (4) of the clinical lots
	CD34+ Cell Identity	(b) (4)	(b) (4)	N/A
Purity and Content	(b) (4)	(b) (4)	(b) (4)	(b) (4) of the clinical lots
	(b) (4)	(b) (4)	(b) (4)	(b) (4)
	Total Cell Concentration	(b) (4)	4 to 30 x 10 <sup>6</sup> total cells/mL	Manufacturing/clinical experience
	(b) (4)	(b) (4)	(b) (4)	Manufacturing/clinical experience
Safety	Sterility	(b) (4)	No Growth	Requirement
	Endotoxin	(b) (4)	(b) (4)	Based on the (b) (4)
	(b) (4)	(b) (4)	None Detected	Requirement
Quality	Appearance	Visual assessment	Colorless to white to red, including shades of white	Based on (b) (4)

		or pink, light yellow, and orange.	
--	--	------------------------------------	--

**Reviewer Comment:** In CMC IR #8, dated 7/1/2022, bluebird bio was asked to tighten the (b) (4), total cell concentration, (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 80 which we agreed with. All updates are reflected in Table 84.

### Sampling time points

Eli-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 85.

**Table 85 - Sampling Points, Matrices and Justifications for eli-cel Release Testing**

Release Test	Sampling Point	Matrix	Justification
(b) (4)	(b) (4)		
CD34+ Cell Identity			
(b) (4)			
(b) (4)			
Total Cell Concentration			
(b) (4)			
Vector Copy Number			
(b) (4)			
%LVV+ Cells			
% ALDP+ Cells			
(b) (4)			
Appearance			
Sterility			
Endotoxin			

**Reviewer Comment:** In CMC IR #7 and #8, dated 6/10/2022 and 7/1/2022, respectively, bluebird bio was asked to include sampling of the DP for (b) (4), (b) (4), Total Cell Concentration, and (b) (4) to confirm on the final formulated DP. In Amendments 66 and 80, 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. The additional validation is anticipated to be

completed in (b) (4) to implement the additional testing in (b) (4). The addition of this testing will be addressed in a PMC bluebird bio agreed to in Amendment 93. Note, bluebird bio still intends to determine eli-cel dose based on the (b) (4) test results to remain consistent with dosing in the clinical trials. Whether this continues to be acceptable will be determined upon review of the data provided in the (b) (4).

#### **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-PL inspection. A follow-up IR (CMC IR #9) was sent on 7/28/2022. Bluebird responded in Amendment 84 that a mechanism to release OOS commercial eli-cel was still in development and until the pathway was implemented, no OOS commercial eli-cel lots would be released. bluebird bio intends to submit a supplement to provide this information once development of the necessary protocols and procedures is complete. This information will be submitted as soon as possible.

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

*Reviewed by AK, MM and DBSQC*

**Reviewer Comment:** Sterility (b) (4) Endotoxin (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 (b) (4) assay and (b) (4) to the (b) (4) test sample to further confirm the absence of contamination. Please see DBSQC review for additional details.

(b) (4)

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

(b) (4)

**Table 88 - Cell Concentration Method Validation**

(b) (4)

(b) (4)

2 pages have been determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

. These data support adequate assay validation.

**Vector Copy Number (VCN)**

To assess VCN, a DP sample,

(b) (4)

**System/Sample Suitability:**

(b) (4)



A summary of the method validation (VAL-VEN-RPT-0399) is provided in Table 90.

**Table 90 - VCN Method Validation**

(b) (4)

(b) (4)

(b) (4)

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

(b) (4)

**System/Sample Suitability:**

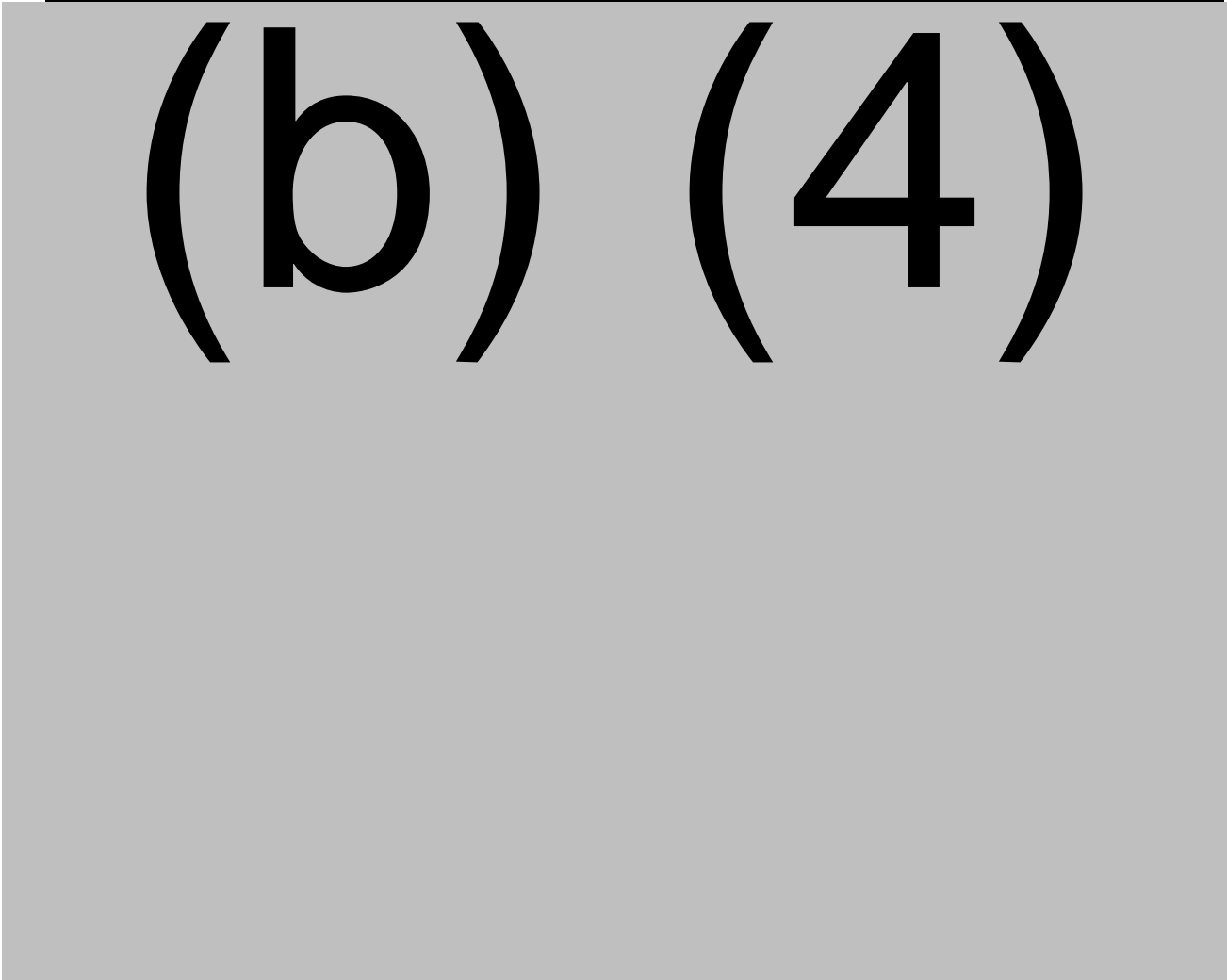
(b) (4)

(b) (4)



A summary of the method validation (VAL-VEN-RPT-0223) is provided in Table 91.

**Table 91 - %LVV Method Validation**



(b) (4)

3 pages have been determined to be not releasable:(b)(4)

(b) (4)

(b) (4)

**% ALDP+ Cells**

The % ALDP+ Cells method determines

(b) (4)

(b) (4)

A summary of the method validation (VEN-RPT-0765, VEN-RPT-0157) is provided in Table 93.

**Table 93 - Summary of % ALDP+ Cells Method Validation**

(b) (4)

6 pages have been determined to be not releasable: (b)(4)

(b) (4)

### 3.2.P.5.4 Batch Analyses

A total of 67 eli-cel lots were manufactured for use in ALD-102 and ALD-104 (b) (4) lots manufactured at (b) (4) lots manufactured at (b) (4) lots manufactured at LHI (b) (4) and (b) (4) lots manufactured at LHI-PL. All lots met the release criteria in place at the time of release and all were administered. At the time of this analysis, of the 67 infused subjects, 3 experienced disease progression, 2 were withdrawn to receive an allogeneic hematopoietic stem cell transplant at the treating physician's discretion and 3 developed myelodysplastic syndrome (MDS). No lot release criteria correlated with disease progression or the development of MDS.

Commercial specifications were established using available data from all (b) (4) eli-cel lots with the exception of the (b) (4) lots manufactured at (b) (4) (for a total of (b) (4) lots) because comparability of the (b) (4) lots to the lots manufactured at LHI or (b) (4) could not be established. Data from the following numbers of lots were used 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):**

- (b) (4), Total Cell Concentration, (b) (4) and VCN: (b) (4) lots
- %LVV+ cells and (b) (4) lots
- %ALDP+ Cells: (b) (4) lots
- (b) (4) lots
- (b) (4) lots

### 3.2.P.5.5 Characterization of Impurities

eli-cel is manufactured from eli-cel DS (b) (4). Characterization of potential impurities in eli-cel DS is discussed in **3.2.S.3.2 Impurities**.

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

Testing of eli-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 (TEB) and Summarized by TS*

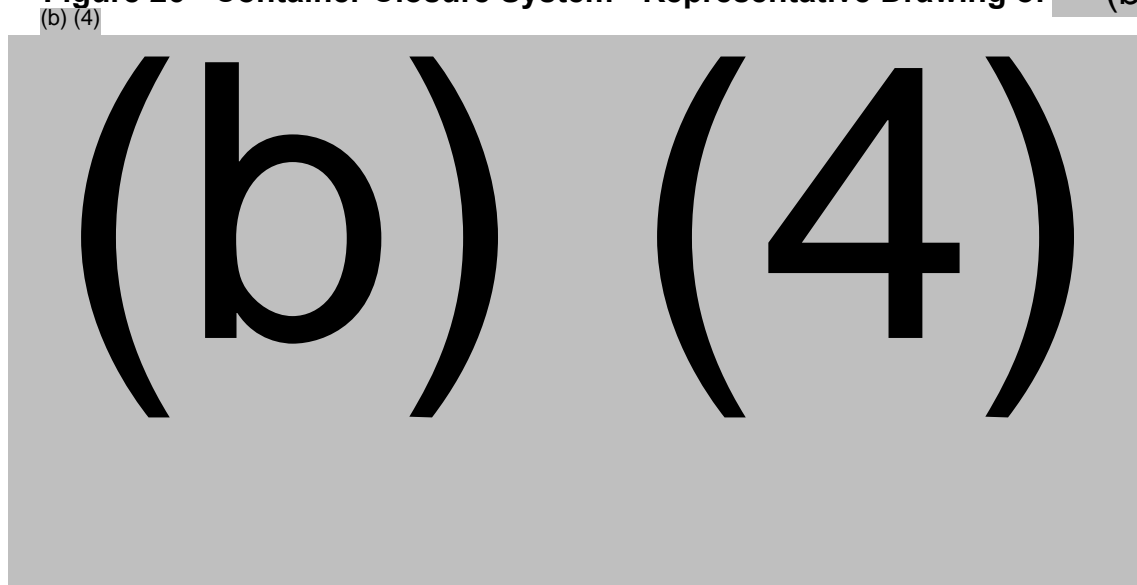
The container closure system consists of a primary package container, the (b) (4) (b) (4) Cryopreservation bag, a secondary package container (b) (4) Overwrap bag), and a tertiary package container (cryocassette). The primary container closure is a 20-mL fluorinated ethylene propylene (FEP) cryopreservation bag with maximum fill volume of (b) (4) mL. The (b) (4) is manufactured by (b) (4) (b) (4). Please see Table 96 and Figure 26

for the specifications and representative drawings of the (b) (4), respectively.

**Table 96 - Container Closure System - Specifications/Technical Information for**

(b) (4) Bag	
Bag material	(b) (4)
Spike port with septum, protected with FEP cover	
Inlet tubing	
Female Luer	
Pinch clamp	
Inside bag dimensions	
Outside bag dimensions (including port and label pouch)	
Working temperature	
(b) (4)	

**Figure 26 - Container Closure System - Representative Drawing of**



**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 extreme temperature conditions and (b) (4) events. This testing is being requested in a PMC. The Applicant agreed to this PMC request on August 17, 2022 (Amendment 93). 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 with this PMR request on August 19, 22 (Amendment 96).*

### **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**

Long-term stability under the recommended long-term storage condition ( $\leq -140^{\circ}\text{C}$ ) was evaluated for (b) (4) eli-cel lots produced at LHI- (b) (4) , and LHI-PL (including the 3 PPQ lots). All eli-cel long-term stability lots were manufactured from healthy donor material and were stored in (b) (4) bags. Table 97 summarizes the long-term stability data provided. Note, in addition to the tests listed in Table 97, all lots were tested for sterility and endotoxin at a minimum at the (b) (4) of the stability study.

**Table 97 – Summary of Available eli-cel Stability Data**

(b) (4)

With three exceptions, all results met the stability acceptance criteria in place at the time the lot was put on stability, and no trends were observed. One VCN OOS result was observed at the (b) (4) -month time point (note, this is beyond the proposed expiry of 9 months) for Lot (b) (4) , with a VCN result of (b) (4) (down from (b) (4) at release). One (b) (4) OOS was observed at the 6-month time point for Lot (b) (4) , with a (b) (4) result of (b) (4) (down from (b) (4) at release). One cell concentration OOS was observed at the 9-month time point for Lot (b) (4) , with a result of  $1\text{e}6$  (down from  $3\text{e}6$  at release). Investigations into the (b) (4) cell concentration OOS results

determined that they were due to inconsistent handling of the stability samples during the thaw procedure. A CAPA was implemented to provide sufficient detail on how to conduct the thaw procedure at the testing site.

Exclusive of the results described above, all results met the proposed commercial lot release acceptance criteria except for the following:

- 2 lots (b) (4) exhibited (b) (4) below (b) (4) (but not below (b) (4), the commercial stability acceptance criterion) at 1-2 time points but then recovered at the next time point. In the case of (b) (4)-01, this may also have been a sample handling issue.
- 3 lots (b) (4) exhibited  $VCN \leq 0.7$  (0.5 – 0.6). However, in all cases the lot had a VCN of 0.6 at release (the clinical acceptance criterion was (b) (4) copies/cell).
- 4 lots (b) (4) exhibited (b) (4) values (b) (4) at one or more time points (1-6 months). Note, lots (b) (4) started with results less than (b) (4) (the clinical acceptance criterion was report result).
- Lot (b) (4) exhibited a %LVV+ cell value of (b) (4) (down from 41% at release) at the (b) (4)-month time point. However, this is beyond the requested expiration date.
- Lot (b) (4) exhibited a (b) (4) value of (b) (4) (down from (b) (4) at release) at the 6-month time point but then recovered at the next time point.
- 5 lots (b) (4) exhibited cell concentrations less than  $4 \times 10^6$  cells/mL at one or more time points. Note, lots (b) (4) started with results less than  $4 \times 10^6$  cells/mL (3, 3.5). (b) (4) started at  $4 \times 10^6$  cells/mL but dropped to  $3 \times 10^6$  cells/mL at 3 months. (b) (4) started at  $5 \times 10^6$  cells/mL but dropped to  $3 \times 10^6$  cells/mL at 6 months. However, no lots dropped below the clinical and commercial stability acceptance criterion of (b) (4).

(b) (4)

(b) (4)

- An additional (b) (4) months of long-term stability data were provided. All prospective acceptance criteria were met and no trends were observed.
  - After (b) (4) cycle, the eli-cel PPQ Lot (b) (4) maintained cell concentration (b) (4) but insufficient cells remained to evaluate (b) (4) or %LVV+ cells. This is acceptable since freeze/thaw cycles are not permitted for eli-cel.
  - Accelerated stability data (b) (4)
- (b) (4)
- bluebird also provided (b) (4) data from the (b) (4) lots manufactured between September 2013 and August 2014 tested using the LHI-PL commercial release methods. No reduction in (b) (4) or (b) (4) was observed. Although comparability of the (b) (4) and LHI-PL methods has not been established, this is supportive of product stability.

A number of attributes were OOS for the commercial lot release specifications at one or more time points. However, in almost all cases the OOS either (1) was not below the commercial stability specifications (b) (4), cell concentration); (2) the attribute began OOS for the commercial specifications because of wider acceptance criteria during clinical investigation (VCN, (b) (4)); (3) recovered at the next time point, indicating that the OOS could be attributed to early assay variability (b) (4)). The remaining OOS observations could easily be attributed to donor variability. Additionally, though the highest concentration of eli-cel being allowed by the commercial specifications is  $30 \times 10^6$  cells/mL, the highest concentration evaluated in the stability

(b) (4)

Although there are very little (b) (4) data, and (b) (4) data as the stability lots were all manufactured from healthy donor material, the remaining data support an eli-cel shelf life of 9 months when stored at the intended long-term storage condition of  $\leq -140^{\circ}\text{C}$ . Additional long-term stability data will be obtained as part of the post-approval stability plan.

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

Post-approval, during each calendar year, one eli-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 98.

**Table 98 - Annual Post-Approval Stability Testing at Long-term Storage Conditions ( $\leq -140^{\circ}\text{C}$ )**

Method	Acceptance Criteria	Time Point (Months)			
		0	3	6	9
Vector Copy Number (VCN)	(b) (4)	P	P	P	P
(b) (4)		P	P	P	P
		P	P	P	P
%ALDP Cells		P	P	P	P
Total Cell Concentration		P	P	P	P
(b) (4)		P	P	P	P
%LVV+ Cells		P	P	NR	P
(b) (4)		P	P	NR	P
(b) (4)		P	P	NR	P
Sterility	No Growth	P	NR	NR	NR
Endotoxin	(b) (4)	P	NR	NR	NR

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

**Reviewer Comment:** In CMC IR#8, dated 7/1/2022, bluebird was asked to i) include %LVV+ cells, (b) (4), and (b) (4) testing to the protocol; ii) update the stability acceptance criteria based on the updates to the lot release specifications; and iii) include (b) (4) testing of any eli-cel lots manufactured from patient material. In Amendment 80, bluebird updated the stability acceptance criteria and agreed to include (b) (4) testing of any eli-cel lots manufactured from patient material that may be placed on stability. Note, (b) (4) cell concentration acceptance criteria is being allowed because lot release testing is currently performed on a (b) (4) sample and some degree of (b) (4)/cell loss is expected upon cryopreservation.

## 3.2.A APPENDICES

### 3.2.A.1 Facilities and Equipment

*Reviewed by DMPQ. Please see DMPQ review for details.*

Additionally, a pre-license inspection of the Lonza Houston, Inc. facility located at 14905 Kirby Drive, Houston, TX 77047 in support of approval of BLA 125755 was conducted between 2/14/2022 and 2/18/2022. No FDA Form 483 items were identified. However, a number of discussion items were conveyed. Please see Establishment Inspection Report for additional details.

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

Information in this section is integrated into section **3.2.S.2.3 Control of Materials - Lenti-D** and section **3.2.S.2.3 Control of Materials**.

#### Viral Clearance Studies

Viral clearance studies were not performed on Lenti-D LVV or the eli-cel DP. However, studies on (b) (4) impurity clearance during the eli-cel manufacturing process was conducted and found acceptable (**3.2.S.2.6 Manufacturing Process Development** and **3.2.S.3.2 Impurities**).

### 3.2.R Regional Information (USA)

#### **Risk Assessment for (b) (4)**

*Reviewed by TS*

Eli-cel is an autologous product not required donor (b) (4) testing. The applicant assessed the potential risk to safety, integrity, strength, purity, and quality (SISPQ) of the unintended expansion of the (b) (4) virus in the eli-cel manufacturing process resulting in the final product with (b) (4) viral load. Based on the assessment performed, there is a low risk of transmission of the (b) (4) virus into patient apheresis material, LVVs or in the DPs. Since (b) (4) used by (b) (4) to enter into target cells, the cells within the apheresis product are an unlikely target for (b) (4) infection. Additionally, the manufacturing process is controlled for adventitious agents and performed using closed systems when 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 controls to reduce the risk of amplified (b) (4) viral load.*

### Executed Batch Records

*Reviewed by TS*

The unexecuted master batch record and an executed batch record for (b) (4), which was manufactured in September 2020, were provided. bluebird bio submitted an updated master batch record incorporating all the changes requested due to various FDA comments provided throughout the BLA review period in Amendment 112 on 9/2/0222.

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

## **Comparability Protocols**

N/A

### **Module 1**

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

bluebird bio is claiming a categorical exclusion under 21 CFR 25.31(c) from the need to prepare an environmental assessment. bluebird bio is not aware of any extraordinary circumstances that would require the preparation of an environmental assessment.

eli-cel is composed of human cells transduced with a LVV. The Lenti-D LVV used is created using a third-generation split HIV-1 genome and plasmid system generating a replication defective, self-inactivating vector. Lenti-D LVV lots are fully tested following manufacturing and clinical trial subjects were monitored after eli-cel administration according to current FDA guidelines. bluebird bio found no evidence of RCL in 20 Lenti-D LVV lots or in samples from more than 50 subjects administered Skysona during investigational studies.

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.

*Reviewer Comment: The categorical exclusion claim is acceptable. No FONSI review required.*

#### **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 eli-cel and there are no licensed biological products that are structurally related to eli-cel for which bluebird bio or one of its affiliates, licensors, predecessors in interest, or related entities are the current or previous license holders.

*Reviewer Comment: The proposed reference product exclusivity period of 12 years is acceptable.*

#### **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 PI provides a detailed and correct description of eli-cel and its mechanism of action. The PI also carefully and correctly describes the receipt and preparation procedures for eli-cel.

**Reviewer Comment:** *There were multiple interactions with the applicant during review of the PI where the applicant was asked to clarify multiple details on the receipt and administration preparation procedures of eli-cel. The applicant agreed to make the requested changes and the changes were found to be adequate.*

#### **Carton and Container Label**

Examples of the eli-cel cassette (Figure 27) and bag (Figure 28) labels as well as the Lot information Sheet (Figure 29) are provided below. All labels contain the required text.

**Reviewer Comment:** *The initial labels provided complied with 21 CFR 610.60-62. However, the statements regarding irradiation, use of an in-line filter, evaluation for infectious substances, and that the cells are genetically modified needed to be included on the bag label. In CMC IR #9 and #10, dated 7/28/2022 and 8/12/2022, respectively, bluebird bio was asked to update the labels with this information as well as the agreed upon cell concentration range. bluebird bio provided the final updated labels below in Amendment 92 (Lot information Sheet and patient identifiers label) and Amendment 108 (cassette and bag labels). The updated labels were acceptable.*

#### **Figure 27 - Eli-cel cassette label**

elivaldogene autotemcel  
skysona™



Suspension for IV infusion  
20 mL containing 4 to 30 x 10<sup>6</sup> cells/mL  
(3.6 to 30 x 10<sup>6</sup> CD34+ cells/mL)

**For autologous use only. For intravenous use only. Rx only.**

Contains autologous hematopoietic stem cells transduced with Lenti-D lentiviral vector 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.**

This medicine contains genetically modified cells.

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: \$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

SKYSONA.com




P/N: XXXXXXX


Label P/N: XXXXXXX



**Figure 28 - Eli-cel bag labels**

elivaldogene autotemcel skysona™		
Suspension for IV infusion 20 mL containing 4 to 30 x 10 <sup>6</sup> cells/mL (3.6 to 30 x 10 <sup>6</sup> CD34+ cells/mL)		<sup>3</sup> NDC 73554-2111-1 <sup>7</sup>
<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.		
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. <b>Dispense with Medication Guide.</b>		
P/N: XXXXXXXX Label P/N: XXXXXXXX	Manufactured for: bluebird bio, Inc. Somerville, MA 02145	

elivaldogene autotemcel skysona™	
Suspension for IV infusion 20 mL containing 4 to 30 x 10 <sup>6</sup> cells/mL (3.6 to 30 x 10 <sup>6</sup> CD34+ cells/mL)	
<b>Confirm Patient Identifiers</b>	
Last Name:	\$LastName\$
First Name:	\$FirstName\$
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 29 – Lot Information Sheet**

elivaldogene autotemcel  
skysona™

Suspension for IV infusion  
20 mL containing 4 to 30 x 10<sup>6</sup> cells/mL  
(3.6 to 30 x 10<sup>6</sup> CD34+ cells/mL)



## LOT INFORMATION SHEET

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

For autologous use only. For intravenous use only.

Confirm patient identifiers. Read the prescribing information before use.

The following lot 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 SKYSONA 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.



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

Manufactured by:  
Lonza, Inc.  
Houston, TX 77047

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

## Modules 4 and 5

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

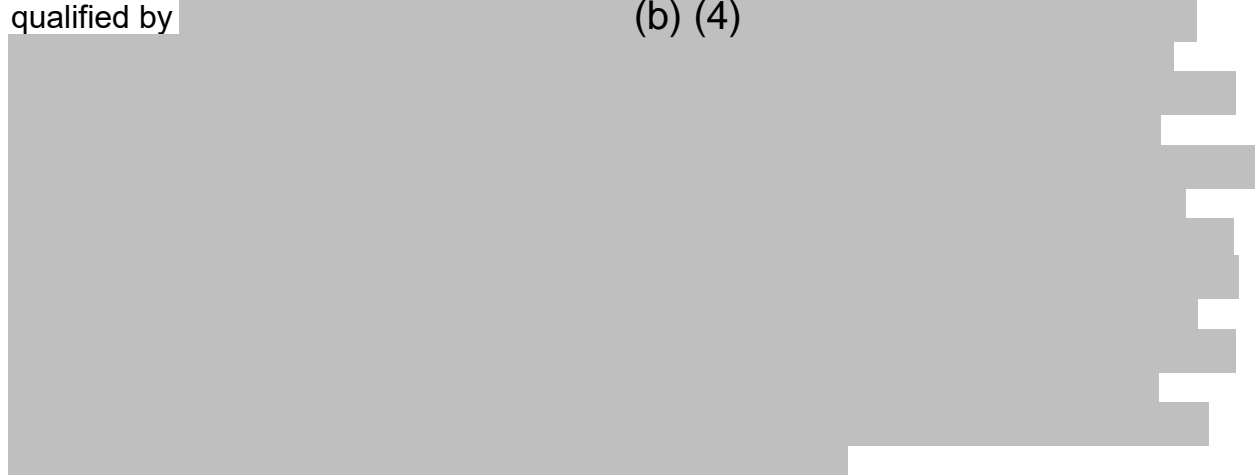
*Reviewed by AK*

#### Vector copy number (VCN)


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



Lenti-D VCN in patient samples was also determined by a qPCR assay performed and qualified by (b) (4)



**Reviewer Comment:** *These assays are considered suitable for the analysis of study/subject samples. In response to the clinical IR (#37), dated 7/11/2022, bluebird bio was asked when the two VCN assays were used. In Amendment 79, bluebird bio indicated that for ALD-102, samples were evaluated at the laboratory at (b) (4). For ALD-104 and LTF-304, samples were evaluated at (b) (4). A bridging study conducted between (b) (4) for the VCN test method. (b) (4) sets of peripheral blood samples from (b) (4) healthy donors were (b) (4)*



(b) (4). The percent difference between calculated VCNs from (b) (4) ranged from (b) (4). The % difference for the (b) (4) was (b) (4), however, the absolute difference was low (b) (4). Additionally, these VCN results are lower than any VCN values observed in any eli-cel study clinical samples. Therefore, this variability was considered acceptable.

#### % ALDP+ Cells

ALDP expression in patient samples was determined by intracellular staining and (b) (4) analysis performed and qualified by the (b) (4)

This assay uses (b) (4)

ALDP expression in patient samples was also determined by intracellular staining (b) (4)

(b) (4)

**Reviewer Comment:** This assay is considered to be suitable for the analysis of study/subject samples. In response to the clinical IR (#37), dated 7/11/2022, bluebird bio was asked when the two %ALDP assays were used. In Amendment 79, bluebird bio indicated that for ALD-102, samples were evaluated at the laboratory at (b) (4). For ALD-104 and LTF-304, samples were evaluated at (b) (4). The ALDP method was optimized, revised, and validated at (b) (4). However, no formal assay transfer study was conducted between the two labs.

Insertion site analysis

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

(b) (4). The assay was qualified for specificity, linearity, accuracy, precision, and range (including LOQ). Samples were analyzed in (b) (4).

The assay was shown to be accurate

(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.