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**CBER CMC BLA Review Memorandum**

**BLA STN 125846**

**etuvetidigene autotemcel  
WASKYRA**

**Reviewers:**

**Laura DeMaster, Ph.D. OTP/OGT/DGT2/GTB4**

**Stella Lee, Ph.D. OTP/OGT/DGT2/GTB4**

**Renuka Miller, Ph.D. OTP/OGT/DGT2/GTB4**

**Teisha Rowland, Ph.D. OTP/OGT/DGT2/GTB4**

**1. BLA#:** STN 125846

**2. APPLICANT NAME AND LICENSE NUMBER**

Fondazione Telethon ETS

License Number: 2378

**3. PRODUCT NAME/PRODUCT TYPE**

Non-Proprietary/Proper/USAN: etuvetidigene autotemcel

Proprietary Name: WASKYRA

Company codename: TLT003

UNII Code: SMN5E7TJ9C

NDC Codes: Pending at time of Approval

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

- a. Pharmacological category: Autologous hematopoietic stem cell-based gene therapy
- b. Dosage form: suspension
- c. Strength/Potency: 1.9E6 – 11.4E6 CD34+ cells per mL with a minimum recommended dose of 7E6 CD34+ cells per kg
- d. Route of administration: Intravenous infusion
- e. Indication(s): Treatment of pediatric patients aged 6 months and older and adults with Wiskott-Aldrich Syndrome (WAS) who have a mutation in the WAS gene and for whom hematopoietic stem cell transplantation (HSCT) is appropriate and no suitable human leukocyte antigen (HLA)-matched related stem cell donor is available.

**5. MAJOR MILESTONES**

Event	Date
Initial IND Submission (IND 18919)	May 15, 2019
IND allowed to proceed	June 14, 2019
Rare Pediatric Disease designation granted	December 1, 2017
Regenerative Medicine Advanced Therapy designation granted	July 11, 2019
DCC Receipt Date	January 10, 2025
First Committee Meeting	January 31, 2025
AOM/Dataset Walkthrough (w/ Applicant)	February 21, 2025
Filing Meeting	February 24, 2025
Mid-Cycle Tcon with Applicant	May 12, 2025
Late-cycle Meeting with Applicant	June 26, 2025
Major Amendment	August 1, 2025
PDUFA Action Date	December 10, 2025

**6. CMC/QUALITY REVIEW TEAM**

Reviewer/Affiliation	Section/Subject Matter
Laura DeMaster, CMC Reviewer/Chair OGT/DGT2/GTB4	etu-cel DP Manufacturing Process and Validation, Comparability; DP Specifications
Stella Lee, CMC Reviewer OGT/DGT2/GTB4	WAS LVV Manufacturing Process and Validation, Comparability
Renuka Miller, CMC Reviewer OGT/DGT2/GTB4	WAS LVV and etu-cel DS: Control of Materials, Control of Excipients, Impurities, Container Closure System; etu-cel DP Stability
Teisha Rowland, CMC Reviewer OGT/DGT2/GTB4	WAS LVV and etu-cel DP: analytical methods; LVV Specifications
Andrey Sarafanov, OTP/OPPT/DH/HB2	Extractables and Leachables Assessment for DP Manufacturing Process

**7. INTER-CENTER CONSULTS REQUESTED**

Not applicable

**8. SUBMISSION(S) REVIEWED**

CMC/ OGT IR #	Date Received	Submission	Comments/ Status
N/A	2/6/2025	STN 125846/2	English versions of executed MBRs for LVV and DP as well as assay SOPs
1	3/26/2025	STN 125846/7 (Part 1)	LVV upstream materials, Comparability PTCs and RPTs, Process-related impurities, Translation certification, Sampling points and storage temperature for safety tests;
	4/1/2025	STN 125846/8 (Part 2)	
2	4/4/2025	STN 125846/9	(b) (4) DP WASP assay test system and control strategy
3	5/8/2025	STN 125846/13	(b) (4) assays validation, Inadequacy of DP stability data, LVV control of materials, (b) (4) (b) (4), LVV PPQ AC, LVV sampling plan, Post PPQ (b) (4) trend, representative label
4	6/9/2025	STN 125846/17	CPP/IPC targets, Justification for CPCP ranges, (b) (4) for microbial testing, Request for normalized WASP data, (b) (4) DP sterility sample storage, TE assay linearity, timelines for (b) (4) assay validation study submissions, submission for DP stability data
	6/9/2025	STN 125846/19	(b) (4) impurity revised batch analysis and lot release AC; sampling point for (b) (4) myco
5	7/15/2025	STN 125846/21	mPB collection centers and labels, DP WASP assay test system, correlative re-analysis to support potency, assay validations for viability, CD34+, and LVV safety assays; (b) (4) (b) (4) product contact and identity testing, info on integration site analysis and clinical WASP assays

6	8/1/2025	STN 125846/23	(b) (4) WASP AC
7	8/20/2025	STN 125846/24	(b) (4) WASP assay test system and validation, (b) (4) assay test system and validation, (b) (4) stability WASP data, support for use of (b) (4) LVV lots in DP manufacturing
8	9/2/2025	STN 125846/24	(b) (4) DP JoS, clarification on data for potency assurance
9	9/10/2025	STN 125846/26	(b) (4) safety assay validation reports, CD34+ and RCL validation information, QC fill path
10	10/10/2025	STN 125846/26	Data supporting transduction with (b) (4) LVV lots
11	11/4/2025	STN 125846/28	(b) (4) (b) (4) incoming testing, (b) (4) endotoxin units, filter compatibility data, DP WASP linearity and range, (b) (4) (b) (4) range, DP JoS figure
12	11/6/2025		Confirm barcode on physical label, (b) (4) (b) (4) for reference material
13	11/12/2025		(b) (4) assay clarification, withdrawal of post-approval comparability protocol
14	11/13/2025		Final requests for physical label
N/A	11/21/2025	STN 125846/29	
15	11/25/2025	STN 125846/30	Exemption request for DSCS provisions

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

Submission Type & #	Holder	Referenced Item	Use in BLA (LVV/DP)	Letter of Cross-Reference	Comments/Status
IND 18919	Fondazione Telethon ETS	Cover letter with product designations; summaries of FDA meetings prior to BLA	N/A	None, since applicant is IND sponsor	No concerns
BB-MF-(b) (4)	(b) (4)	(b) (4)	DP	yes	Suitable for commercial manufacturing CMC: Iain Farrance CBER/OTP/OCTHT/DCT1/CTB1
MF-(b) (4)	(b) (4)	(b) (4)	DP	yes	Suitable for commercial manufacturing CMC: Jaikumar Duraiswamy CBER/OTP/OCTHT/DCT1/CTB2
MF-(b) (4)	(b) (4)	(b) (4)	DP	yes	Suitable for commercial manufacturing CMC: Archana Siddam

					CBER/OTP/OCTHT/DCT1/CTB1
MF-(b) (4)	(b) (4)	(b) (4)	DP	yes	Suitable for commercial manufacturing CMC: Jaikumar Duraiswamy CBER/OTP/OCTHT/DCT1/CTB2
MF (b) (4)	(b) (4)	(b) (4)	DP	yes	Suitable for commercial manufacturing CMC: Fatima Abassi CBER/OTP/OCTHT/DCT1/CTTB
MF(b) (4)	(b) (4)	(b) (4)	LVV	yes	Suitable for commercial manufacturing CMC: Christelle Mbondji and Timothy Kamaldinov CBER/OTP/OGT/DGT2/GT B4
MF-(b) (4)	(b) (4)	(b) (4)	LVV	yes	Suitable for commercial manufacturing CMC: Stella Lee CBER/OTP/OGT/DGT2/GT B4

## 10. REVIEWER SUMMARY AND RECOMMENDATION

### A. EXECUTIVE SUMMARY

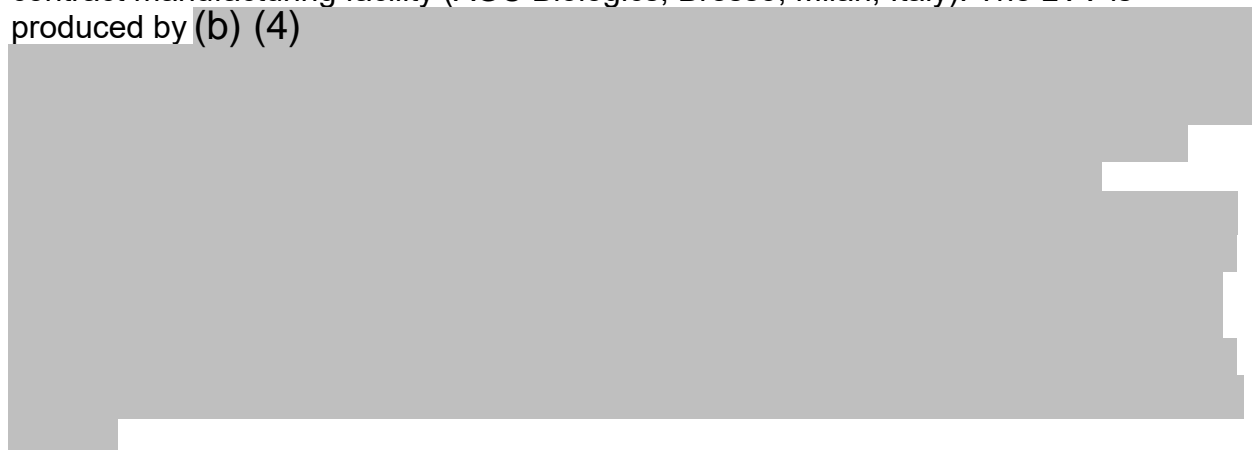
*Written by LKD*

The FDA CMC Review Team concludes that the manufacturing process, test methods, and control measures for etuvetidigene autotemcel (etu-cel, WASKYRA) are capable of yielding autologous products with consistent quality attributes determined acceptable for commercial manufacturing under this BLA.

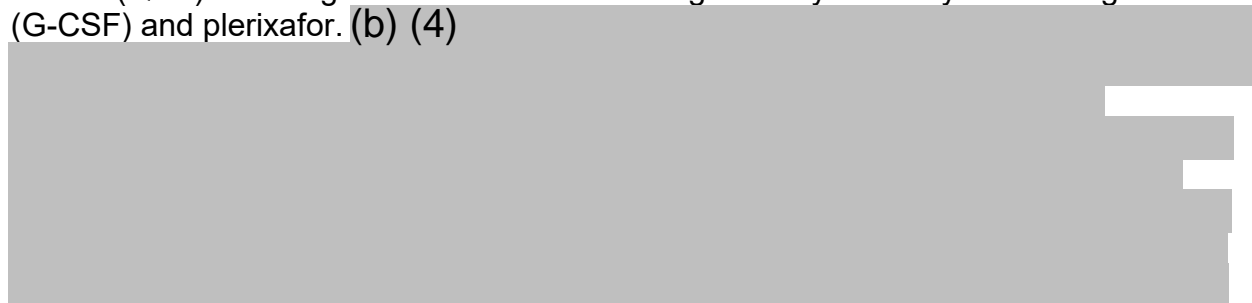
Etu-cel is an autologous gene therapy product for the treatment of Wiskott Aldrich Syndrome (WAS) in patients aged 6 months and older who have a mutation in the WAS gene and for whom no suitable human leukocyte antigen (HLA)-matched related hematopoietic stem cell donor is available. Etu-cel consists of a cell population originating from autologous hematopoietic stem and progenitor cells (HSPCs) enriched for CD34+ cells and transduced with a lentiviral vector (LVV) encoding the cDNA for WAS protein (WASP). The proposed mechanism of action involves engraftment of transduced CD34+ cells in the bone marrow and differentiation into lymphoid and myeloid progenitors whose progeny express WASP. WASP is a cytoskeletal regulator that promotes actin polymerization in lymphoid and myeloid-lineage cells.

During etu-cel manufacturing, the WAS gene sequence is transferred into the patient's CD34+ cells by the WAS LVV. WAS LVV is a non-replicating, self-inactivating lentivirus based on a 3<sup>rd</sup> generation HIV-1-derived vector, which is (b) (4). (b) (4) WAS LVV is manufactured at a

contract manufacturing facility (AGC Biologics, Bresso, Milan, Italy). The LVV is produced by (b) (4)



Etu-cel is manufactured from autologous mobilized peripheral blood (mPB) starting material. mPB is collected by apheresis from each patient at a Qualified Treatment Center (QTC) following HSPC mobilization with granulocyte-colony stimulating factor (G-CSF) and plerixafor. (b) (4)



(b) (4) cryopreservation medium (0.9% w/v sodium chloride with 5% v/v DMSO and 7% w/v HSA). The harvested cells are resuspended at a target concentration of 2E6 to (b) (4) E6 cells per mL in 10 – 20 mL of total volume per EVA bag. Final drug product may be composed of 1 – 8 total bags and is stored at <-130°C.

The etu-cel control strategy includes raw material and reagent qualification, in-process and lot-release testing. Lot release assays are suitably validated or verified, and product specifications are adequate to ensure commercial etu-cel quality is consistent with product lots used in clinical studies.

FDA exerted regulatory flexibility in several areas of the review given that WAS is a rare disease primarily impacting a pediatric population and considering the importance of ensuring that children have access to these therapies. The potency assurance strategy for commercial etu-cel is based on WASP expression assay lot release criteria and an interim analysis demonstrating correlation of WASP expression values in the final product with increases in platelet count between baseline and Day 180 (a relevant clinical outcome) rather than a DP functional potency assay. In the case of this submission, patient cellular material is necessary to develop and validate a DP potency assay, and, as patient material was severely limited, development of a potency assay measuring transgene function was challenging. Considering the clinical benefit observed in the clinical trials, FDA agreed that it was not appropriate to delay access of this therapy for a rare disease pediatric population in order to implement a validated

functional potency assay for lot release when product potency could be assured with the currently validated WASP expression release assay based on the interim correlation analysis. However, given the limited data set available for the correlative analysis at the time of approval, 2 PMCs were issued related to potency assurance. The applicant committed to either confirm the correlation from the interim analysis or implement a potency test for DP release. Additionally, a PMC was issued for implementation of an assay measuring WASP expressed by (b) (4). The applicant also committed to validate the assays in both of these PMCs, as validated analytical assays for licensed products are required by cGMPs.

Regulatory flexibility was also exerted in the data used to support assay validations for several LVV and DP lot release tests. The applicant utilized data from a similar genetically modified CD34+ cell product; to support etu-cel assay validations based on the justification that it is sufficiently representative of etu-cel for these purposes. FDA agreed that it is reasonable to leverage these data for certain lot release assays given that the same (b) (4) assay methods are used to test the same attributes for both products and the similarity between the manufacturing processes for the products. Of note, product-specific assays were validated using etu-cel.

The commercial manufacturing process has been adequately validated, and continuous process verification is in place. FDA exerted regulatory flexibility in the nature of the study data used to support manufacturing changes and the number of representative etu-cel lots used to support the shelf-life. Etu-cel stability in vapor phase liquid nitrogen ( $\leq -130^{\circ}\text{C}$ ) was determined to be 6 months. Chain of Identity and Chain of Custody are established and maintained throughout the manufacturing process and administration. Etu-cel drug product is supplied as a frozen suspension of cells for intravenous infusion.

## **B. RECOMMENDATION**

### **I. APPROVAL**

This biological license application (BLA) provides an adequate description of the manufacturing process and characterization of etuvetidigene autotemcel (WASKYRA). The CMC review team has concluded that the manufacturing process along with the associated test methods and control measures are capable of yielding a product with consistent quality characteristics. This information along with postmarketing commitments (PMCs) and a post-marketing requirement (PMR) listed below satisfy the CMC requirements for biological licensure per the provisions of section 351(a) of the Public Health Service (PHS) Act controlling the manufacturing and sale of biological products. Based on the information provided in the BLA submission and subsequent amendments, the CMC review team recommends approval of this BLA.

#### **Drug Substance and Drug Product Manufacturing Facilities:**

Lentiviral Vector and Drug Product: AGC Biologics S.p.A.; Via Meucci 3 Openzone  
20091 Bresso (Milan) Italy

FEI: 3020270660, DUNS: 338913341



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

Responses were received in Amendment 28:

1. Fondazione Telethon ETS commits to implement and validate an assay measuring (b) (4) . The final validation study report will be submitted as a “Postmarketing Commitment – Final Study Report” by December 31, 2026.
2. Fondazione Telethon ETS commits to implement and validate a drug product (DP) assay measuring (b) (4) .  
The final report will be submitted as a “Postmarketing Commitment – Final Study Report” by December 31, 2026.
3. Fondazione Telethon ETS commits to perform a (b) (4) .  
under the intended conditions as described in BLA 125846. The final report will be submitted as a “Postmarketing Commitment – Final Study Report” by May 31, 2026.
4. Fondazione Telethon ETS commits to re-validate the (b) (4) assay to include the range of the commercial lot release criterion or implement and validate an alternative assay. The final validation study report will be submitted as a “Postmarketing Commitment – Final Study Report” by December 31, 2026.
5. Fondazione Telethon ETS commits to validate the updated (b) (4) test and reassess the criteria for drug product lot release. The final report will be submitted as a “Postmarketing Commitment – Final Study Report” by December 31, 2025.
6. Fondazione Telethon ETS commits to validate the following assays for robustness: (b) (4) . The final study reports will be submitted as a “Postmarketing Commitment - Final Study Report” by March 31, 2026.
7. Fondazione Telethon ETS commits to conduct a study measuring (b) (4) . The final study report will be submitted as a “Postmarketing Commitment - Final Study Report” by September 30, 2026.
8. Fondazione Telethon ETS commits to perform additional (b) (4) . The final report will be submitted as a “Postmarketing Commitment – Final Study Report” by March 31, 2026.

9. Fondazione Telethon ETS commits to perform a study assessing the impact of the (b) (4) [REDACTED] LVV release. The final study report will be submitted as a “Postmarketing Commitment – Final Study Report” by May 31, 2026.
10. Fondazione Telethon ETS commits to perform an additional in-use DP stability study that includes an administration set equipped with a filter and assesses the viability of DP under the administration conditions described in the BLA. The final study report will be submitted as a “Postmarketing Commitment – Final Study Report by November 30, 2026”

**Post-Marketing Requirement (PMR):**

An adequate leachables safety assessment for the TLT003 drug product (DP) through its manufacturing process, storage, and in-use conditions. The assessment must include both elemental and organic leachables from the formulation, storage and in-use preparation product-contacting components appearing cumulatively in final DP. The leachables study can be conducted without active ingredient by simulating the DP manufacturing process from the (b) (4) step through in-use preparation steps of the simulated DP. Such study should use maximal hold times and temperatures at respective manufacturing process steps to assess cumulative leachables in the DP from the (b) (4) through product freezing, shelf-life, storage, thawing, and in-use processing. A final study report and toxicological risk assessment should be provided.

Final Protocol Submission: March 31, 2026

Study Completion Date: September 30, 2026

Final Report Submission: December 31, 2026

**II. COMPLETE RESPONSE (CR)**

Not Applicable

**III. SIGNATURE BLOCK**

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Laura DeMaster, PhD, Chair, CMC Reviewer, OTP/DGT/GTB4	Concur	
Stella Lee, PhD, Chair, CMC Reviewer, OTP/DGT/GTB4	Concur	
Teisha Rowland, PhD, Chair, CMC Reviewer, OTP/DGT/GTB4	Concur	
Anna Kwilas, PhD, Branch Chief OTP/DGT/GTB4	Concur	
Kimberly Schultz, PhD, Director OTP/OGT/Division 2	Concur	
Denise Gavin, PhD, Director OTP/Office of Gene Therapy	Concur	

## Review of CTD

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*Overall Reviewer's Assessment of Section 3.2.P.7: This information is acceptable and supports the use of (b) (4) freezing bags as the container closure for TL003*

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**Module 3**

**3.2.S DRUG SUBSTANCE – (b) (4)**

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### 3.2.P DRUG PRODUCT - etuvetidigene autotemcel (herein TLT003 DP)

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

*Reviewed by LKD*

TLT003 is a cryopreserved product supplied in a single dose cell suspension for intravenous administration in 1 – 8 (b) (4) EVA bag(s). TLT003 cells are formulated in 5% v/v DMSO, 7% w/v HSA in 0.9% w/v saline solution, with a concentration of 2- (b) (4) E6 viable cells/mL in a volume of 10 – 20mL of total volume per bag. The number of total cells and CD34+ cells varies, but is controlled by lot release limits on %CD34+ cells. No dilution of the DP occurs at the clinical site.

### 3.2.P.2 Pharmaceutical Development

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

##### 3.2.P.2.1.1 Drug Substance

(b) (4)

##### 3.2.P.2.1.2 Excipients

*Reviewed by RM*

The excipients dimethyl sulfoxide (DMSO), human serum albumin (HSA), and sodium chloride solution are the excipients used in TLT003 formulation. A list of these excipients is provided in Table 50.

**Table 50 Excipients Present in TLT003 (adapted from applicant information)**

Component	Quantity	Reference to Standard
Autologous CD34 <sup>+</sup> cell enriched haematopoietic stem and progenitor cells transduced <i>ex vivo</i> with a lentiviral vector encoding the human Wiskott Aldrich Syndrome (WAS) gene	2-11.4 x 10 <sup>6</sup> viable cells/mL <sup>a</sup>	In-house
Sodium Chloride Infusion	0.9% w/v	Medicinal product
Dimethylsulfoxide (DMSO)	5% v/v	(b) (4)
Human Serum Albumin Solution	7% w/v	Medicinal Product

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

*Reviewed by LKD*

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

Early TLT003 (i.e. lots manufactured with process versions (b) (4) ) were formulated as a fresh DP in 0.9% w/v sodium chloride solution. Preliminary studies to identify appropriate cryoformulation screened excipients using (b) (4) cells due to the limited availability of primary HSPCs. The applicant pursued DMSO as the primary cryoprotectant based on positive results in the screening study and its wide use in the field of stem cell transplantation.

Confirmatory studies assessing the CQAs following cryopreservation were performed with surplus patient cells collected from the mPB of (b) (4) subjects who participated in the hospital exemption program. As no significant advantage was observed with (b) (4) DMSO compared to 5% DMSO (Table 51), the applicant chose 5% DMSO, 7% w/v HSA, 0.9% saline solution as the final formulation for the commercial manufacturing process (A4).

Table 51: Data Comparing 5% (b) (4) DMSO DP formulations (adapted from applicant table)

(b) (4)

*Reviewer Comment: Data from the fresh formulation arm of the study is omitted here, since the change from fresh to cryopreserved formulation is reviewed in [3.2.S.2.6 Manufacturing Process Development, Comparability](#). The commercial cryoformulation is adequately supported.*

### 3.2.P.2.2.2 Overages

There are no overages used for TLT003 DP.

*Reviewer Comment: Acceptable*

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

The properties of TLT003 DP are the (b) (4) (b) (4) (b) (4). Attributes that are critical for DP function are tested as part of lot release as 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 LKD*

Process development studies for the DS and DP were performed during clinical development to support the comparability of TLT003 across manufacturing changes, including the change in (b) (4) source, DP formulation (fresh to cryopreserved) and manufacturing site. These changes are reviewed in [3.2.S.2.6 Manufacturing Process Development, Comparability](#). The justification for implementation of a (b) (4) DP wash step and change in container closure (b) (4) EVA bag) are reviewed below.

#### (b) (4) DP Wash

(b) (4)

(b) (4)

Table 52: (b) (4) Data on Day (b) (4) Wash (adapted from applicant table)

(b) (4)

(b) (4)

**Reviewer Comment:** (b) (4) (b) (4) (b) (4) (b) (4) (b) (4)  
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### 3.2.P.2.4 Container Closure System

*Reviewed by RM*

The primary container closure system for TLT003 DP is a sterile, single use, ethylene vinyl acetate (EVA) (b) (4) Freezing Bag 50 manufactured by (b) (4)

(b) (4) This EVA bag has CE Class II status in the EU and 510k clearance (No. (b) (4)) in the US as a medical device for use with hematopoietic progenitor cells.

The filled primary EVA bag will be sealed and packaged into an overwrap EVA bag before cryopreservation. After cryopreservation the bag with overwrap will be placed into metal cassettes for storage in vapor phase liquid nitrogen. The applicant has deemed this container suitable because it is designed for the manipulation and storage of cellular material.

*Reviewer comments: The choice of container closure for TLT003 is acceptable. Please see the DMPQ review by Jared Greenleaf for additional details.*

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

*Reviewed by RM*

TLT003 is a cryopreserved DP which is administered to the patient immediately after thaw and therefore must be free from microbiological contamination. To ensure that the drug product is sterile, tests for microbiological contaminants (b) (4), bacterial endotoxins (b) (4) and Mycoplasma (b) (4) are included in the DP release specification. The primary container closure was included in aseptic process simulation studies which simulated all aseptic manipulations in the manufacturing process of TLT003. The ability of the container closure to prevent microbial contamination was addressed in the container closure integrity testing performed by AGC Biologics.

*Reviewer comments: This information is acceptable to support container closure integrity. Please see the DMPQ review by Jared Greenleaf for additional details.*

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

At the qualified treatment center TLT003 is thawed and directly administered to the patient from the container closure system using an intravenous (IV) administration set equipped with a filter.

*Reviewer comments: As stated in [3.2.P.2.4 Container Closure System](#), the container closure system has 501k clearance for the commercial use. The CCS is suitable, and the in-use stability data, reviewed in Section [3.2.P.8.1 Stability Summary and Conclusion](#), supports stability 2 hours stability post-thaw.*

*In CMC IR#11 FDA requested data on the in-use stability (i.e. viability) of DP when used in conjunction with an administration set filter, as such administration sets are described in the PI. No data were provided, but the applicant agreed in amendment 28 to conduct a study assessing post-thaw viability of DP that includes an administration set equipped with a filter as a PMC. Given that the clinical protocol indicated that administration sets with 200uM filters should be used for TLT003, FDA considers the benefit observed during clinical studies together with the PMC to be acceptable. The WASKYRA PI is reviewed in Section [C. Labeling Review](#).*



## Extractables and Leachables Testing

A risk assessment was conducted to identify potential leachables in the TLT003 manufacturing process. The applicant determined that the (b) (4) step represented the highest risk step for leachables and related contact components. (b) (4) high-risk materials (b) (4) ) were subjected to leachables studies using exaggerated conditions (i.e. (b) (4) Samples were analyzed using (b) (4) respectively.

***Reviewer comments:** The extractables and leachables assessment was reviewed by Andrey Sarafanov (OTP/OPPT/DH/HB2). Analytical Evaluation Thresholds (AETs) were set for organic compounds based on the Threshold of Toxicological Concern (TTC) of 120 ug/day and an Uncertainty Factor of (b) (4). Compounds at levels that exceeded the TTC (n=3) were evaluated for toxicological risk. While a potential health risk was identified for (b) (4), testing confirmed that the compound is adequately cleared through TLT003 (b) (4) (b) (4)*

*The deficiencies in the design of the leachables study are discussed in detail in the review memo by Dr. Sarafanov. Briefly, the study only included organic compounds and assessed each of the (b) (4) high-risk components individually. Therefore, the leachables analysis is incomplete because it does not assess cumulative leachables profile in final DP. Moreover, the assessment lacks components in the TLT003 formulation (0.9% NaCl, 7% HSA, (b) (4) DMSO) and elemental compounds.*

***Overall Reviewer's Assessment of Section P.2.6:** Overall, the extractables and leachables assessment is not adequate, and the applicant committed to a leachables assessment as a PMR including a toxicology report discussing any compounds identified, safety thresholds, and potential risks to patient safety.*

## 3.2.P.3 Manufacture

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

*Reviewed by LKD*

TLT003 DP is manufactured and tested at the sites listed in Table 54.

**Table 54: Manufacturing and Testing Facilities for TLT003 Drug Product (applicant table)**

Facility	Address	FEI	DUNS	Responsibilities
AGC Biologics S.p.A.	Via Meucci 3 Openzone 20091 Bresso (Milan) Italy	3020270660	338913341	Drug product manufacture Drug product primary and secondary packaging QC testing for the following test methods: <ul style="list-style-type: none"> <li>• Cell count and viability</li> <li>• (b) (4)</li> <li>• Mycoplasma</li> <li>• Immunophenotype CD34+</li> <li>• WASP assay</li> <li>• Vector copy number</li> <li>• Transduction efficiency</li> <li>• Sterility</li> <li>• Endotoxin</li> </ul>

**3.2.P.3.2 Batch Formula***Reviewed by LKD*

The TLT003 batch formulation is described in Table 55. The minimum recommended dose is  $7 \times 10^6$  CD34+ cells/kg. TLT003 is provided in up to 8 bags, each of which contains  $2 - (b) (4) \times 10^6$  cells/mL in a volume of 10 – 20 mL. No overage is included.

**Table 55: Drug Product Batch Formulation (adapted from applicant information)**

Component	Quantity	Reference to Standard
Autologous CD34+ cell enriched haematopoietic stem and progenitor cells transduced ex vivo with a lentiviral vector encoding the human Wiskott Aldrich Syndrome (WAS) gene	$2-11.4 \times 10^6$ viable cells/mL <sup>a</sup>	In-house
Sodium Chloride Infusion	0.9% w/v	Medicinal product
Dimethylsulfoxide	5% v/v	(b) (4)
Human Serum Albumin Solution	7% w/v	Medicinal Product

<sup>a</sup> The target concentration is  $2 - (b) (4) \times 10^6$  viable cells/mL. The final DP must meet recommended dose (AC is (b) (4) CD34+ cells) and minimum transduction efficiency of (b) (4).

<sup>a</sup> Registered as medicinal product in EU

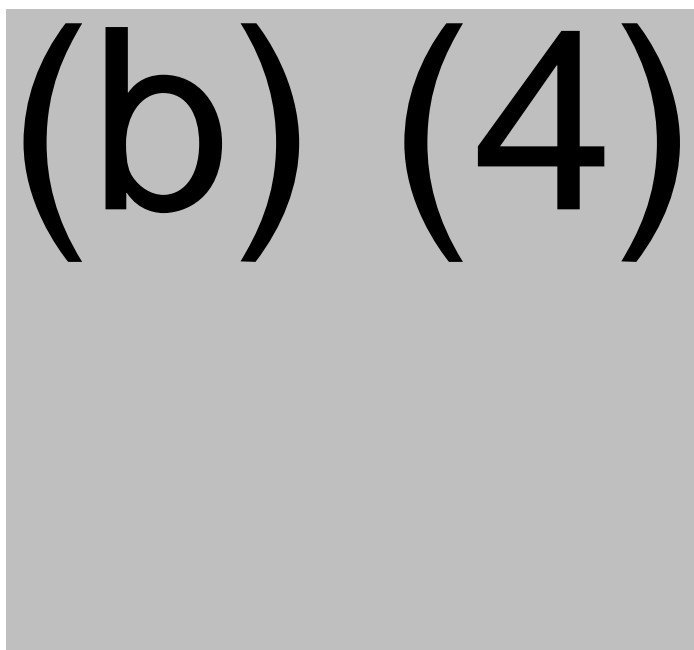
**Overall Reviewer's Assessment of Sections 3.2.P.3.1 and 3.2.P.3.2:** *The information provided on TLT003 DP manufacturer/testing sites and the batch formula are acceptable.*

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

*Reviewed by LKD*

The TLT003 manufacturing process is (b) (4) (b) (4) (b) (4) steps as shown in Figure 17. All available cells are formulated.

**Figure 17: Process Flow Diagram for DP Manufacturing (Wash, Formulation, Fill Steps)**



(b) (4)

[Redacted text block]

#### **Formulation and fill**

Based on the results of in-process testing in Step (b) (4) the (b) (4) concentration by resuspension to a final formulation containing 0.9% w/v saline, 7% w/v HSA, and 5% v/v DMSO as described in Table 55. In batches that cannot support the minimum fill volume of 10mL at (b) (4) E6 cells/mL, a lower cell concentration (down to 2E6 cells/mL) is used. Stability data, reviewed in [3.2.P.8.1 Stability Summary and Conclusion](#), support the minimum cell concentration. The theoretical infusion dose is confirmed to be at least 7E6 CD34+ cells/kg (the minimum recommended dose) based on the post-selection %CD34+ IPC and the patient weight.

QC samples (maximum of (b) (4) for each lot) are collected (b) (4) (b) (4)

(b) (4)

Visual inspection (VI) of all filled bags is performed by a trained GMP operator in a Grade (b) (4) to confirm the appearance of DP and the integrity of the primary container. The bag is gently (b) (4) observed for defects, aggregates, and particulates for at least (b) (4). Following (b) (4) passing bags are transferred to a Grade (b) (4) area for labeling, (b) (4) sealing and (b) (4), and an additional visual check of the primary container. Each labeled cryobag is placed into a labeled secondary EVA overwrap bag per batch record instructions.

The DP bags are transferred to a non-classified area, and the secondary overwrap bags are sealed. Packaged DP and QC samples are cryopreserved in parallel using a (b) (4). Following cryopreservation, packaged DP bags are placed into labeled metal cassettes and stored at <-130°C in the vapor phase of liquid nitrogen. QC samples are frozen, with the exception of samples for sterility testing.

#### **Reviewer comments:**

- *Additional information on sampling points for QC tests was requested in CMC IR#10 (amendment 26). In response to IR the applicant provided clarification that the (b) (4). While a (b) (4) is then used to (b) (4) this approach aligns with 21 CFR 610.1. Acceptable.*
- *In response to CMC IR#10 the applicant indicated that cell loss was observed for some patient DPs (n<sup>(b) (4)</sup>) during the (b) (4) step resulting in lower yields than observed during (b) (4) PPQ runs. FTE justified that the variability in cell yields across (b) (4) is a result of biological heterogeneity in WAS patient material compared to DPs made from (b) (4) starting material. All DPs used in clinical studies had viability ≥80%, and the minimum dose of TLT003 was 7 x 10<sup>6</sup> viable CD34+ cells/kg. FDA considers the TLT003 manufacturing process to be adequately controlled, as discussed in 3.2.P.3.5 Process Validation and/or Evaluation. No concerns.*
- *The appearance lot release test for commercial DP will be performed simultaneously with visual inspection. This is reviewed in 3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s).*
- *Initially the applicant proposed to test for mycoplasma on a final DP sample that undergoes (b) (4). The reasoning for this approach was provided in CMC IR#1 (amendment 7), in which the applicant explained that (b) (4) interferes with the assay test system. FDA had multiple additional communications with the applicant (DBSQC IRs documented in amendments 11 and 18 as well as the Mid-Cycle meeting) and agreed to temporarily accept results from the (b) (4). The applicant commits to validation per (b) (4) as a PMC issued by DBSQC. Once validation is successful, (b) (4)*

*material will be used for mycoplasma lot release testing. Additional information is provided in the DBSQC reviewer memo.*

**Packaging, and Shipping:** DP is stored at AGC Biologics until batch release activities are complete. FTE Quality Assurance receives the CoA and batch certificate (certified by the Qualified Person at AGC Biologics). The transport provider generates an Air Waybill (AWB) prior to pickup. COC handover is recorded and documentation is maintained by FTE. The DP is shipped frozen using a validated process reviewed in [3.2.P.3.5 Process Validation and/or Evaluation](#).

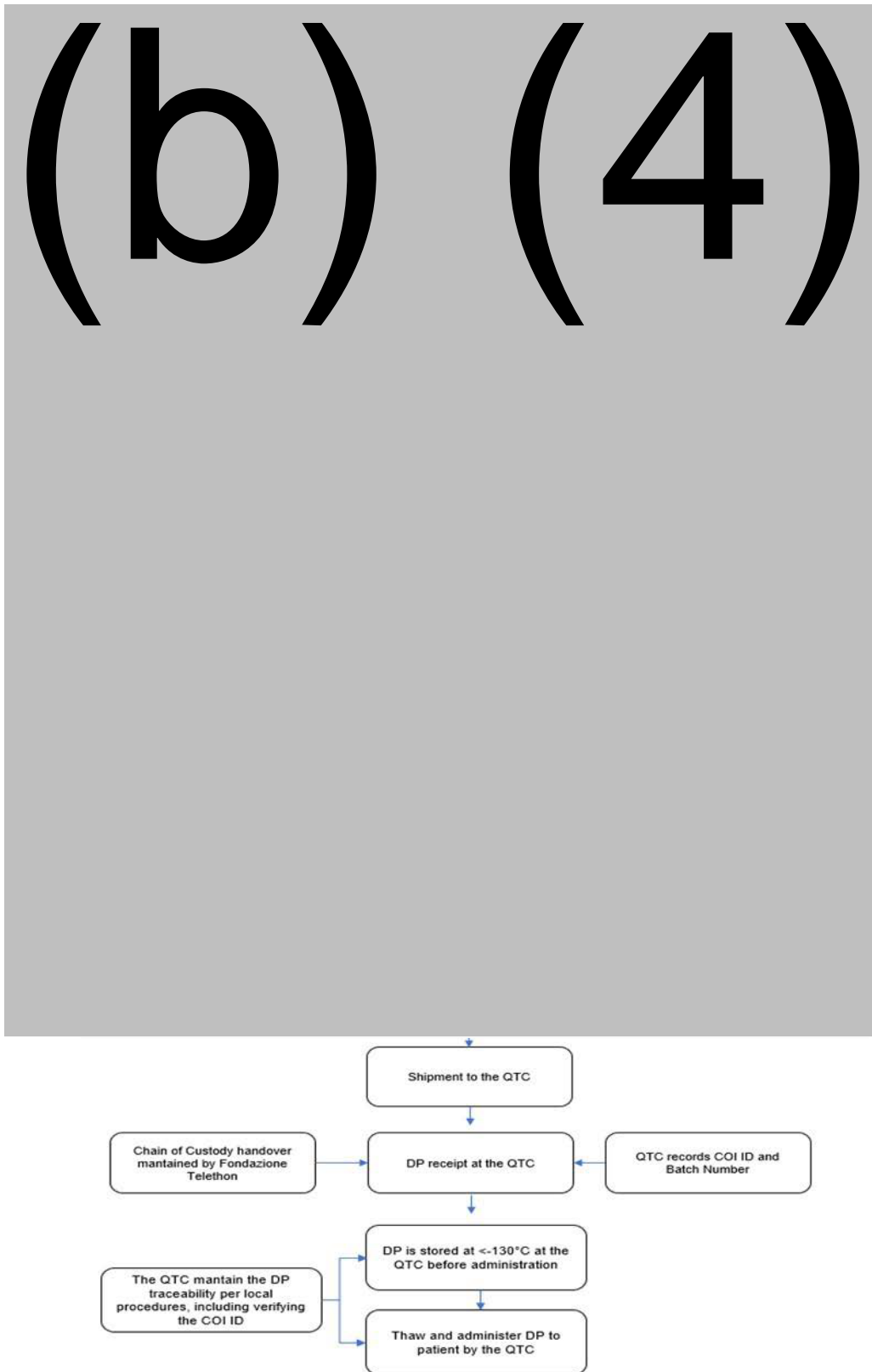
### **Chain of Identity and Chain of Custody**

Chain of identity (COI) and chain of custody (COC) controls have been implemented from the initiation of apheresis through administration of the product to the patient. The applicant's COI/COC system is paper-based and is comprised of a series of sequential controls and documents (Figure 18) to support traceability and identity. Traceability ensures that raw materials and all other substances that contact with TLT003 cells, as well as TLT003 material itself, can be traced through the sourcing, manufacturing, packaging, storage, transport and delivery to patient.

FTE assigns each patient a unique COI identifier (COI ID). Each COI ID is associated with a unique donor identification number (DIN), which is issued by the collection center to identify patient mPB material. Both identifiers are recorded and tracked on the COC/COI form. The COI ID and DIN are recorded upon receipt at the TLT003 manufacturing site. Tracking and transport of mPB starting material is reviewed in [Section 3.2.S.2.3 Control of Starting \(i.e., Source\) Material\(s\)](#). A COC handover is documented at the manufacturing site. TLT003 DP batches are assigned lot numbers as described in [3.2.S.2.2 Description of Manufacturing Process](#). The lot number is used during manufacturing activities, and both the DP lot info and the DIN are recorded in the batch record. The primary DP label, secondary overwrap bag label, and metal cassette label include the DP lot number, COI ID, and DIN and are reviewed in [C. Labeling Review](#).

TLT003 DP is stored at the manufacturing facility at  $\leq -130^{\circ}\text{C}$  until the CoA and QP batch certificate is provided for the lot to the FTE Quality Assurance department. The released DP lot is prepared for transport to the treatment site, and the manufacturing site records COI ID and lot number prior to pickup by the transport provided. A Lot Information Sheet is included in the shipment. The COC handover is recorded and maintained by FTE.

**Figure 18: Overview of TLT003 COI/COC**



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

*The manufacturing information is adequate; no CMC concerns.*

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

CPPs and IPCs for the DP manufacturing process are listed in Table 56 and Table 57. The criticality of each parameter was established as described in Section [3.2.S.2.4 Controls of Critical Steps and Intermediates](#).

**Table 56: CPPs for TLT003 DP Manufacturing (adapted from applicant table)**

(b) (4)

**Table 57: In-process Controls for TLT003 DP Manufacturing (adapted from applicant table)**

Unit Operation	Parameter	Action
(b) (4)	(b) (4)	(b) (4)

**Reviewer comment:** *Actions taken in the event of a failure of the microbiological testing IPCs are described in [Section 3.2.S.2.4 Controls of Critical Steps and Intermediates](#). The fate of the batch is determined based on the results of the sterility test for lot release. DP lots that fail to meet lot release AC are not released for infusion.*

**Overall Reviewer's Assessment of Section 3.2.P.3.4:** *CPPs are reasonable. With regard to IPCs, the control strategy is adequate for commercial manufacture.*

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

*Reviewed by LKD*

TLT003 process performance qualification (PPQ) was performed at the AGC Biologics (Bresso, Milan) facility in 2020. PPQ consisted of 3 consecutive runs with (b) (4) mPB material ((b) (4)). The applicant provided a comparison of historical TLT003 DPs made with (b) (4) versus patient material and noted that CQAs tested after (b) (4) trend lower in (b) (4) DPs compared to patient DPs and established PPQ AC accordingly.

**Reviewer comment:** *The applicant has sufficiently justified the use of (b) (4) material based on comparison of quality attributes between (b) (4) and patient DPs. Considering the limited availability of patient cellular material and the higher sampling needed for PPQ, the use of (b) (4) mPB starting material is reasonable and acceptable. The applicant justified PPQ AC based on manufacturing history and the rationale that (b) (4) have been shown to have a growth advantage. The justification and PPQ AC are reasonable.*

An overview of the PPQ lots is provided in Table 58. Additional process validation activities supporting transport validation, extractables and leachables assessment, aseptic processing, and microbial hold times are also reviewed below. Definitions for process terms (e.g. IPC, CPP, PAR, Action Limit) are provided in [Section 3.2.S.2.4 Controls of Critical Steps and Intermediates](#). The change of manufacturing suites for TLT003 post-PPQ is reviewed in [3.2.S.2.6.2 Manufacturing Process Development, Comparability](#).

**Table 58: Batch Details for DP A4 PPQ (adapted from applicant information)**

(b) (4)
---------

The validation study was sponsored by (b) (4), and the PPQ protocol was developed based on a PPQ Master Plan. The PPQ study protocol and reports were



provided in the application. Data for CPPs and IPCs are shown in Table 59, and release test results are provided in Table 60.

**Table 59: DP PPQ Process Parameter and In-Process Control Data (adapted from applicant table)**

(b) (4)

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**Table 60: CQA Results for TLT003 PPQ Batches (adapted from applicant table)**

(b) (4)

Minor deviations occurred during PPQ runs and were investigated as follows:

- (b) (4)



(b) (4)

Results related to the performance of the shipper and the integrity of the primary container are shown in Table 61. No impact to DP CQAs were observed (data not shown).

**Table 61: DP Transport Validation Summary: (b) (4) Performance and Transit (applicant table)**

(b) (4)

*Reviewer comment: The use of (b) (4) DPs as well as TLT003 DP is reasonable given that the same CCS and transport shipper are used. (b) (4)*

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

(b) (4)

(b) (4)

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

*. Acceptable.*

### Continued Process Verification (CPV)

A CPV program has been established to monitor the commercial DP manufacturing process. The program includes trending of DP attributes using control charts and process capability metrics. Initially, historical data from clinical lots are used to establish control limits. Once sufficient commercial manufacturing experience is gained, control limits will be calculated based on a statistical analysis of process variability. Trend violations and out-of-limit results are investigated as deviations and documented within the periodic CPV report (every (b) (4) batch or (b) (4) year at minimum).

### 3.2.P.4 Control of Excipients

#### 3.2.P.4.1 Specifications

*Reviewed by RM*

TLT003 is formulated with DMSO, HSA and 0.9% sodium chloride solution (Table 62). All are held to licensed and/or (b) (4) standards.

**Table 62: Quality of TLT003 Excipients (adapted from applicant information)**

Material	Grade	Supplier	Internal testing (method)		Acceptance Criteria
(b) (4) Human Serum Albumin	(b) (4)	(b) (4)	(b) (4)		Meets specification;
		HSA (b) (4)	(b) (4)		Conforms to reference
	(b) (4) /licensed	(b) (4)	(b) (4)		
DMSO	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
			Sterility	(b) (4)	Pass
			Endotoxin	(b) (4)	
0.9% w/v Sodium Chloride	(b) (4)	(b) (4)	Verification of marketing authorization		Verified
			(b) (4)	(b) (4)	(b) (4)
			(b) (4)	(b) (4)	(b) (4)

***Reviewer comment:** DMSO and 0.9% sodium chloride are (b) (4) reagents used in the final formulation of TLT003 DP. In-house identity testing is performed on all raw materials listed using (b) (4) methods and the manufacturer's specifications except for in house (b) (4) determination of 0.9% sodium chloride solution. The (b) (4) acceptance range set for sodium chloride is (b) (4) than the manufacturer's specification and is based on historic testing of sodium chloride batches.*

The DMSO and HSA have (b) (4) specifications. The sodium chloride solution is a medicinal product licensed by a (b) (4) and is used routinely in clinical settings (0.9% w/v Sodium Chloride Infusion). Endotoxin and sterility are confirmed by the manufacturer's certificate of analysis. Additional in-house tests for (b) (4) and (b) (4) are performed using (b) (4) methods and the acceptance criteria listed in Table 63.

**Table 63: Tests and AC for sodium chloride excipient (compiled from applicant information)**

(b) (4)

***Reviewer Comment:** This is acceptable.*

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

Sodium Chloride Infusion (0.9% w/v) is used in the formulation of TLT003 DP. In-house testing of this product includes (b) (4) and using an (b) (4). The test methods for (b) (4) are (b) (4) and therefore do not require additional validation.

***Reviewer Comment:** This is acceptable.*

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

The (b) (4) specification is based on historic testing of sodium chloride batches during manufacturing of TLT003 and similar products manufactured by AGC Biologics S.p.A. The specification for (b) (4) is based on the manufacturer's specification.

***Reviewer Comment:** This is acceptable.*

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

Human Serum Albumin (HSA) is the only excipient of human origin used in the formulation of TLT003. A review of the quality and safety of (b) (4) HSA used in final formulation is provided in 3.2.S.2.3 Control of Materials

### 3.2.P.4.6 Novel Excipient

No novel excipients are used to manufacture TLT003.

### 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 LKD*

Data include (b) (4) total lots administered to patients in Study 201228, OTL-103-4 as well as Expanded Access Programs (EAPs) Hospital Exemption (HE) 205030 and Compassionate Use Program (CUP) 206527.

Commercial lot release acceptance criteria for TLT003 DP are provided in Table 64 and are based on up to (b) (4) clinical, EAP/HE, and early access lots, which were administered to patients and demonstrated efficacy per the FDA Clinical review team. Not all release tests included in the commercial specification were tested throughout clinical development. Moreover, lot inclusion for each attribute was impacted by the process version used for each DP (e.g. viability was based only on process versions with cryopreserved DP) and is described in more detail below for each test. For attributes with an AC based on calculated statistical intervals, the number of lots supporting the AC is provided in Table 64. Additional review of the clinical DP lot data provided in the submission is reviewed in [3.2.P.5.4 Batch Analyses](#).

The patient who received DP lot (b) (4), (b) (6) died 4.5 months after treatment due to deterioration of a pre-existing neurological condition unrelated to TLT003. As this patient was not followed for sufficient follow-up to determine the efficacy of TLT003, this DP lot was excluded from analyses to justify release AC.

#### *Reviewer comments:*

- Commercial DP specifications were modified during the review cycle compared to those submitted by the applicant initially. The considerations and justifications for the AC (e.g. lot inclusion, calculation of statistical interval, etc) are discussed in the section below. Summary statistics for quantitative lot release results are provided in [3.2.P.5.4 Batch Analyses](#). Lot release test values for individual DP lots are plotted in run charts in [3.2.S.2.6 Manufacturing Process Development, Comparability](#). Inclusion of lot (b) (4), (b) (6) lot release values would not have changed calculated TIs or AC for any CQA, as all values for this DP were well within manufacturing experience.*
- A typical commercial strategy for potency assurance relies upon LVV and DP lot specifications specific to the products mode of action. Due to challenges with assay development for TLT003, however, neither a DP potency assay measuring transgene function nor an LVV activity assay are included for lot release. Instead, the potency assurance strategy for TLT003 is based primarily on DP WASP protein production, measured for lot release and the correlations of this DP attribute with meaningful clinical platelet-related parameters as discussed below in the section on the (b) (4) by (b) (4) test. Other potency-related CQAs,*



*which are tested for lot release and described below, further support the potency assurance strategy for TLT003.*

- The final agreed-upon lot release AC are shown in Table 64.*

**Table 64: DP Lot Release Specifications (adapted from applicant table and information)**

Attribute (unit)	Test	Sample Type	AC	Justification
<b>General</b>				
Viable cell concentration (cells/mL)	DP		2.0 - 11.4 x 10 <sup>6</sup>	Lower limit based on range tested for stability; upper limit based on max experience (b) (4)
Appearance	DP prior to (b) (4)		A cloudy to clear, colourless to yellow or pink dispersion of cells	Based on (b) (4)
<b>Identity/Purity</b>				
Immunophenotype CD34+ (%)	DP		(b) (4)	(b) (4)
<b>Potency</b>				
Viability (%)	DP		(b) (4)	(b) (4)
Vector Copy Number (VCN) (vector copies per cell)	(b) (4)		(b) (4)	Lower limit based on min experience; upper limit based on (b) (4)
VCN (b) (4)	(b) (4)		(b) (4)	Lower limit based on (b) (4) upper limit based on max experience
Transduction Efficiency (%)	DP		(b) (4)	Lower limit based on min experience (b) (4)
(b) (4)	DP		(b) (4)	(b) (4)
(b) (4)	DP		(b) (4)	(b) (4)
(b) (4)	DP		(b) (4)	(b) (4)
<b>Potency/Identity</b>				
Transgene Expression (b) (4)	(b) (4)		(b) (4)	(b) (4)

Safety			
Sterility	DP	No growth	Requirement
Bacterial Endotoxins (b) (4)	DP	(b) (4)	Based on experience
Mycoplasma (b) (4)	DP (b) (4)	Not detectable	Requirement


<sup>a</sup>FTE noted an error in 2 lot release values in Amendment 25 and provided revised Batch Analysis and Justification of Specification sections. Lowest WASP value among DPs used in clinical studies is (b) (4).

<sup>b</sup>As discussed in the section on potency assurance below, (b) (4) is the minimum value associated with efficacy in clinical trials. See the correlative analysis between WASP (b) (4) and increase and platelet count between baseline and Day 180.

[illegible]

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

(b) (4)

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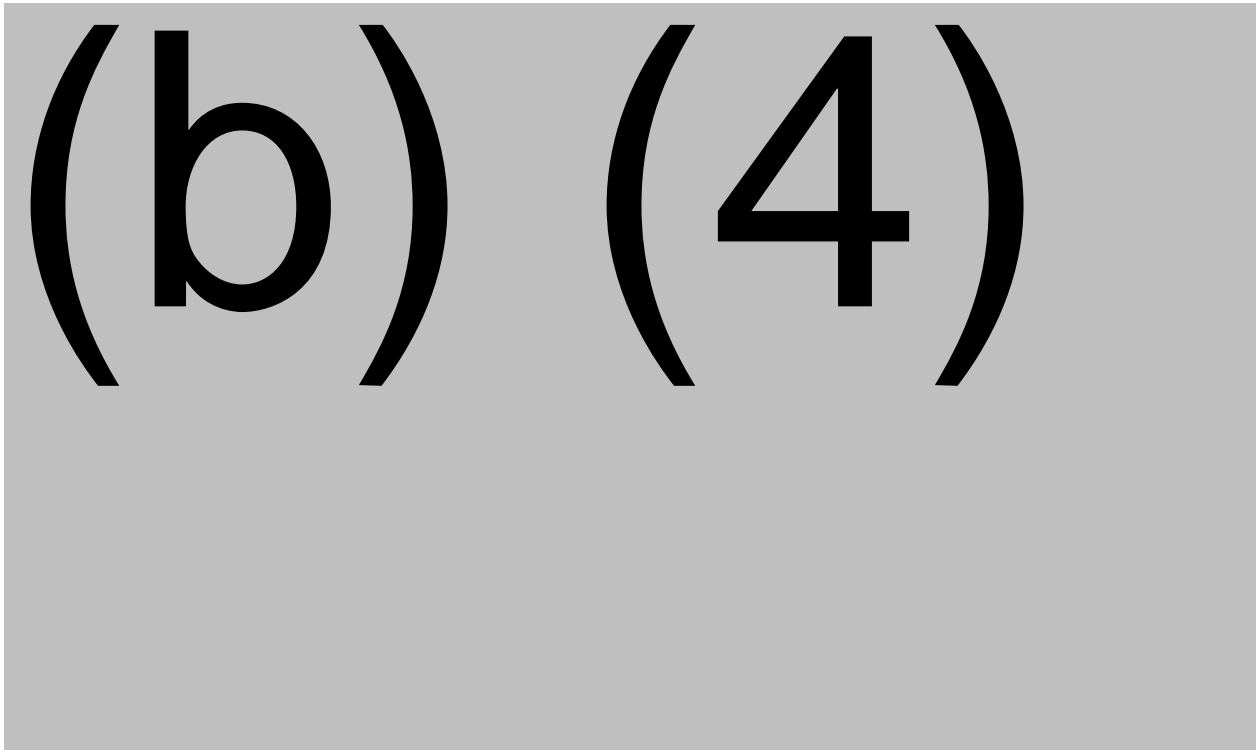
*The justifications for the commercial lot release AC are acceptable.*

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

*(Reviewed by TR)*

An overall summary of the validation procedures and parameters evaluated for each assay for TLT003 DP is shown in **Table 65**.

(b) (4)

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

(b) (4)

*Overall Reviewer's Assessment of Section 3.2.P.5.2 and 3.2.P.5.3: The information provided is acceptable. The data provided in the BLA and through various amendments (as specified in the comments on the method assessed) supports appropriate validation for the majority of the analytical methods used for the assessment of the DP. FTE agreed to include information that remains insufficient as PMCs. Please refer to the PMC details.*

### 3.2.P.5.4 Batch Analyses

*Reviewed by LKD*

Table 72 summarizes batch analysis data for TLT003 DP batches that were administered to patients and shown to be efficacious. Data for the DP lot administered to the patient who died post-treatment were not included in the analysis. See [3.2.P.5.1 and 3.2.P.5.6 Specification\(s\) and Justification of Specification\(s\)](#) for additional information.

**Table 72: TLT003 DP Batch Analysis (FDA analysis)**

(b) (4)

#### **Reviewer Comments:**

- Given the limitations of the current %TE assay test system that is unable to quantify %TE above the ULOQ of (b) (4), values for this attribute are not included in Table 72. In summary, quantitative %TE values are reported in the BLA for all DPs measured prior to the assay validation study, whereas DPs with TE values

above (b) (4) that were measured after assay validation are reported as >ULOQ. The assay test system is further reviewed in [3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures](#), and %TE data are plotted for clinical DPs in Figure 15.

- The batch analysis data provided were adequate. Consistency of product quality is reviewed in [3.2.S.2.6 Manufacturing Process Development, Comparability](#). No concerns were identified.

### 3.2.P.5.5 Characterization of Impurities

*Reviewed by RM*

TLT003 product related impurities include (b) (4)

TLT003 process related impurities derived from (b) (4) is discussed in Section [3.2.S.3.2 Impurities](#) (b) (4). Table 73 outlines potential process related impurities derived from the TLT003 manufacturing process.

**Table 73. Potential TLT003 process related impurities (adapted from applicant information)**

(b) (4)

(b) (4)

*Reviewer comments:* (b) (4) process related impurity testing was not extensive and was mainly assessed through 3 PPQ runs. Impurities appear to be reduced to safe levels by (b) (4). In IR #8, the applicant was asked to clarify the baseline (b) (4) levels in (b) (4) that were referenced to justify safety and elaborate on the evaluation of (b) (4). In their response on 01 Apr 2025, the applicant addressed these issues by citing specific values from the references for (b) (4) safety and provided a more comprehensive explanation of the risk of (b) (4). Based on the justification and information provided by the applicant, control of product related impurities is acceptable.

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

There are no reference standards or materials for the DP. Some DP release assays use material as positive controls; however no release testing results are normalized to a references standard and each positive control must meet system suitability controls for each assay to be valid. The assay level control was deemed acceptable for use as positive controls.

### 3.2.P.7 Container Closure System

*Reviewed by RM*

#### Manufacturer's Description, Construction, and Testing

(b) (4) freezing bags are CE-marked and Class IIa compliant in the EU and have 510k clearance in the US. The bags are manufactured in a Class (b) (4) clean room and



(b) (4) using a (b) (4) compliant process. The individual components of the (b) (4) freezing bags were chosen based on biocompatibility and suitability for (b) (4). The raw materials used to manufacture the freezing bags can withstand exposure of up to (b) (4). The manufacturer's recommendation for filling is 10 – 20 mL. Bags should be stored at (b) (4) and once filled can be cryopreserved down to (b) (4) and thawed at 37°C. (b) (4) freezing bags are designed for a single cycle of freezing and thawing. (b) (4) freezing bags are considered biocompatible with blood products based on the conformance to (b) (4) and the analysis of extractable substances.

(b) (4) performs a series of physical tests on the bags including a (b) (4)

(b) (4), (b) (4)

### Secondary and Tertiary Packaging

The filled and sealed (b) (4) freezing bag is placed into an EVA overwrap bag which is also sealed before freezing. The materials used for secondary overwrap bag are (b) (4) compliant. After freezing, the overwrapped bag is placed within a metal cassette for storage and transportation.

Extractables and Leachables are described in Section 3.2.P.2.6 Compatibility, TLT003 Dispersion for Infusion.

*Overall Reviewer's Assessment of Section 3.2.P.7: This information is acceptable and supports the use of (b) (4) freezing bags as the container closure for TLT003*

### **3.2.P.8 Stability**

#### **3.2.P.8.1 Stability Summary and Conclusion**

*Reviewed by RM*

TLT003 DP shelf life was established with multiple studies. All samples on stability were cryopreserved TLT003 DP stored at <130°C in vapor phase liquid nitrogen. Due to the limited availability of patient material, most lots were manufactured using (b) (4) material. The applicant states that the use of (b) (4) material is supported by process development data demonstrating comparability between patient derived and (b) (4) TLT003 DP. The analytical testing methods and acceptance criteria described in Table 75 were in place at beginning of stability testing and were used to assess stability samples. TLT003 lots and related samples used to evaluate stability are outlined in Table 74. The following conditions were tested using developmental lots and (b) (4) (b) (4) :

- Long term and 2-hour in use stability
- Long term and 45 minute in-use stability
- Long term stability: tested immediately after thawing
- DP material from clinical lot (b) (4), (b) (6) tested immediately after thawing

**Table 74. TLT003 stability study lots (compiled from applicant tables)**

Batch	Source/ Mfg site	CC	Volume	Concentratio n (cells/mL)	Held 2h post- thaw	Held 45 min post- thaw	Immediately post-thaw
(b) (4), (b) (6)	mPB/ (b) (4)	50 mL (b) (4)	(b) (4)	(b) (4) x 10 <sup>6</sup>	0, 2, 6	0, 1, 2, 3, 6	
		(b) (4)					
	BM/ (b) (4)	50 mL (b) (4)	(b) (4)	(b) (4) x 10 <sup>6</sup>	0, 6	0, 6	
		(b) (4)					
	mPB/ Bresso	50 mL (b) (4)	(b) (4)	(b) (4) x 10 <sup>6</sup>	0, 2, 6	0, 1, 2, 3, 6	
		(b) (4)					
	mPB/ Bresso	50 mL (b) (4)	10 mL	2 x 10 <sup>6</sup>			0, 2, 6
		(b) (4) (b) (4)	(b) (4)				0, 2, 6
	mPB/ Bresso	(b) (4) (4)					
	mPB/ Bresso						
	mPB/ Bresso						
	mPB/ Bresso						

Lots (b) (4), (b) (6) and the analytical methods in Table 75 were included in the assessment of the applicant's proposed shelf life.

**Table 75. Analytical methods and acceptance criteria used for stability study (adapted from applicant information)**

Analytical method	Test	Acceptance Criterion
Potency	Total viable cell concentration (cells/mL)	Report Results
Identity/Purity	Immunophenotype (% CD34+)	Report Results
Potency	Viability (%)	Report Results
	Vector Copy Number (copies/cell)	Report Results
	(b) (4)	Report Results
	Transduction Efficiency	Report Results
Potency/Identity	Transgene WAS presence (b) (4)	Report Results
Safety	Sterility	Negative
	(b) (4)	Report Results
	(b) (4)	Report Results

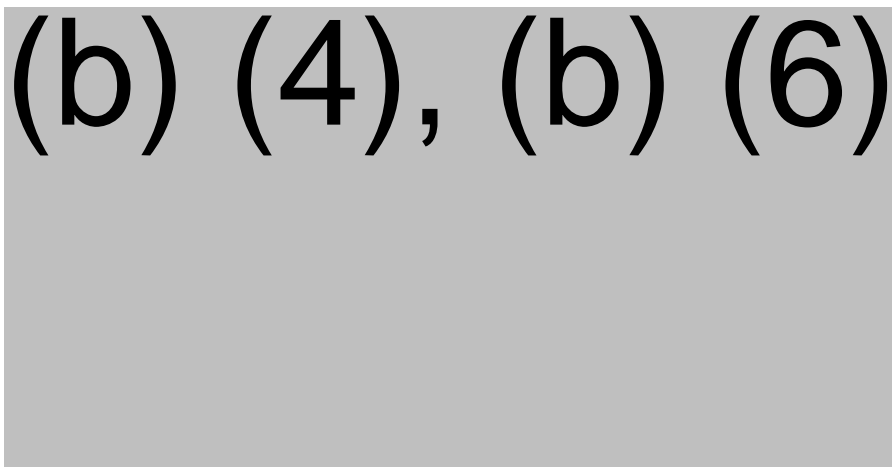
Lot (b) (4), (b) (6) was filled into 50 mL (b) (4) freezing bags at a concentration of (b) (4)  $\times 10^6$  cells/mL. To maximize available DP material, each sample cryobag was filled to (b) (4). Since the minimum recommended volume of a 50 mL (b) (4) is 10 mL, the bag size was reduced to an (b) (4) total volume by (b) (4) to accommodate a smaller volume and maintain the product to bag surface area ratio. Stability samples of (b) (4), (b) (6) were cryopreserved for the 2 hour and 45-minute hold study. Samples used in the 2-hour study were tested at 0, 2, and 6 months, and samples used in the 45 minute hold study were tested at 0, 1, 2, 3, and 6 months. Cryobags were thawed at 37°C using a (b) (4), then held for the appropriate time at room temperature before testing.

Lot (b) (4), (b) (6) was filled into 50 mL (b) (4) (b) (4) freezing bags at a concentration of  $2 \times 10^6$  cells/mL. (b) (4) 50 mL bags were filled with 10 mL of the DP, cryopreserved, and stored until thawing for 0, 2, and 6 months. At the appropriate time point, the bags were thawed and analyzed immediately.

### Summary of Results

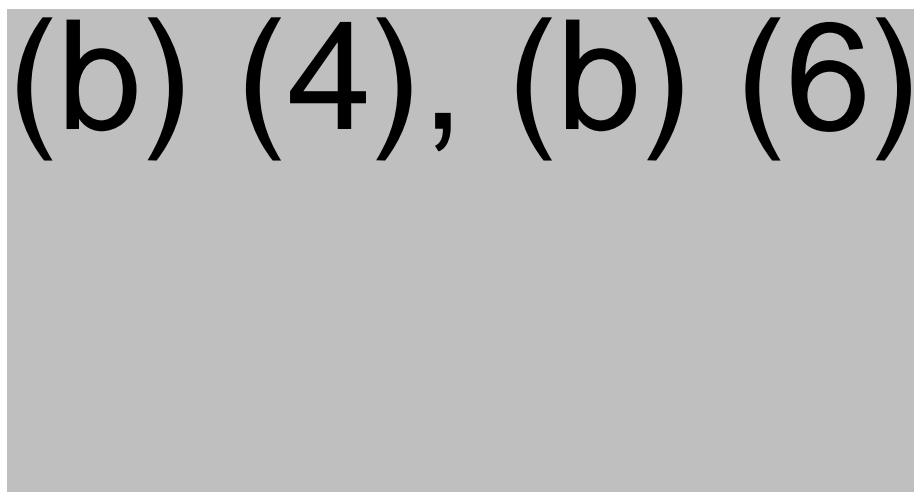
Viability after thaw was  $\geq 83\%$  in all samples tested. Figure 20 shows the % viable cells of the thawed (b) (4), (b) (6) samples from time 0 to 6 months. No downward trend in cell viability was observed.

**Figure 20: Cell viability of TLT003 DP samples thawed from 0 - 6 months**



Viable cell concentration after thaw is shown in Figure 21 for (b) (4), (b) (6) samples. No downward trend was observed in any lots tested.

**Figure 21: Viable cell concentration of post thaw TLT003 DP from 0 - 6 months**



CD34+ cell percentage after thaw in samples (b) (4), (b) (6) is shown in Figure 22. Stability samples thawed at month 1-6 were comparable to T0 and contained  $>99\%$  CD34+ cells. No downward trend was observed with the frequency of CD34+ cells.

**Figure 22: Percent CD34+ cells in post thaw TLT003 DP from 0-6 months**

(b) (4), (b) (6)

(b) (4), (b) (6)

Transduction efficiency measures provirus integration. Results of all samples analyzed are shown in Figure 23. For lots (b) (4), (b) (6), analysis for transduction efficiency was performed on samples at 0, 2, and 6 months. No meaningful downward trend for any of the tested lots was observed.

**Figure 24: Transduction efficiency of thawed TLT003 DP samples from 0 - 6 months**

(b) (4), (b) (6)

Vector Copy Number (VCN) measured the (b) (4) per (b) (4) VCN results for lots (b) (4), (b) (6) are shown in Figure 25. VCN values for these lots were lower than historic values observed with TLT003 manufactured with (b) (4) leukapheresis. However, samples tested at 0, 2, and 6 month time points were comparable and no downward trend was observed.

**Figure 25: Vector copy number of thawed TLT003 samples from 0 - 6 months**

(b) (4), (b) (6)

(b) (4), and sterility were also evaluated. (b) (4) were tested on the 45-minute hold time samples of lot (b) (4), (b) (6) and samples of PPQ lot (b) (4), (b) (6). (b) (4) was consistent with a range of (b) (4) for samples of (b) (4), (b) (6), and (b) (4) for samples of lot (b) (4), (b) (6). The range for (b) (4) was (b) (4) in all samples. Sterility testing was performed on all samples of PPQ lot (b) (4), (b) (6) and was negative at all time points.

The data suggest that there are no differences in DP attributes when cryopreserved and stored in the proposed 50 mL (b) (4) freezing bag for up to 6 months. The applicant proposes a 6-month shelf life for TLT003 DP. No post-approval stability studies are planned.

**Reviewer comments:** *Stability lots (b) (4), (b) (6) were not included in the assessment of the applicant's proposed shelf life because these lots were manufactured at the (b) (4) site and were not representative of the proposed commercial manufacturing process. Stability lots (b) (4), (b) (6) and supplemental data from lots (b) (4), (b) (6) were also not included in the assessment because these samples were cryopreserved in (b) (4) which is not the proposed DP container closure. Therefore, developmental lot (b) (4), (b) (6) were the only primary lots used to assess the proposed shelf life.*

*Samples of lot (b) (4), (b) (6) were cryopreserved for analysis in the 2 hour and 45 minute hold study. To maximize the amount of DP material available, DP was filled into 50 mL (b) (4) freezing bags at (b) (4). The nominal volume recommended by the manufacturer is 10 mL, so the surface area of the bag was reduced by (b) (4) to maintain the recommended volume to surface area ratio. In response to CMC IR #3 on 08 May 2025, the applicant provided a detailed description on the methods used to reduce the freezing bag with calculations to demonstrate the surface area ratio is maintained.*

*Based on the data provided, the DP appears stable with comparable attributes from 0 to 6 months. Sterility testing of lot (b) (4), (b) (6) was negative for samples up to 6 months. We agree to the proposed 6-month shelf life.*

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

No post approval stability protocol has been described by the applicant. Stability studies supporting the commercial shelf-life have been completed.

**Reviewer comment:** *In addition to the primary stability lots (i.e. (b) (4), (b) (6)), data on stability in cryobags were provided for (b) (4) DP lots (b) (4), (b) (6) made with the commercial manufacturing process unit operations at the (b) (4) (i.e. clinical) facility. Data on stability of TLT003 in (b) (4) were provided for the patient DP lot (b) (4), (b) (6), which was manufactured at the commercial manufacturing facility. FDA considers data from these additional stability lots to be supportive, and the lack of a post-approval stability plan for TLT003 is acceptable.*

## 3.2.A APPENDICES

### 3.2.A.1 Facilities and Equipment

*Reviewed by LKD*

AGC Biologics S.p.A (Bresso, Milan) is a contract facility that manufactures multiple products, including viral vectors and other cell and gene therapy products. The most recent inspection of the facility was a pre-license inspection (PLI) conducted by CBER

from November 9 – 20, 2023. Thus, the PLI for this submission was waived. Areas used for (b) (4) WAS LVV process operations are also used for production of LVV for the FDA-licensed product (b) (4). In addition to these areas, the applicant proposes to use (b) (4) WAS LVV (b) (4) as described in Sections [3.2.S.2.5.1 Process Performance Qualification \(PPQ\) study](#) and [3.2.S.2.6.3 Manufacturing changes implemented after PPQ runs](#). The suite proposed for TLT003 manufacture, (b) (4) is located on the same corridor as the suites inspected, reviewed, and approved for (b) (4). Product quality data supporting the introduction of this suite post-PPQ is reviewed in [Section 3.2.S.2.6.2 Manufacturing Process Development, Comparability](#).

*Overall Reviewer's Assessment of Section 3.2.A.1: No outstanding CMC concerns for facilities and equipment information. Additional review of facility information is provided in the DMPQ reviewer's memo (b) (5), (b) (7)(E)*

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

#### *Reviewed by RM*

To mitigate risk of contamination from non-viral and viral adventitious agents, strategies such as the appropriate selection, handling, and testing of raw materials, maintaining aseptic practices during manufacturing, and testing for adventitious agents at various stages of production have been implemented.

Biological materials of human or animal origin are used to manufacture TLT003 (b) (4). For quality information and relevant testing procedures, refer to 3.2.S.2.3 Control of Materials, TLT003 (b) (4) and 3.2.S.2.3 Control of Materials, TLT003 (b) (4). Manufacturing and testing performed on (b) (4) is also described in 3.2.S.2.3 Control of Materials, TLT003 (b) (4).

Procedures are in place to maintain aseptic practices and containment during manufacturing unit operations. Product contact materials are disposable and single use. Non disposable equipment is sanitized or sterilized prior to use according to established internal procedures. Media and buffers are prepared aseptically from sterile raw materials or sterilized after preparation. Testing for non-viral contamination, (e.g. sterility, mycoplasma) are included in the release specifications for the (b) (4) (b) (4) material and TLT003 DP.

*Reviewer comments: Risk mitigation for contamination by adventitious agents is appropriate. Adequate procedures are in place for raw material handling, internal testing of materials, and procedures to track supplier recalls and trace to patient lots. Aseptic manufacturing practices and microbial safety tests for TLT003 DS and DP are adequate.*

#### ❑ Viral Clearance Studies

Viral clearance studies were not performed on (b) (4) TLT003 drug product. However, (b) (4) in the DP were evaluated during the



process validation using (b) (4) and (b) (4) assays. Results of the PPQ batches demonstrated a (b) (4) as reviewed in [3.2.P.5.5 Characterization of Impurities](#).

**Reviewer comments:**

- (b) (4) : *Risk of viral contamination is low based on quality of (b) (4) (e.g. (b) (4) ) and production system (i.e. (b) (4) rather than use of a (b) (4) ). Moreover, (b) (4) process includes (b) (4) steps (e.g. (b) (4) ) that would remove viral contaminants based on differences in (b) (4) . Acceptable.*
- *TLT003 DP: Data from the PPQ lots suggest adequate viral clearance of the replication incompetent vector. Acceptable.*

### 3.2.A.3 Novel Excipients

No novel excipients are used in TLT003 DP.

### 3.2.R Regional Information (USA)

#### ❑ Executed Batch Records

Master batch records (MBRs) as well as executed MBRs are provided in English. The commercial batch record for WAS LVV is composed of 3 documents (parts A, B, and C). Executed MBRs for lot (b) (4), (b) (6) were submitted. The DP batch record is a single document. The applicant provided the executed MBR for lot (b) (4), (b) (6), the most recently manufactured DP lot for an Early Access patient.

#### ❑ Method Validation Package

Method validation study reports were provided for product-specific methods. Validation studies are reviewed in detail in Sections [3.2.S.4.2 Analytical Procedures and 3.2.S.4.3 Validation of Analytical Procedures](#) and [3.2.P.4.2 and 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures](#) for WAS LVV and TLT003 DP, respectively.

#### ❑ Combination Products

Not applicable

#### ❑ Comparability Protocols

No comparability protocols were included in the BLA to support future changes.

**Reviewer comment:** *The two documents originally submitted as PACMPs were withdrawn by the applicant because they either lacked sufficient detail to support a planned change (regarding implementation of the (b) (4) to measure WASP) or described changes that should be handled through existing control procedures and*

*submitted as part of an annual report (implementation of (b) (4) and new version of the (b) (4)). The documents were withdrawn in amendments 7 and 28, respectively, in response to CMC IRs #1 and #13.*

**Overall Reviewer's Assessment of 3.2.R documents:** *The batch records are adequate for commercial manufacturing of LVV and DP.*

## **Other eCTD Modules**

### **Module 1**

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

A categorical exclusion is submitted under 21 CFR 25.31 (c) on the grounds that genetically modified human cells are considered to be substances that occur naturally in the environment because they have stringent nutritional requirements for survival and replication. Therefore, they are not viable in the environment and are degraded into naturally occurring substances.

*Reviewer comment: FDA agrees with the claim of categorical exclusion.*

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

Not applicable

#### **C. Labeling Review**

##### **Full Prescribing Information (PI):**


The following sections of the PI were reviewed: Section 2 (Dose and Administration), Section 3 (Dosage Forms and Strengths), Section 11 (Description), Section 12 (Clinical Pharmacology, Mechanism of Action), and Section 16 (How supplied/storage and handling). The PI provides a detailed and correct description of TLT003, its mechanism of action, and the receipt and preparation procedures for DP at the authorized treatment center.

*Reviewer comment: Details of receipt, administration, and preparation procedures were requested during review of the PI, and the applicant provided adequate information. During PI review, FDA also requested data on the in-use stability (i.e. viability) of DP when used in conjunction with an administration set filter, as such administration sets are described in the PI. No data were provided, but the applicant agreed to conduct a study assessing post-thaw viability of DP that includes an administration set equipped with a filter as a PMC. FDA CMC also discussed the description of administration sets in the clinical protocol with FDA Clinical reviewers. Given that the clinical protocol indicates that administration sets with 200uM filters should be used for TLT003, the PI language is considered acceptable based on the experience gained during clinical studies, even though evaluation of viability was not provided in the BLA.*


DP container labels including infusion bag label, overwrap label, and metal cassette label are shown in Figure 26.

**Figure 26. DP container closure physical labels: (A) infusion bag label (B) overwrap label (C) metal cassette label**



**A.**

<b>etuvetidigene autotemcel</b> <b>Waskyra</b> <b>1.9 -11.4 x 10<sup>6</sup> CD34+ cells /mL dispersion for infusion</b> <b>Rx only. Intravenous use. For autologous use only.</b> cryopreservative solution containing 5% DMSO Store and transport frozen (<-130°C)		 0 000000 000000 0
Lot: _____ DIN: _____ Bag ID: _____ COI ID: _____ EXP: _____		NDC: xxxxxxxxxxxx  10-20 mL of cell dispersion per bag. Read the package leaflet before use. Do not irradiate. No US standard for potency.  Manufactured for: Fondazione Telethon ETS Via Varese 16/B, 00185 - Rome, Italy  Manufactured by: AGC Biologics S.p.A. Via Meucci 3, 200091-Bresso (MI), Italy

**B.**

<b>etuvetidigene autotemcel</b> <b>Waskyra</b> <b>1.9 -11.4 x 10<sup>6</sup> CD34+ cells /mL dispersion for infusion</b> <b>10-20 mL</b>	
<b>Rx only. For intravenous use. For autologous use only. Keep out of the sight and reach of children.</b> An autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells (HSPC) transduced ex vivo using a lentiviral vector encoding the human Wiskott-Aldrich Syndrome (WAS) gene. Also contains 5% dimethylsulfoxide, 7% human serum albumin and 0.9% sodium chloride. Read the package leaflet before use. One bag needs to be completely infused. Do not use a lymphodepleting filter. Do not irradiate. Store and transport frozen (<-130°C). Keep infusion bag in the metal cassette until ready for thaw and administration. Do not unseal the overwrap bag until after thaw. Once thawed do not re-freeze. Shelf-life after thawing: 2 hours at room temperature (20°C-25°C.) This medicine contains human blood cells. Unused medicine or waste material must be disposed of in compliance with the local guidelines on handling of waste of human-derived material.	
Manufactured for: Fondazione Telethon ETS Via Varese 16/B, 00185 - Rome, Italy  Manufactured by: AGC Biologics S.p.A. Via Meucci 3, 200091-Bresso (MI), Italy  US License #XXXX NDC: xxxxxxxxxxxx	DIN: _____ COI ID: _____ Lot: _____ Bag ID: _____ EXP: _____   0 000000 000000 0

C.

etuvetidigene autotemcel Waskyra 1.9 -11.4 x 10 <sup>6</sup> CD34+ cells /mL dispersion for infusion	
<b>Rx only</b>	
An autologous CD34+ cell enriched population that contains haematopoietic stem and progenitor cells (HSPC) transduced ex vivo using a lentiviral vector encoding the human Wiskott-Aldrich Syndrome (WAS) gene.	
Also contains 5% dimethylsulfoxide, 7% human serum albumin and 0.9% sodium chloride.	
10-20 mL.	
Read the package leaflet before use. See Lot Information Sheet for number of infusion bags and CD34 <sup>+</sup> cells per bag for this patient. One bag needs to be completely infused.	
Do not use a lymphodepleting filter. Do not irradiate.	
Keep out of the sight and reach of children.	
<b>For intravenous use. For autologous use only.</b>	
<b>Store and transport frozen (&lt;-130°C).</b> Keep infusion bag in the metal cassette until ready for thaw and administration. Do not unseal the overwrap bag until after thaw. Once thawed do not re-freeze.	
<b>Shelf-life after thawing:</b> 2 hours at room temperature (20°C-25°C)	
This medicine contains human blood cells.	
Unused medicine or waste material must be disposed of in compliance with the local guidelines on handling of waste of human-derived material.	DIN: _____
Manufactured by: AGC Biologics S.p.A.	COI ID: _____
Via Meucci 3, 20091, Bresso, Italy.	Lot: _____
US License #XXXX	Bag ID: _____
NDC:xxxxxxxxxxx	EXP: _____
 <b>Fondazione Telethon</b>	
Fondazione Telethon ETS Via Varese 16/B 00185 Rome Italy	0 00000 00000 0

*Reviewer comments: Revisions to physical labels were submitted in amendments 21, 23, and 29. All concerns were addressed. Physical labels are acceptable.*

#### **Modules 4 and 5**

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

*Reviewed by RM*

#### **Vector Copy Number (VCN) by qPCR (b) (4)**

VCN was originally analyzed by qPCR at SR-TIGET to support preclinical studies. The assay was validated in (b) (4) runs using an (b) (4) system and assessed ULOQ-LLOQ, specificity, linearity, accuracy, and intra and inter assay precision. All acceptance criteria were met, and the assay was successfully validated.

Later in development, a study was performed to qualify the (b) (4) system to (b) (4) VCN assay from (b) (4) to support preclinical and clinical studies. (b) (4)

(b) (4) and successfully established assay precision, LLOQ-ULOQ, specificity, accuracy, and the stability of test samples. The results demonstrated comparability between the two methods with regard to VCN and the (b) (4). The (b) (4) assay was validated once more to quantify VCN in (b) (4). Because the (b) (4) method was previously validated, this study assessed

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