

**DBSQ/OCBQ ANALYTICAL METHOD REVIEW MEMO**

**To:** The file: STN 125785/0

**From:**

| Reviewer               | Role          | Date finalized | Stamp | Supervisor                  | Stamp |
|------------------------|---------------|----------------|-------|-----------------------------|-------|
| M. Nahid Parvin, Ph.D. | Lead Reviewer | 12/07/2023     |       | Muhammad Shahabuddin, Ph.D. |       |
| Tao Pan, Ph.D.,        | Reviewer      | 12/15/2023     |       | Kenneth S. Phillips, Ph.D.  |       |
| Hyesuk Kong, Ph.D.     | Reviewer      | 12/13/2023     |       | James L. Kenney, D.Sc.      |       |

**Through** Maryna Eichelberger, Ph.D.  
Division Director, DBSQ/OCBQ

**Applicant:** Vertex Pharmaceuticals Incorporated

**Subject:** Review of Analytical Methods used for Exagamglogene autotemcel (exa-cel) Critical Gene Editing Reagents and Drug Product (DP) Lot Release

**Recommendation:** Approval

**Executive Summary:**

The following analytical methods used for release of critical gene editing reagents (Cas9 and SPY101) and drug product (DP) of Exagamglogene autotemcel (exa-cel) and the associated analytical method validations and qualifications, were reviewed:

1. (b) (4) (M. Nahid Parvin)
2. (b) (4) (M. Nahid Parvin)
3. (b) (4) (M. Nahid Parvin)
4. (b) (4) (M. Nahid Parvin)
5. (b) (4) (Tao Pan)
6. (b) (4) (Tao Pan)
7. (b) (4) (Tao Pan)
8. (b) (4) (Tao Pan)
9. (b) (4) (Tao Pan)

10. (b) (4) (Tao Pan)
11. (b) (4) (Tao Pan)
12. (b) (4) (Tao Pan)
13. (b) (4) (Tao Pan)
14. (b) (4) (Tao Pan)
15. (b) (4) (Tao Pan)
16. Appearance of DP (Tao Pan)
17. (b) (4) (Hyesuk Kong)
18. Sterility of (b) (4) DP (Hyesuk Kong)
19. Endotoxin of (b) (4) DP (Hyesuk Kong)
20. Mycoplasma of DP (Hyesuk Kong).

**Conclusion:** The analytical methods and their validations or qualifications reviewed for release of Exagamglogene autotemcel (exa-cel) were found to be adequate for their intended use.

#### **Documents Reviewed:**

Information in sections of the original submission that describe control of DS (critical gene editing reagents; Cas9 and SPY101) and DP (3.2.S.4 and 3.2.P.5, respectively), including descriptions of the specifications, analytical procedures of Cas9, SPY101, and DP and validation or qualification of analytical procedures were reviewed. Additional information in amendments #125785/0.17, #125785/0.28, #125785/0.31, #125785/0.43, and #125785/0.66 received on May 18, June 02, June 23, August 30, and October 30, 2023, were also reviewed.

#### **Background:**

Vertex Pharmaceuticals, Inc. submitted original BLA on March 31, 2023, STN 125785 for exagamglogene autotemcel (exa-cel), formerly CTX001, for the treatment of transfusion-dependent  $\beta$ -thalassemia (TDT), a rare, debilitating, and life-shortening disorder caused by genetic defects affecting the production of hemoglobin. Exa-cel holds the following designations: fast track, orphan drug (DRU-2019-7143), and regenerative medicine advance therapy. Exa-cel is indicated for the treatment of TDT in patients 12 years and older.

Exa-cel is a CRISPR/Cas9 modified autologous CD34+ human hematopoietic stem and progenitor cell (hHSPC) cellular therapy product. The cells have been edited to express fetal hemoglobin (HbF) protein levels in adult erythroid cells. Exa-cel is administered as a single dose consisting of  $\geq 3 \times 10^6$  CD34+ cells/kg of patient weight; the DP contains approximately  $4\text{-}13 \times 10^6$  cells/mL suspended in (b) (4) cryopreservation medium containing 5% DMSO and Dextran 40. Edited cells from more than one vial and more than one lot may be used to provide a complete patient dose through intravenous administration (IV). Exa-cel is packaged in 20 mL (b) (4) Vials at 1.5 - 20 mL per vial in liquid nitrogen vapor ( $\leq -135$  °C). The filled infusion vials are clearly labeled for autologous use only and linked to the recipient using a unique identifier for each patient.

Exa-cel is manufactured using a continuous culture process and therefore, there is no distinct drug substance. The manufacturing of the exa-cel DP involves the electroporation of autologous CD34+ cells with Ribonucleoprotein (RNP) complex. The RNP complex consists of two critical gene editing components, Cas9 and SPY101 single guide RNA (sgRNA). Cas9 is a functional protein for gene editing and is manufactured (b) (4), SPY101 sgRNA is a 100-mer synthetic oligonucleotide manufactured (b) (4); both are shipped for exa-cel DP manufacturing at (b) (4)

The following facilities perform the analytical methods listed above reviewed and mentioned below in parenthesis:

(b) (4)

The standard operating procedures (SOPs) for (b) (4) were not initially submitted by the sponsor. An information request sent to the sponsor to provide the SOPs. Sponsor provided the requested information on May 18, 2023 in amendment #125785/0.17. The SOPs and attached supplementary documents for each SOPs were developed in German and are effective. English translations of the SOPs were reviewed.

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

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

(b) (4)

## 16. Appearance of Exa-cel (DP)

The appearance of Exa-cel DP is determined by visual inspection, and its specification for both release and stability is “Translucent cell suspension, practically free of visible foreign particles”; the lot release test is performed and verified at (b) (4)

### Method

The appearance method for Exa-cel DP is a visual assessment of translucency and presence or absence of visible foreign particles of (b) (4) DP ((b) (4) SOP-0043: Visual Inspection, Appearance and Particulate Testing of a Reagent or Product). In brief, a final container of (b) (4) DP is visually inspected for the translucency of its cell suspension and presence or absence of visible foreign particles in the cell suspension at (b) (4). The description of the method is acceptable with sufficient details.

Method Verification

The appearance of Exa-cel DP method was verified at (b) (4) (b) (4): Qualification/Validation Summary Report Appearance Operator Training Enhancement) and (b) (4) (AVR-58939: Appearance Precision Assessment Report for Exa-cel DP Release and Stability Testing at (b) (4)).

For the verification, in (b) (4), (b) (4) different analysts inspected (b) (4) lots of DP, (b) (4) vials each, on different days for their appearance, and all the inspected vials, (b) (4) in total, met the release specification; in (b) (4), (b) (4) analysts inspected total (b) (4) lots of DP, and all met the release specification. The precision of the method was demonstrated, and the method was verified at both sites.

Conclusion

Based on information provided, the appearance method has been verified for its intended use and is suitable for release testing of Exa-cel DP.

(b) (4)

(b) (4)

(b) (4)

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(b) (4)

(b) (4)

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

**18. Sterility of (b) (4) DP**

Introduction

Sterility testing is performed on (b) (4) exa-cel DP testing is performed at (b) (4) (b) (4) Acceptance criteria of 'No Growth' must be met for the lot release of (b) (4) exa-cel DP.

Method

The membrane filtration sterility test is used in accordance with (b) (4) . Test samples are (b) (4)

The (b) (4) sterility test is used for various liquids, solids, and device in accordance with (b) (4) . Test samples are (b) (4)

The (b) (4) sterility test methods are described in more detail below together with the tests that were performed to determine suitability of these test methods.

The original qualification reports for sterility lacked sufficient information to complete the review: 1) sterility testing of DP was performed using (b) (4) at (b) (4) , 2) a small sample volume i.e., (b) (4) in (b) (4) of media was used at the (b) (4) site, and 3) the qualification studies performed at (b) (4) did not include evaluation of environmental isolate; therefore, IRs were sent requesting: 1) a qualification study be performed with at least (b) (4) lots, 2) a reduced media volume in order to have an appropriate sample to media ratio to increase the sensitivity of the method, or to perform an additional sterility test using (b) (4) mL of (b) (4) to provide assurance for the use of their small sample volume. Responses were received on June 2, 2023 (Amendment 28), June 23, 2023 (Amendment 31) and August 30, 2023 (Amendment 43), which were found acceptable and reviewed as part of the DP sterility testing below.

In addition, Vertex initially contracted their sterility test for exa-cel DP out to (b) (4) and their sterility qualification was also found incomplete since it was performed using (b) (4) . An IR was sent out on May 18, 2023, requesting the missing information described above with an additional sterility supplemental qualification. In amendment 43, received on August 30, 2023, Vertex withdrew the (b) (4) sterility testing site, as they were unable to initiate the study in time due to equipment issues.

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

Conclusion

The method suitability tests including supplemental sterility test using (b) (4) were performed and compliant with (b) (4) and the test results indicate there is no product inhibition of microorganism growth, thus indicating the (b) (4) sterility test methods are appropriate under the actual conditions of use.

**19. Endotoxin of (b) (4) DP**

Introduction

Endotoxin testing for (b) (4) is performed at (b) (4), respectively, while exa-cel DP testing is performed at (b) (4). Acceptance criteria of: (b) (4), and (b) (4) must be met for release of exa-cel DP.

Method

(b) (4)

(b) (4)

(b) (4)

The original qualification report for the endotoxin study did not include information required for completion of the review; therefore, an IR was sent requesting missing information and

responses were received on June 2, 2023 (Amendment 28), June 23, 2023 (Amendment 31), and August 30, 2023 (Amendment 43), which were found acceptable and explained below.

(b) (4)



(b) (4)

Conclusion

The method suitability test was performed and compliant with (b) (4) and the test results indicate there is no product interference from (b) (4) DP test samples, thus indicating the (b) (4) BET test method is appropriate under the actual conditions of use.

**20. Mycoplasma of DP**

Introduction

Mycoplasma testing is performed using (b) (4) method in exa-cel final harvest cell suspension, which was initially validated at (b) (4) and later transferred to (b) (4)

Acceptance criteria of 'Negative' must be met for the release of the exa-cel final harvest cell suspension.

Method

(b) (4)

The validation reports for mycoplasma test submitted in the original submission lacked sufficient information to complete the review; therefore, an IR was sent requesting data and clarification to fulfill these deficiencies. A response was received on June 2, 2023 (Amendment 28), which was found acceptable and explained below.

(b) (4) Mycoplasma Test Validation for DP

(b) (4) performed detailed validation studies using their (b) (4) mycoplasma test for exa-cel DP that covered specificity, limit of detection (LOD), and robustness as well as a comparability study with (b) (4) mycoplasma method to determine if the (b) (4) method provides assurance equal to or greater than the (b) (4) method in accordance with (b) (4). In addition, (b) (4) performed an additional product-specific qualification (PSQ) and a product-specific bridging study of (b) (4) method vs. (b) (4) method on exa-cel patient and HD cells to demonstrate the comparability between the two methods for exa-cel DP as reported in validation report # AVR-57579 (Amendment 28).

Specificity is the ability of the method to (b) (4)

Limit of Detection is the (b) (4)

(b) (4)

Robustness is the ability of a test method to

(b) (4)

Comparability of (b) (4) to (b) (4) Method

(b) (4)

|

(b) (4)

Conclusion

The method validation tests for the DP were performed and compliant with (b) (4) and the test results indicate there is no product interference from the test sample. The test was shown to provide assurance equal to or greater than the (b) (4) methods. Therefore, the mycoplasma test method is appropriate under the actual conditions of use at (b) (4) facility for exa-cel DP.