



## DBSQ/OCBQ ANALYTICAL METHOD REVIEW MEMO

**To** The file: STN 125807/57

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**Applicant** Abeona Therapeutics Inc.

**Subject** Review of Mycoplasma, Endotoxin, Sterility, (b) (4) Analytical  
Methods used for prademagene zamikeracel (ZEVASKYN).

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**Recommendation:** Approval

### Executive Summary:

The mycoplasma, endotoxin, sterility, and (b) (4) analytical methods used for testing and release of ZEVASKYN and the associated analytic method qualifications, were reviewed.

**Conclusion:** The analytical methods and their qualifications reviewed during the resubmission for ZEVASKYN (b) (4) drug product were found to be adequate for their intended use.

### Documents Reviewed

Information in sections of the resubmission that describe control of the (b) (4) (b) (4) (b) (4) Drug Product (DP) (3.2.S.4 and 3.2.P.5, respectively), including descriptions of (b) (4) DP specifications, analytical procedures of (b) (4) DP, and qualifications of these analytical procedures were reviewed. In addition, the responses to CBER's Information Requests (IRs) received on November 21, 2024 (Sequence No. 0060) and January 17, 2025 (Sequence No. 0062) and were also reviewed as mentioned below.

### Background

On September 25, 2023, Abeona Therapeutics Inc. submitted a Biologics Licensing Application (BLA) for ZEVASKYN. After a complete review of the submission, FDA issued a complete response (CR) letter to Abeona on April 16, 2024, which stopped

the review clock until Abeona addressed their multiple deficiencies. Abeona Therapeutics, Inc. resubmitted their BLA on October 29, 2024, for continued review.

ZEASKYN is a genetically engineered autologous cell therapy intended for the treatment of wounds associated with recessive dystrophic epidermolysis bullosa (RDEB). RDEB is caused by mutations in the COL7A1 gene. It is a severe form of epidermolysis bullosa (EB) and is a rare, life threatening, autosomal recessive form of EB with symptoms present at birth. Mutations in COL7A1 gene lead to reduced, or absent levels of, biologically active collagen protein (C7) and result in lack of anchoring fibrils (AF). Because of this lack of AF, RDEB is characterized by mechanical fragility of the skin and other epithelial lined or surface tissues, resulting in chronic wounds, restrictive scarring, and aggressive squamous cell carcinoma (SCC).

ZEASKYN contains functional copies of the COL7A1 transgene that is transduced ex vivo into the patient's own keratinocyte cells using a LZRS-COL7A1 vector to form gene-corrected keratinocyte sheets with C7 expression. Upon viral transduction, the COL7A1 transgene integrates into the host-cell genome, resulting in durable expression and secretion of collagen protein, which addresses the underlying mechanism of the disease.

## 1. Mycoplasma (DP)

### Introduction

Mycoplasma testing for ZEASKYN DP is performed at (b) (4) in (b) (4). Specification of 'Not Detected' for DP must be met for release of ZEASKYN.

There were two CR issues pertinent to mycoplasma testing addressed in the resubmission:

- 1) For the mycoplasma validation, FDA requested the applicant conduct the specificity study for the (b) (4) method using their (b) (4)   
 (b) (4)
- 2) Adequate data for the mycoplasma validation method was not provided to demonstrate equivalency of the (b) (4) and the proposed (b) (4) (b) (4) system. Without the comparability / equivalency data, the (b) (4) (b) (4) mycoplasma assay validation is not complete. Since the (b) (4) method is proposed to replace the (b) (4) method, comparability

testing on the product must be performed to provide assurance that the sensitivity of the (b) (4) method is equal to or greater than the (b) (4) method (b) (4) and is suitable under the actual condition of use.

While the review was on hold, a Type A meeting was held August 8, 2024. CBER was able to further discuss acceptance criteria for Abeona's comparability study between the (b) (4) method and the mycoplasma (b) (4) method per (b) (4).

#### Mycoplasma Test Validation for DP

Abeona performed detailed validation studies for the (b) (4) mycoplasma method that covered robustness, ruggedness, and LOD in the original submission. The comparability study report for mycoplasma DP lacked sufficient information to complete the review. Therefore, an IR was sent requesting data on the comparability study as per 21 CFR 610.9 and (b) (4) to fulfill the deficiency. A response was received on November 21, 2024 (Sequence No. 0060), which was reviewed and explained below.

Abeona performed validation testing using (b) (4) lots of ZEVASKYN (i.e., (b) (4) (b) (4)

The sample (b) (4) for the (b) (4) mycoplasma test session is composed of (b) (4) (b) (4)

#### Specificity

Assessment of specificity of the (b) (4) method was not performed using the ZEVASKYN product (b) (4). Specificity testing was performed in (b) (4) (b) (4) (b) (4) (b) (4) (b) (4) (b) (4)

as recommended in (b) (4). This data was found to be acceptable. FDA rescinded the request to review the applicants study due to the (b) (4) demonstrating specificity for the (b) (4) method.

Comparability with the (b) (4) method

(b) (4) evaluated comparability by performing (b) (4)

(b) (4)

Conclusion: The method validation tests were performed and compliant with (b) (4) (b) (4) and the test results indicate there is no product interference from the test sample. The (b) (4) test method was demonstrated to provide assurance equal to or greater than the (b) (4) method and is appropriate under the actual conditions of use.

## 2. Endotoxin (DP)

### Introduction

Endotoxin testing for ZEVASKYN DP is performed at Abeona Therapeutics, Inc. (Abeona) in Cleveland, Ohio. Specification of (b) (4) for the DP must be met for release of ZEVASKYN.

There were two CR issues pertinent to endotoxin testing addressed in the resubmission:

- 1) A DP (b) (4) study will need to be submitted to show media (b) (4) with a (b) (4) DP is sufficient to indicate the method is appropriate for its intended use.
- 2) Bacterial Endotoxin testing is performed on a sample testing dilution that is equal to the (b) (4) of the test. The (b) (4) determines how much a test material may be diluted and still detect endotoxin in the sample at the specification limit. At dilutions beyond the (b) (4) a negative may have been a positive as the endotoxin present has been (b) (4) beyond the sensitivity of the (b) (4) used in the test. Therefore, testing at the (b) (4) should only be performed if dilutions (b) (4) (b) (4) do not provide valid

test results. Please provide your test for interfering factors results showing positive product control recoveries for test dilutions (b) (4) (b) (4). If one of these dilutions provides valid test results, please indicate which dilution will be used as your test dilution to ensure your test sample has not been diluted beyond the sensitivity of the test.

An informal meeting was held May 14, 2024 to discuss Abeona's deficiency regarding their bacterial endotoxin test method. CBER requested Abeona use a (b) (4) (b) (4) to test the excipient media and to establish a new specification. During the Type A meeting held August 8, 2024, CBER agreed to the updated test parameters and acceptance criteria made to the test method.

#### Method

The (b) (4) is a (b) (4) assay that is based on the (b) (4)


The ZEVASKYN (b) (4) is performed using the (b) (4)

The qualification report for endotoxin DP lacked sufficient information to complete the review. Therefore, an IR was sent requesting clarification to fulfill the deficiency. A response was received on January 17, 2025 (Sequence No. 0062) which was reviewed and explained below.

#### Endotoxin: Sample (b) (4) Validation Report (DP)


The objective of this validation was to verify the (b) (4) (b) (4) with the P1 packaged PZ DP needed to recover a level of gram-negative microorganism that was detectable in the endotoxin assay.

(b) (4)




(b) (4) Qualification for DP

Abeona qualified their (b) (4) method using ZEVASKYN DP to determine if the method is suitable under the actual conditions of use. The (b) (4) (b) (4) of DP was calculated to be (b) (4). The (b) (4) requirements for a medical device specification is (b) (4) therefore, Abeona (b) (4) (b) (4) which was (b) (4). The endotoxin limit of (b) (4) is then (b) (4).



(b) (4)



The (b) (4) concentration results for the DP samples found during the qualification testing were all within the release specification of (b) (4) for ZEVASKYN DP and were found acceptable.

Conclusion

The method suitability test was performed and compliant with (b) (4) and (b) (4). The test results indicate the (b) (4) method is appropriate under the actual condition of use.

### 3. Sterility (b) (4) DP)

#### Introduction

Due to the nature of ZEVASKYN, Abeona has adopted the use of the (b) (4) (b) (4) for rapid detection of microbial contamination at (b) (4) sample points. A (b) (4) (b) (4) (b) (4) rapid sterility test conducted on sample from drug product harvest day. In the resubmission, Abeona has submitted a validation for the rapid (b) (4) for sterility testing.

Sterility testing for ZEVASKYN on the (b) (4) DP is performed at Abeona Therapeutics, Inc. (Abeona) in Cleveland, Ohio. Acceptance criteria of 'No Growth Detected' must be met for ZEVASKYN.

There were two CR issues pertinent to sterility testing addressed in the resubmission:

- 1) A DP (b) (4) study will need to be submitted to show media (b) (4) with a (b) (4) DP is sufficient to indicate the method is appropriate for its intended use.
- 2) Abeona proposed to conduct rapid sterility testing (b) (4) on (b) (4) (b) (4) on final drug product (DP) samples using the (b) (4) (b) (4). However, data to demonstrate the suitability of these (b) (4) sterility methods were not provided.

During the Type A meeting held August 8, 2024, Abeona further discussed their rapid sterility test method, along with their method protocols and proposed validations for the resubmission.

#### Method

(b) (4)

Sterility: Sample (b) (4) Validation Report (DP)

The objective of this validation was to verify the hold time sensitivity for the sterility assay when the (b) (4)

(b) (4)

To assess the sensitivity of a (b) (4)

CBER finds this acceptable.

The original validation reports for sterility lacked sufficient information to complete the review. Therefore, an IR was submitted to Abeona requesting additional data. A response was received on November 21, 2024 (Sequence No.0060) which was found acceptable and explained below.

(b) (4)



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

(b) (4)

(b) (4) Rapid Sterility Test Method Validation for DP

Abeona performed the same validation study on their final DP. (b) (4) (b) (4) will be collected from P1 packaged ZEVASKYN DP. Identical microorganisms, incubation conditions, and test parameters were evaluated.

Conclusion

The method suitability tests were performed and compliant with (b) (4) and (b) (4) (b) (4) and the test results indicate there is no product inhibition of microorganism growth, thus indicating the rapid (b) (4) sterility test method is appropriate under the actual conditions of use and provides the same level of assurance as the (b) (4) (b) (4) method.

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