

**CBER CMC BLA Resubmission Review Memorandum
Complete Response**

BLA STN 125807/57

**ZEVASKYN
Prademagene zamikeracel**

Reviewers

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1. **BLA#:** STN 125807

2. **APPLICANT NAME AND LICENSE NUMBER**

Abeona Therapeutics, Inc., License # - n/a

3. **PRODUCT NAME/PRODUCT TYPE**

Non-Proprietary/Proper/USAN: Prademagene zamikeracel
Proprietary Name: ZEVASKYN
NDC Codes: 84103-007-01

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

- a. **Established Pharmacological Class (EPC):** Autologous cell sheet-based gene therapy
- b. **Dosage Form:** The recommended dose of ZEVASKYN is based on the surface area of the wound. One sheet of ZEVASKYN covers an area of 40 cm².
- c. **Strength/Potency:** The potency of the product is measured by the amount of Collagen VII Trimer protein expressed by transduced cells
- d. **Route of Administration:** Cellular sheets are topically applied to skin wounds
- e. **Indication:** Recessive Dystrophic Epidermolysis Bullosa (RDEB)

5. **MAJOR MILESTONES**

Initial IND Submission (BB-IND 13708)	May 18, 2008
IND allowed to proceed	August 28, 2009
Orphan Drug Designation granted	May 25, 2017
Breakthrough Therapy Designation granted	August 16, 2017
RMAT Designation granted	January 26, 2018
Pre-BLA Meeting	August 23, 2023
Original BLA Submission	September 25, 2023
Complete Response (CR) Letter Sent	April 16, 2024
Response to CR Letter Received	October 28, 2024
PDUFA Due Date	April 29, 2025

6. CMC/QUALITY REVIEW TEAM

Reviewer/Affiliation	Section/Subject Matter
Bao-Ngoc Nguyen (BNN), PhD, OTP/OCTHT/DCT2/TEB1	Complete Response Letter (CRL) CMC Deficiency #1d Section 3.2.P.5.3 Section 3.2.P.3.3 CRL Deficiency #4 Section 3.2.S.1.3 (EB-101) Section 3.2.S.3.1 (EB-101) Section 3.2.S.2.4 (EB-101) Section 3.2.P.5.1-4, 6 CRL Deficiency #8 CRL Additional Comment #13 Section 1.14.1.1-3 Additional Changes Reviewed Section 3.2.R Section 3.2.P.5.2 Section 3.2.P.7
Joshua Kufera (JTK), PhD, OTP/OGT/DGT2/GTB5	CRL Deficiency #7 Section 3.2.S.2.3 (LZRSE-Col7A1 RVV) CRL Deficiency #8 CRL Additional Comment #15 Section 3.2.S.4.1, 5 (LZRSE-Col7A1 RVV) CRL Additional Comment #16 Section 3.2.S.2.4, 6 (LZRSE-Col7A1 RVV) CRL Additional Comment #17 Section 3.2.S.7 (LZRSE-Col7A1 RVV) CRL Additional Comment #18 Section 3.2.S.2.3 (LZRSE-Col7A1 RVV) Additional Changes Reviewed Section 3.2.S.2.1-3 (LZRSE-Col7A1 RVV)
Mo Liu (ML), PhD OTP/OGT/DGT2/GTIB	CRL Deficiency #2 Section 3.2.S.4.2, 3 (LZRSE-Col7A1 RVV) CRL Deficiency #3 Section 3.2.S.4.3 (LZRSE-Col7A1 RVV)
Ileana Marrero-Berrios (IMB), PhD, OTP/OCTHT/DCT2/TEB1	CRL Deficiency #6 CRL Deficiency #7 Section 3.2.S.2.3 (EB-101)
Carolina Panico (CP), MD, PhD, OTP/OCTHT/DCT2/TEB2	CRL Deficiency #5 Section 3.2.P.5.2.3 CRL Additional Comment #14 Section 3.2.P.8

7. INTER-CENTER CONSULTS REQUESTED

None.

8. SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/Status
January 17, 2025	Amendment 60	Response to IR #41 (CMC IR #14)
February 14, 2025	Amendment 63	Response to IR #45 (CMC IR #15)
February 24, 2025	Amendment 64	Response to IR #46 (Regulatory/Labeling)
March 5, 2025	Amendment 67	Response to IR #50 (CMC IR #16)
March 12, 2025	Amendment 69	Response to IR #52 (CMC IR #17)
March 13, 2025	Amendment 71	Response to IR #54 (CMC IR #18)

March 18, 2025	Amendment 72	Response to IR #55 (CMC IR #19)
March 21, 2025	Amendment 74	Response to IR #57 (CMC IR #20)
April 3, 2025	Amendment 79	Response to IR #60 (CMC IR #21)

9. REFERENCED REGULATORY SUBMISSIONS

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
(b) (4)	(b) (4)	(b) (4)	Yes	Information regarding (b) (4) (b) (4)
		(b) (4)	Yes	Module 3.2.S. and specific media related modules for (b) (4) (b) (4)
		(b) (4)	Yes	Module 3.2.S. and specific media related modules for Keratinocyte (b) (4) catalog numbers (b) (4) (b) (4) (Custom #'s (b) (4)
		(b) (4)	Yes	Module 3.2.S. and specific media related modules for (b) (4) (b) (4) catalog number (b) (4)
		Gene Therapy Testing	Yes	Information pertinent to LZRSE-Col7A1 RVV Manufacturing Process Development was reviewed by JTK and incorporated into Module 3.2.S.2.6
		Retroviral Manufacturing	Yes	Information pertinent to LZRSE-Col7A1 RVV Manufacturing Process Development was reviewed by JTK and incorporated into Module 3.2.S.2.6
		(b) (4)	Yes	Information pertinent to (b) (4) testing of the LZRSE-Col7A1 RVV (b) (4) was reviewed by JTK and incorporated into Module 3.2.S.2.6

10. REVIEWER SUMMARY AND RECOMMENDATION

Executive Summary

After review of the Complete Response (CR) Resubmission by Abeona Therapeutics, the Chemistry, Manufacturing, and Controls (CMC) review team concludes that the manufacturing process for prademagene zamikeracel described in Biologics License Application BL125807 contains adequate controls to ensure product safety. Therefore, the CMC review team recommends approving this BLA, with two (2) post-market commitments. More details may be found below in section B.I. Post marketing Commitments.

Regulatory History – Prior Interactions with the Agency

The original product, Autologous Keratinocytes Transduced with (b) (4) (b) (4) was submitted by (b) (4) from (b) (4) (b) (4) in (b) (4) for a Phase 1 Study. The IND was transferred to Abeona Therapeutics in 2019, who conducted the pivotal studies that would support the safety and efficacy of the marketing application. A pre-BLA meeting was held on August 23, 2023.

Original Submission (OS) BLA Review Cycle

BL125807 was submitted by Abeona Therapeutics on September 25, 2023 to request approval of prademagene zamikeracel (PZ) for the treatment of recessive dystrophic epidermolysis bullosa (RDEB). The BLA was reviewed under priority review.

During the OS BLA review, several safety issues related to product quality were identified by CMC OTP, DBSQC, and DMPQ reviewers. There were no concerns raised by other review disciplines (e.g., preclinical, clinical, biostatistics). These issues were communicated to the applicant as CR Deficiencies on April 16, 2024, as outlined below:

1. You did not demonstrate adequate suitability of the microbiological test methods listed below for your final drug product, prademagene zamikeracel (PZ).
 - a. Sterility Test Method: You proposed to conduct (b) (4) rapid sterility testing (b) (4) on final drug product (DP) samples using the (b) (4). However, data to demonstrate the suitability of these (b) (4) sterility methods were not provided. Without adequate validation of the sterility methods, the sterility of your PZ DP is not assured. To demonstrate that each method can reproducibly detect appropriate levels of microbial contamination, you should provide a report that includes the (b) (4) as well as acceptance criteria (AC) with justification, for at least (b) (4) DP lots. The impact of any deviations that occurred during testing should be described in the report. In addition, method validation should be performed in accordance with

(b) (4) To demonstrate your rapid sterility test methods, provide assurance of effectiveness equal to or greater than the assurances provided by the (b) (4) sterility test method under the actual condition of use, you should provide data from comparability studies.

- b. Mycoplasma Test Method: The data you provided does not demonstrate the suitability of your mycoplasma test method because assay specificity and equivalency to (b) (4) methods were not adequately demonstrated. To complete demonstration of assay specificity, you should conduct a specificity study for the (b) (4) method using your (b) (4)

(b) (4) To demonstrate comparability/equivalency of the (b) (4) and the proposed (b) (4) system, data from a comparability study must be provided to assure that the sensitivity of the (b) (4) method is equal to or greater than the (b) (4) method (b) (4) under the actual condition of use.

- c. Bacterial Endotoxin Test: The test sample dilution (b) (4) you proposed for determining endotoxin concentration is not acceptable because it is at the limit of the test, i.e., the (b) (4). Testing at the (b) (4) may detect endotoxin in the sample at the specification limit; however, testing at the (b) (4) should only be performed if dilutions (b) (4) (b) (4) do not provide valid test results. To identify the appropriate sample dilution to use in the endotoxin test, you need to provide data from a test for interfering factors that show positive product control recoveries for a series of test dilutions (b) (4) (b) (4). As per (b) (4) the dilution equation must include the media volume. You should use this information to identify the dilution that provides optimal recovery and use as the sample dilution in release assays.
- d. Supporting (b) (4) Studies: You did not provide adequate evidence of endotoxin or microorganism recovery in media that has been in (b) (4) (b) (4) with DP after assembly of the P1 packaging (i.e., clamshell inside (b) (4) bag). Data should be provided for (b) (4) studies for all assays that will utilize this media as a test sample, including endotoxin, (b) (4) (b) (4), and all sterility tests. The (b) (4) tests should be designed to demonstrate that the proposed (b) (4) time allows for adequate detection and recovery of appropriate levels of contaminants in the DP and use an (b) (4) (b) (4) volume that is appropriate for the intended test method. The (b) (4) studies for bacterial endotoxin must comply with (b) (4) for bacterial endotoxin (b) (4) volume and (b) (4) process. A full test report including a description of the test methods, acceptance criteria (AC) with justification, and sensitivity of the evaluated assays with the media samples, is needed to demonstrate your endotoxin, (b) (4) and rapid sterility testing methods are appropriate for their intended use.

(b) (4)

4. You proposed to conduct additional identity testing on your final DP as part of lot release testing to assess and confirm the cell populations in your DP. You propose to utilize a (b) (4) to “detect the presence of keratinocytes” in your product. However, you did not provide an adequate method description, protocol, or validation report for your proposed identity assay. In order to ensure the identity and purity of your final DP, a validated identity assay is necessary. Please submit a method validation report demonstrating that your proposed method can adequately detect and identify the cell populations present in your DP. The report should include the AC, with justification, description of the test method, including test sample and sample size, discussion of results, and deviations, if any.
5. As part of the final product lot release testing, you conduct (b) (4) visual inspection on the DP. You indicate that these (b) (4) methods are

qualified and operators are adequately trained to conduct these visual tests. However, you did not provide adequate validation reports for these methods to demonstrate that these assays can be consistently and accurately performed. Specifically, we identified the following issues in the method validation reports provided in Amendment 45, received on March 28, 2024:

- a. (b) (4) identified a contaminant (hemoclip) in the DP during visual inspection that the other (b) (4) operators missed during the (b) (4) validation run. The discrepancy described resulted in out-of-specification results of the visual inspection.
- b. (b) (4) that identified the contaminant (hemoclip) did not perform (b) (4) as the (b) (4) tests were performed (b) (4). This resulted in (b) (4) (b) (4) the visual inspection validations to be conducted on (b) (4) DP validation runs.
- c. A discrepancy was described where all operators missed a tear in the cell sheet during visual inspection.

To address these issues, you indicated that you intend to make additional protocol changes for the visual inspection validation protocol, after which you plan to manufacture an (b) (4) to complete the (b) (4) validation run that did not pass specifications. However, with a revised visual inspection protocol, (b) (4) (b) (4) is inadequate to sufficiently validate both visual testing methods. Therefore, please utilize at (b) (4) DP lots to validate the final validation protocols for the (b) (4) visual inspection test. Please provide validation reports including the AC, with justification, description of the test methods, including test sample and sample size, discussion of results, and deviations, if any. Please also provide a justification for the changes implemented in the protocols.

6. You manufacture several media and reagents at your manufacturing facility which are used in the aseptic manufacturing of PZ DP. (b) (4) of the reagents, (b) (4)
- (b) (4)
- However, as this proposed change was made after completion of your process performance qualification (PPQ), you did not provide adequate data to support the change in reagent. Therefore, in order to replace the (b) (4) reagent with a (b) (4) alternative, please submit data to demonstrate that the reagent change does not impact the final DP.
7. You describe your control of materials in Section 3.2.S.2.3, indicating that as part of your raw material qualification program, all incoming reagents are, (b) (4)
- (b) (4) Additionally, you perform material qualification and requalification testing of the incoming materials to ensure that they meet the QC requirements. Regarding these activities, we have the following

comments:

- a. You state that (b) (4) testing is conducted on all incoming materials. However, you did not provide a list of these (b) (4) tests and did not provide information to demonstrate the testing is performed using appropriately qualified or validated assays. In order to assess the (b) (4) of your reagents, you should utilize (b) (4) tests which are appropriately qualified or validated to (b) (4) of all incoming materials. Please provide a list of all (b) (4) assays performed as part of your raw material qualification program, including a description of the assay/method, as well as the validation method protocols and reports. If using (b) (4) methods, then providing qualification protocols and reports would be sufficient.
 - b. In Table 2 of Section 3.2.S.2.3, you state that certain testing is conducted on incoming materials as part of the material qualification requirements, including minimum incoming QC tests and (b) (4) re-qualification testing performed on (b) (4) to verify the reagent's certificate of analysis (CoA). However, you did not specify the methods or assays used to perform these qualifications/re-qualifications. Per 21 CFR 211.82(d)(2), you should establish "the reliability of the supplier's analyses through appropriate validation of the supplier's test results at appropriate intervals." Therefore, you should provide additional details regarding your raw material qualification program, including the methods or assays used to perform these qualifications/re-qualifications, and how you are validating the reagents' CoA at appropriate intervals.
8. Your corrections to FDA's inspectional observations issued to you at the conclusion of the inspection conducted between February 19 and March 1, 2024, of your Cleveland, OH facility are still ongoing.

(b) (4)

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

(b) (4)

12. To support the container closure integrity of the PZ drug product, you performed a (b) (4) test per (b) (4)

The integrity of the container closure should be demonstrated to ensure sterility is maintained and to prevent contamination of the drug product (21 CFR 211.94b Drug Product Containers and Closures). The studies provided do not satisfy the requirement of ensuring the final drug product packaging is integral.

We requested additional CCIT studies through an information request (IR) on March 11, 2024. In your response to the IR submitted on March 27, 2024, you indicated that this study will be performed. However, the studies could not be provided in sufficient time to review prior to the action due date.

Please provide a CCIT study for the PZ DP primary containers per (b) (4) and include details of the test method performed with an established sensitivity of the method (b) (4)

The letter also included 6 additional CMC comments that did not rise to the level of CR deficiencies. These comments were addressed by the applicant in the CR resubmission and are summarized below:

13. We reserve comment on the proposed labeling until the application is otherwise acceptable. We may have comments when we see the proposed final labeling.
14. In your BLA submission, you provided stability data in support of a proposed shelf-life of 84 hours for the PZ DP. However, the data you provided is insufficient to support your proposed shelf-life. Specifically, you did not provide adequate data to demonstrate robust viability and sheet integrity in a sufficient number of samples. For example, you indicated that cell viability results were not available for (b) (4) (b) (4) out of (b) (4) tested DP lots at the 60 hour timepoint due to equipment failure. In addition, the AC you proposed for (b) (4) is that (b) (4) (b) (4) (b) (4) (b) (4). However, at some timepoints you tested (b) (4) (b) (4) per (b) (4). Thus, the (b) (4) acceptance criterion is not adequate because you did not demonstrate you have enough product (i.e., (b) (4)) to treat a patient from (b) (4) at all timepoints. Therefore, to demonstrate the stability of your product, we recommend the AC for the (b) (4) be revised to show that at (b) (4) (b) (4) per (b) (4) at any given timepoint remain intact. Please also refer to comment 5 above related to the validation of (b) (4).
15. In response to information requests (IRs) during the review cycle, you proposed modified AC for (b) (4) lot release testing. Please note that AC for (b) (4) lot release testing will be finalized upon review of your complete response. We reserve comment on your proposed AC until the application is otherwise acceptable.
16. In response to IRs during the review cycle, you changed criticality designations for several process parameters in (b) (4) manufacturing. Please note that criticality designations for in-process parameters and AC for in-process testing of your (b) (4) (b) (4) will be finalized upon review of your complete response.
17. In section 3.2.S.7 of your BLA submission, you propose a shelf-life of (b) (4) for your LZRSE-Col7A1 RVV. Your proposed LZRSE-Col7A1 RVV shelf-life is still under review, pending FDA receipt of additional stability data. In your resubmission, please include any stability data collected prior to the resubmission date, including but not limited to the following:
- a. Data collected from (b) (4) stability studies you proposed in amendment 33 received on March 1, 2024, which was expected to be submitted to the FDA by June 30, 2024.
 - b. Any additional long-term stability data collected according to your stability protocols STA-DS-000001 and STA-DS-000003.
18. In amendment 21 received on January 22, 2024, you noted that a report of (b) (4) testing of your (b) (4)

(b) (4) (b) (4) would be submitted to the FDA by April 30, 2024. Please include this report in your resubmission.

Post-Action Interactions

After the CR Letter was sent to the applicant on April 16, 2024, there were several additional communications between the FDA and the applicant:

- On May 14, 2024, FDA held an informal CMC meeting with the applicant to discuss identity and endotoxin testing. Specifically, the applicant proposed (b) (4) to perform identity testing and address Deficiency #4 in the CR Letter. The applicant also requested feedback regarding their endotoxin test method and FDA suggested the use of a more sensitive test (b) (4).
- On May 28, 2024, the applicant submitted an amendment to the BLA (Sequence 54), providing additional information about RCR testing requested under CR Deficiency #2. FDA indicated on June 17, 2024 in a communication to the Applicant that there were no additional comments.
- On June 17, 2024, the applicant requested an informal meeting, which was held on June 28, 2024, to discuss mycoplasma and sterility testing methods. Information discussed at the meeting was used to answer CR Deficiency #1a).
- A Type A meeting was held on August 8, 2024 to discuss rapid sterility testing for the DP (CR Deficiency #1a), mycoplasma validation testing (CR Deficiency 1b), bacterial endotoxin validation testing (CR Deficiency #1c), (b) (4) validation testing (CR Deficiency #3), identity testing (CR Deficiency #4), (b) (4) identity testing (CR Deficiency #6), (b) (4) validation testing on (b) (4) (CR Deficiency #9), (b) (4) validation testing on (b) (4) (CR Deficiency #10), and shelf-life of the DP (CR Additional Comment #14).
- On August 28, 2024, an informal CMC meeting was held with the applicant to discuss the acceptance criteria of the identity testing to address CR Deficiency #4.

Product Summary

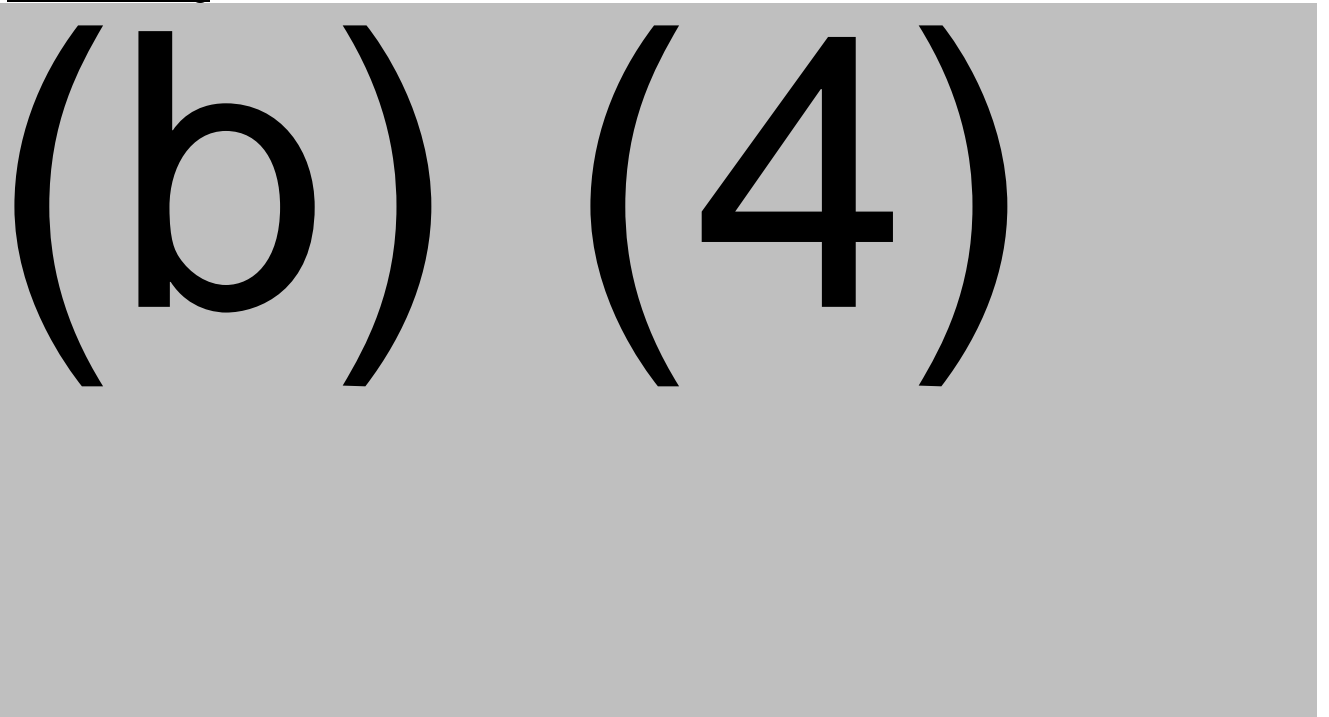
Prademagene zamikeracel (proprietary name: ZEVASKYN, also referred to throughout this review as PZ) is a biologic/device combination product intended for treatment of Recessive Dystrophic Epidermolysis Bullosa (RDEB). The drug product consists of autologous cell sheets which have been genetically modified via *ex vivo* transduction with a replication-incompetent gamma retroviral vector (RVV) carrying a human collagen VII A1 (COL7A1) transgene. Transgene integration into the host cell genome allows for constitutive COL7A1 expression by transduced cells. Three COL7A1 alpha chains trimerize to form a collagen VII fibril, which facilitates wound healing in patients who receive PZ. PZ is manufactured starting with an autologous skin biopsy. The

autologous cells are transduced with the LZRSE-Col7A1 RVV and then grown into ~40 cm² sheets. Each sheet is secured to sterile petrolatum gauze using sterile ligation clips. Up to (b) (4) PZ sheets may be manufactured per autologous skin biopsy, of which up to 12 may be surgically administered. PZ sheets are surgically applied to the wound beds using absorbable sutures, and covered with additional wound dressings and gauze. PZ sheets remain on the wound for up to 3 weeks, after which the petrolatum gauze and ligation clips dissociate, leaving behind the cell sheet that integrates with surrounding tissue. Based on clinical data, a single PZ treatment has provided in some cases provide long-term (>5 years) wound healing, including decreased pain and itching.

Therapeutic Rationale

People living with RDEB do not express collagen VII in skin cells due to mutations in the *COL7A1* gene. These mutations cause dysfunctional collagen VII synthesis or anchoring, which in turn leads to poor epidermal-dermal adherence and disease pathology. Clinically, RDEB patients present with slow-healing and often repeated scarring in epithelial wounds. RDEB wounds are associated with chronic inflammation and increased risk for squamous cell carcinoma. Transduced PZ cells secrete soluble trimeric collagen VII fibrils which function to anchor the epidermis to the dermis to facilitate wound healing.

Manufacturing



The PZ manufacturing process begins with obtaining autologous skin biopsies from RDEB patients. Chain of identity/chain of custody (COI/COC) procedures are established once the biopsy material has been collected. After receipt at the manufacturing site, the biopsies are (b) (4)

(b) (4)

(b) (4)

PZ DP assembly begins. The assembly process involves enzymatically lifting the cell sheets from the plates and attaching them to a petrolatum gauze with titanium ligation clips. The assembled PZ DP is then visually inspected for holes or tears before being packaged in a custom-made clamshell container, which is placed with excipient media inside a sterile (b) (4) bag, which acts as the sterile barrier packaging. The DP can be stored for up to 84 hours at room temperature. After release of the assembled PZ DP, they are hand delivered to the clinical site for administration to the skin wounds of RDEB patients.

PZ DP is manufactured in a closed system from raw materials and reagents that meet acceptable quality standards. Human and animal derived raw materials are appropriately controlled to ensure absence of microbial and viral contaminants. Samples for lot release testing are collected at the following stages during manufacture to accommodate the testing limitations on the assembled DP and its short shelf-life: samples for potency testing are taken after viral transduction; (b) (4) (b) (4) (b) (4) (b) (4) the mycoplasma testing sample is taken on the day of harvest; final product endotoxin and (b) (4) test samples are taken on the day of harvest of P1 packaging (b) (4) bag containing the a clamshell tray with the final DP); final product rapid sterility testing and (b) (4) sterility testing is taken from P1 packaging (b) (4) after harvest (with results available after administration and (b) (4) after release); visual testing and (b) (4) are conducted on a quality control (QC) DP sample prior to release and again on each DP (i.e., cell sheet) at the clinical site prior to administration.

The device constituents of this combination product consist of the petrolatum gauze, petroleum jelly, and titanium ligation clips. These devices are 510(k)-cleared (ligation clips) or preamendment devices (gauze and petroleum jelly). The ligation clips (cleared under (b) (4)) are utilized per the general cleared indication for use ("Ligating Clips are intended for use in procedures involving vessels or anatomic structures for which the surgeon determines ligating clips are the best choice. Surgeons should select the size, type and material of the clip based upon their experience, judgment and needs."). Adequate testing, design controls, and clinical experience provide appropriate assurance of their safety and effectiveness as part of the PZ DP combination product.

PZ DP Stability

The applicant submitted transport and stability studies that support the 84-hour shelf-life claim.

11. RECOMMENDATION

After review of the CR resubmission and information provided through interactive review, we recommend approving this BLA for prademagene zamikeracel for the treatment of Recessive Dystrophic Epidermolysis Bullosa (RDEB) with two (2) CMC post-marketing commitments (PMCs).

I. Post-Marketing Commitments:

1. Abeona Therapeutics commits to re-evaluate the acceptance criteria (AC) for release testing of the LZRSE-Col7A1 retroviral vector based on manufacturing experience when data from (b) (4) total commercial batches are available and revise the AC, if appropriate. AC will be re-evaluated for the (b) (4)

(b) (4) release tests. The re-evaluation report will be submitted as a “PMC Submission-Final Study Report.”

Report submission date: June 30, 2027.

2. Abeona Therapeutics commits to providing the results of an updated (b) (4) study in a Final Study Report Submission by July 29, 2025.

Final Study Report Submission: October 29, 2025

12. SIGNATURE BLOCK

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Bao-Ngoc Nguyen, Ph.D., CMC Reviewer, Chair OTP/OCTHT/DCT2/TEB1	Concur	
Joshua Kufera, Ph.D., CMC Reviewer, OTP/OGT/DGT2/GTB5	Concur	
Mo Liu, Ph.D., CMC Reviewer OTP /OGT/DGT2/GTIB	Concur	
Ileana Marrero-Berrios, Ph.D., CMC Reviewer, OTP/OCTHT/DCT2/TEB2	Concur	
Carolina Panico, M.D., Ph.D., CMC Reviewer OTP/OCTHT/DCT1/TEB1	Concur	
Zehra Tosun, Ph.D., Branch Chief OTP/OCTHT/DCT2/TEB1	Concur	
Graeme Price, Ph.D. Branch Chief OTP/OGT/DGT2/GTB5	Concur	
Laura Ricles, Ph.D., Division Director OTP/OCTHT/DCT2	Concur	
Kimberly Schultz, Ph.D., Division Director OTP/OGT/DGT2	Concur	
Denise Gavin, Ph.D., Office Director OTP/OGT	Concur	
Steven Oh, Ph.D., Deputy Office Director OTP/OCTHT	Concur	
Rachael Anatol, Ph.D., Office Director (acting) OTP/OCTHT	Concur	

**Review of CTD
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RESUBMISSION REVIEW

Complete Response (CR) Deficiencies

Reviewer Comment: The following comments were communicated to the applicant in a Complete Response (CR) Letter on April 15, 2024. The applicant provided a complete response to the deficiencies on October 29, 2024 (in *italics*) and reviewer assessments are provided in blue below.

Deficiency 1

You did not demonstrate adequate suitability of the microbiological test methods listed below for your final drug product, prademagene zamikeracel (PZ).

- a. Sterility Test Method: You proposed to conduct (b) (4) (b) (4) (b) (4) well as rapid sterility testing (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. Without adequate validation of the sterility methods, the sterility of your PZ DP is not assured. To demonstrate that each method can reproducibly detect appropriate levels of microbial contamination, you should provide a report that includes the (b) (4) (b) (4) (b) (4) as well as acceptance criteria (AC) with justification, for at least (b) (4) DP lots. The impact of any deviations that occurred during testing should be described in the report. In addition, method validation should be performed in accordance with (b) (4). To demonstrate your rapid sterility test methods provide assurance of effectiveness equal to or greater than the assurances provided by the (b) (4) sterility test method under the actual condition of use, you should provide data from comparability studies.

Summary of Applicant's Response: *Outlined below are significant interactions regarding rapid sterility testing, leading up to resubmission:*

- In the Type A Meeting Briefing Book submitted on July 8, 2024, Abeona provided the Agency with the summary of the study protocols (MTH-VAL-PRO-000033 and MTHVAL- PRO-000013) in which the rapid sterility test method (MTH-000094) is performed for the detection and numeration of viable microorganisms for the (b) (4) method and the (b) (4) release rapid sterility test method.*
- In the preliminary response received from the Agency on August 6, 2024, the Agency provided the recommendations in Abeona's approach to rapid sterility. CRL Item 1a, rapid sterility, was discussed at the Type A Meeting held on August 8, 2024.*

In this resubmission, Abeona therapeutics is addressing CRL Item 1a, rapid sterility, by providing the Agency with the following updated Module 3 sections, including the (b) (4) rapid sterility test methods and validation information.

To address the Agency's request to include comparability study results between the rapid sterility tests and (b) (4) Abeona is providing this information within the respective method validations. These method validation results and the supporting reports referenced in these sections demonstrate that the rapid sterility methods are suitable for their intended use and that they provide assurance of effectiveness equal to the assurances provided by the (b) (4) sterility test method. The information provided in this resubmission fully addresses all requests related to CRL Item 1a, rapid sterility testing, received to date.

Reviewer Comment (BNN): The method suitability tests were performed and compliant with (b) (4) and (b) (4) and the test results indicate there is no product inhibition of microorganism growth, thus indicating the rapid (b) (4) test method is appropriate under the actual conditions of use. Please refer to DBSQC memo for additional information.

- b. Mycoplasma Test Method: The data you provided does not demonstrate the suitability of your mycoplasma test method because assay specificity and equivalency to (b) (4) methods were not adequately demonstrated. To complete demonstration of assay specificity, you should conduct a specificity study for the (b) (4) method using your (b) (4) To demonstrate comparability/equivalency of the (b) (4) and the proposed (b) (4) system, data from a comparability study must be provided to assure that the sensitivity of the (b) (4) method is equal to or greater than the (b) (4) method (b) (4) under the actual condition of use.

Summary of Applicant's Response: For context, interactions leading up to resubmission are detailed below:

- Reference is made to Agency responses received on June 28, 2024, and July 1, 2024, where FDA rescinded the request to have a specificity study submitted for the (b) (4) method using the (b) (4)
- In the Type A Meeting Briefing Book submitted on July 8, 2024, Abeona provided the Agency with the summary of the comparability study protocol between the Abeona (b) (4) method and mycoplasma (b) (4) (b) (4) method per (b) (4)

- *In the preliminary response received from the Agency on August 6, 2024, the Agency provided recommendations in Abeona's approach to mycoplasma testing.*
- *CRL Item 1b, mycoplasma testing, was discussed at the Type A Meeting held on August 8, 2024.*

In this resubmission, Abeona therapeutics is addressing CRL Item 1b, mycoplasma testing, by providing the Agency with the revised comparability study results using 3 lots. In addition, Abeona would like to clarify that the sample type utilized for the comparability study was consistent with the typical Drug Product release matrix for MTH-000018. The sample was prepared from (b) (4)

An updated Module 3, Section 3.2.P.5.3 is being submitted as part of the resubmission to address CRL Item 1b. The updated section will include information demonstrating the comparability of the (b) (4) and the proposed (b) (4) (b) (4) system. As such, Abeona considers all requests related to CRL Item 1b, mycoplasma comparability testing, to be fully addressed. No information related to specificity is included, since the Agency rescinded the request for a specificity study for the (b) (4) method in a follow-up correspondence received on July 1, 2024.

Reviewer Comment (BNN): The method validation tests were performed and compliant with (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. Please refer to DBSQC memo for additional information.

- c. Bacterial Endotoxin Test: The test sample dilution (b) (4) you proposed for determining endotoxin concentration is not acceptable because it is at the limit of the test, i.e., the (b) (4). Testing at the (b) (4) may detect endotoxin in the sample at the specification limit; however, testing at the (b) (4) should only be performed if dilutions (b) (4) do not provide valid test results. To identify the appropriate sample dilution to use in the endotoxin test, you need to provide data from a test for interfering factors that show positive product control recoveries for a series of test dilutions (b) (4). As per (b) (4) the dilution equation must include the media volume. You should use this information to identify the dilution that provides optimal recovery and use as the sample dilution in release assays.

Summary of Applicant's Response: For context, the interactions leading up to resubmission are detailed below:

- An informal meeting was held on May 14, 2024, to discuss CRL Item 1c and CRL Item 4. As a result of the meeting, The Agency and Abeona aligned on the following proposal for CRL Item 1c:
 - Regarding CRL Item 1c – Bacterial Endotoxin, The Agency suggested using a more sensitive (b) (4) to test the excipient media.
- In the Type A Meeting Briefing Book submitted on July 8, 2024, Abeona provided the Agency with a justification of our revised specification in alignment with (b) (4) and FDA feedback utilizing a more sensitive (b) (4)
- In the preliminary response received from the Agency on August 6, 2024, the Agency agreed with the updated test parameters and acceptance criteria submitted in the Type A Meeting Briefing Book, which includes the use of a more sensitive (b) (4) to test the excipient media that was requested at the May 14, 2024 CMC Informal Meeting.
- To fully address CRL Item 1c, bacterial endotoxin, Abeona is providing the updated MTH- 000017 (endotoxin test) and its validation report, MTH-VAL-RPT-000022, and updated the following Module 3 BLA sections:
 - Module 3, Section 3.2.P.5.1 Specifications
 - Module 3, Section 3.2.P.5.2 Analytical Procedures
 - Module 3, Section 3.2.P.5.3 Validation of Analytical Procedures
 - Module 3, Section 3.2.P.5.6 Justification of Specifications

Reviewer Comment (BNN): The method suitability test was performed and compliant with (b) (4). The test results indicate the (b) (4) test method is appropriate under the actual condition of use. Please refer to DBSQC memo for additional information.

- d. Supporting (b) (4) Studies: You did not provide adequate evidence of endotoxin or microorganism recovery in media that has been in (b) (4) with DP after assembly of the P1 packaging (i.e., clamshell inside (b) (4) bag). Data should be provided for (b) (4) studies for all assays that will utilize this media as a test sample, including endotoxin, (b) (4) and all sterility tests. The (b) (4) (b) (4) tests should be designed to demonstrate that the proposed (b) (4) time allows for adequate detection and recovery of appropriate levels of contaminants in the DP and use an (b) (4) volume that is appropriate for the intended test method. The (b) (4) studies for bacterial endotoxin must comply with (b) (4) for bacterial endotoxin (b) (4) volume and (b) (4) process. A full test report including a description of the test methods, acceptance criteria (AC) with justification, and sensitivity of the evaluated assays with the media samples, is needed to demonstrate your endotoxin, (b) (4) and rapid sterility testing methods are appropriate for their intended use.

Summary of Applicant's Response: Abeona is providing the Agency with the endotoxin hold-time study report (STUDY-RPT-000013), gram stain hold-time study report (STUDY-RPT-000012), rapid-sterility hold time study report (STUDY-RPT-000014), and the updated Module 3, Section 3.2.P.5.3 and Module 3, Section 3.2.P.3.3 to fully address CRL Item 1d, regarding the drug product (b) (4) studies. These documents demonstrate that the now proposed (b) (4) time of (b) (4) (b) (4) (submitted with BLA 125807, Sequence 0037, as supplemental response to FDA IR #26, CMC IR #11 Question 4) allows for adequate detection and recovery of appropriate levels of contaminants in the DP and use an (b) (4) volume that is appropriate for the intended test method.

Reviewer Comment (BNN): During review of the OS BLA, there were extensive discussions with the applicant regarding their DP sterility assurance plan. Due to the short shelf-life of the product (84 hours), the final product must be conditionally released prior to the availability of (b) (4) and endotoxin results. Therefore, several of the test samples used for sterility testing (rapid sterility) are taken from (b) (4) final packaging steps that have had minimal contact with the DP. The applicant's original sterility testing plan for the final DP included media samples taken from the P1 packaging after (b) (4) of (b) (4) with the DP. During formal and informal meetings with the applicant following issuance of the CR letter, the hold time was eventually revised to (b) (4) to allow to (b) (4) time between the excipient media and the DP. However, to demonstrate that the sterility assays could adequately detect any contaminants in samples with such minimal DP contact, the applicant was asked to perform (b) (4) (b) (4) studies. In the resubmission, the applicant provided hold-time studies for the proposed rapid endotoxin assay, rapid sterility assay, and (b) (4) testing. Please refer to the DBSQC memo for assessment of these (b) (4) hold studies and their protocols.

As part of the endotoxin (b) (4)

(b) (4)

This change is acceptable.

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

(b) (4)

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

(b) (4)

Deficiency 4

You proposed to conduct additional identity testing on your final DP as part of lot release testing to assess and confirm the cell populations in your DP. You propose to utilize a (b) (4) to “detect the presence of keratinocytes” in your product. However, you did not provide an adequate method description, protocol, or validation report for your proposed identity assay. In order to ensure the identity and purity of your final DP, a validated identity assay is necessary. Please submit a method

validation report demonstrating that your proposed method can adequately detect and identify the cell populations present in your DP. The report should include the AC, with justification, description of the test method, including test sample and sample size, discussion of results, and deviations, if any.

Summary of Applicant's Response: *In this resubmission, Abeona Therapeutics is addressing CRL Item 4, identity testing, by providing the Agency with MTH-000093 (Keratinocyte Identity Test for EB-101 by (b) (4) and its validation report (MTH-VAL-RPT-000017), which demonstrate that the method is suitable for its intended use, and characterization reports STUDY-RPT-000005 and STUDY-RPT-000006, which demonstrate that the (b) (4) sampling point is representative of the drug product stage.*

Briefly, the applicant provided protocol MTH-000093, which describes the method to (b) (4)

(b) (4)

(b) (4)

(b) (4)

*The applicant also provided a validation report of the identity testing assay under MTH-VAL-RPT-17. The report provided validation of the assay with regard to linearity, precision, specificity, robustness sample. **Table 4** summarizes the validation parameters assessed, along with the testing design, acceptance criteria, and results.*

(b) (4)

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

(b) (4)

Reviewer Comment (BNN): During review of the validation of the identity assay (described in validation report MTH-VAL-RPT-000017), additional information was requested from the applicant to explain their method to calculate the accuracy of the assay, specifically how the expected (b) (4) values and observed (b) (4) values were determined to calculate the accuracy parameter (theoretical – observed). Briefly, the accuracy is calculated using (b) (4)

Adequate explanations with sample calculations were provided in response to IR #41 (CMC IR #14) under Amendment 60.

Additionally, in the original validation report MTH-VAL-RPT-000017, the applicant indicated that robustness testing was incomplete due to unavailability of a (b) (4) (b) (4)

but that the testing would be updated. Upon request of the additional data as part of IR #41 (CMC #14), the applicant indicated in

Amendment 60, that an updated validation report would be provided by February 13, 2025. The updated validation report was provided under Amendment 63 with additional robustness data included. The new data included a (b) (4)

(b) (4) to assess the robustness of the assay. The applicant confirmed in Amendment 58, received March 27, 2025, that the alternate vendor was adequately qualified and that sufficient studies were completed to show that the (b) (4) (b) (4) did not change the validation results. The new data supports the validation of robustness of the identity assay and is acceptable.

There are no additional concerns with the validation report for the identity assay of the DP.

Furthermore, the applicant provided a characterization study (DEV-0000068) to demonstrate that the (b) (4) used for the identity assay is representative of the cells in the final DP. The applicant justified that due to the short shelf-life of the DP, identity testing could not be performed on the final product, but instead would be performed on (b) (4) In the characterization study, the (b) (4)

(b) (4)

Reviewer Comment (BNN): The applicant developed and validated an identity assay to demonstrate that the majority of the cells (b) (4) in the final DP PZ are keratinocytes. The applicant provided adequate characterization studies (STUDY-RPT-000005, STUDY-RPT-000006, Study DEV-000068 provided in 3.2.S.3.1 Elucidation of Structure and Other Characteristics) (b) (4)

(b) (4) to demonstrate that the sampling point (b) (4) suspension of transduced cells) is representative of the final DP (cells formed into a cell sheet). While this identity assay is adequate to demonstrate that there are keratinocytes in the final DP, the acceptance criteria (b) (4) indicates that there may be other cells present as well (not characterized by the applicant). Therefore, as part of the product labeling, the product is not described as a keratinocyte sheet but instead as a cellular sheet. The applicant's response to CR Deficiency #4 is adequate. This assay, along with robust chain of identity (COI) processes in place, the applicant is providing adequate assurance of identity of the DP.

Deficiency 5

As part of the final product lot release testing, you conduct (b) (4) visual inspection on the DP. You indicate that these (b) (4) methods are qualified and operators are adequately trained to conduct these visual tests. However, you did not provide adequate validation reports for these methods to demonstrate that these assays can be consistently and accurately performed. Specifically, we identified the following issues in the method validation reports provided in Amendment 45, received on March 28, 2024:

- a. (b) (4) identified a contaminant (hemoclip) in the DP during visual inspection that the other (b) (4) operators missed during the (b) (4) validation run. The discrepancy described resulted in out-of-specification results of the visual inspection.
- b. (b) (4) that identified the contaminant (hemoclip) did not perform (b) (4) as the (b) (4) tests were performed concurrently. This resulted in (b) (4) the visual inspection validations to be conducted on only (b) (4) DP validation runs.

A discrepancy was described where all operators missed a tear in the cell sheet during visual inspection.

To address these issues, you indicated that you intend to make additional protocol changes for the visual inspection validation protocol, after which you plan to manufacture (b) (4) to complete the (b) (4) validation run that did not pass specifications. However, with a revised visual inspection protocol, (b) (4) (b) (4) is inadequate to sufficiently validate both visual testing methods. Therefore, please utilize (b) (4) DP lots to validate the final validation protocols for the (b) (4) (b) (4) visual inspection test. Please provide validation reports including the AC, with justification, description of the test methods, including test sample and sample size, discussion of results, and deviations, if any. Please also provide a justification for the changes implemented in the protocols.

Summary of Applicant's Response: *To address CRL Item 5, Abeona is providing MTH-VAL-RPT-000006 (validation report for visual inspection) and MTH-VAL-RPT-000007 (validation report for (b) (4) that covers additional validation on (b) (4) lots for the (b) (4). The validation reports for (b) (4) (b) (4) visual inspection testing on DP for final product release aim to demonstrate that these assays are consistent and accurately performed. Module 3, Section 3.2.P.5.3 has been updated to align with the reports to address this request. In addition, the justifications for change implemented in the protocols can be found in MTHVAL-PRO-000005 and MTH-VAL-PRO-000004. The information provided in this resubmission fully addresses all requests related to CRL Item 5 on (b) (4) (b) (4) visual inspection for final product lot release testing.*

Reviewer Comment (CP): The Applicant had provided validation reports for (b) (4) and visual inspection in the original submission; however, major discrepancies were identified that resulted in the validations not being adequate to support the reliability of

(b) (4) (b) (4) visual inspection for release testing and stability (please refer to Deficiency 5 for details on the discrepancies). In the resubmission, the Applicant provided results from (b) (4) additional validation runs for the (b) (4) the visual inspection using the same acceptance criteria for precision and robustness. The training steps for (b) (4) Visual Inspection were modified to include a (b) (4) step by a coordinator to ensure that all testing steps were performed consistently, including more consistent and precise preparation of the positive controls. The validation protocols included precision and robustness assessments of the testing that included (b) (4)

(b) (4) No major or significant deviations were reported for the validation runs. The validation results were compared against acceptance criteria described in the validation protocol and reported as passed. CP found the results included in the MTH-VAL-RPT-000006 (visual inspection) and MTH-VAL-RPT-000007 (b) (4) adequate to support the consistent and reliable use of (b) (4) visual inspection for DP stability and release testing. This information adequately addresses CR Deficiency #5.

Deficiency 6

You manufacture several media and reagents at your manufacturing facility which are used in the aseptic manufacturing of PZ DP. (b) (4) of the reagents, (b) (4)

(b) (4) However, as this proposed change was made after completion of your process performance qualification (PPQ), you did not provide adequate data to support the change in reagent. Therefore, in order to replace the (b) (4) reagent with a (b) (4) alternative, please submit data to demonstrate that the reagent change does not impact the final DP.

Applicant Response: On July 8, 2024, Abeona submitted a Type A Meeting package detailing the protocol implementation and results for the (b) (4) validation, and testing plans for (b) (4) for their (b) (4). The Agency provided feedback on August 6, 2024, agreeing that the (b) (4) validation and testing activities for (b) (4) will be acceptable. However, to assess the adequacy of the Applicant's proposal, the totality of the data for these tests had to be submitted to the BLA for review. In the Complete Response to Letter Comments submitted as STN 125807/0.57 (eCTD #0058), the Applicant provided the (b) (4) validation protocol and results. Regarding (b) (4) lot release testing includes (b) (4). According to the Applicant, their data demonstrated that the reagent is adequately evaluated for (b) (4) (b) (4)

(b) (4)

(b) (4)

(b) (4)

Deficiency 7

You describe your control of materials in Section 3.2.S.2.3, indicating that as part of your raw material qualification program, all incoming reagents are, (b) (4) (b) (4). Additionally, you perform material qualification and requalification testing of the incoming materials to ensure that they meet the QC requirements. Regarding these activities, we have the following comments:

- a. You state that (b) (4) testing is conducted on all incoming materials. However, you did not provide a list of these (b) (4) tests and did not provide information to demonstrate the testing is performed using appropriately qualified or validated assays. In order to assess the (b) (4) of your reagents, you should utilize (b) (4) tests which are appropriately qualified or validated to (b) (4) of all incoming materials. Please provide a list of all (b) (4) assays performed as part of your raw material qualification program, including a description of the assay/method, as well as the validation method protocols and reports. If using (b) (4) methods, then providing qualification protocols and reports would be sufficient.
- b. In Table 2 of Section 3.2.S.2.3, you state that certain testing is conducted on incoming materials as part of the material qualification requirements, including minimum incoming QC tests and (b) (4) re-qualification testing performed (b) (4) to verify the reagent's certificate of analysis (CoA). However, you did not specify the methods or assays used to perform these qualifications/re-qualifications. Per 21 CFR 211.82(d)(2), you should establish "the reliability of the supplier's analyses through appropriate validation of the supplier's test results at appropriate intervals." Therefore, you should provide additional details regarding your raw material qualification program, including the methods or assays used to perform these qualifications/re-qualifications, and how you are validating the reagents' CoA at appropriate intervals.

Summary of Applicant's Response: RVV Module 3, Section 3.2.S.2.3 and EB-101 Module 3, Section 3.2.S.2.3 have been updated to include the following:

- A list of all qualified/validated (b) (4) test methods used to (b) (4) of incoming raw materials.
- A list of (b) (4) test methods' qualification/validation reports.

Reviewer Comment: The information provided below related to the manufacturing reagents used for the LZRSE-Col7A1 RVV was reviewed by JTK and found to be acceptable.

(b) (4)

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

(b) (4)

Deficiency 8

Your corrections to FDA's inspectional observations issued to you at the conclusion of the inspection conducted between February 19 and March 1, 2024, of your Cleveland, OH facility are still ongoing.

Summary of Applicant's Response: *At the conclusion of the Pre-Licensing Inspection (PLI) conducted between February 19 and March 1, 2024, at the Cleveland, OH facility, Abeona was issued five observations in the FDA Form 483. Abeona responded to these observations in compliance with the 15 business day requirement (form-fda-483-bla-1258070-abeona-pli-responses) on March 15, 2024, contained within BLA 125807, Sequence 0037.*

On March 27, 2024, Abeona received an IR (DMPQ #6) that included Agency feedback to Abeona's response to the PLI items submitted on March 15, 2024. Subsequently, Abeona submitted BLA 125807, Sequence 0048 on April 3, 2024, that included responses to DMPQ IR#6 (responses-to-fda-questions-fda-ir-35-dmpq-ir-6-response-docu) to address the Agency feedback.

In this BLA resubmission, Abeona is providing an update on the resolution of the FDA Form 483 observations in alignment with the Agency's feedback.

Reviewer Comment (BNN): The applicant provided a summary of their responses to the FDA Form 483 items. Their acceptability was assessed as part of DMPQ's 483 Response Memo and found adequate. Refer to DMPQ's 483 Response Memo and Establishment Inspection Report (EIR) for details.

(b) (4)

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

(b) (4)

Deficiency 12

To support the container closure integrity of the PZ drug product, you performed a (b) (4) (b) (4) test per (b) (4)

The integrity of the container closure should be demonstrated to ensure sterility is maintained and to prevent contamination of the drug product (21 CFR 211.94b Drug Product Containers and Closures). The studies provided do not satisfy the requirement of ensuring the final drug product packaging is integral.

We requested additional CCIT studies through an information request (IR) on March 11, 2024. In your response to the IR submitted on March 27, 2024, you indicated that this study will be performed. However, the studies could not be provided in sufficient time to review prior to the action due date.

Please provide a CCIT study for the PZ DP primary containers per (b) (4) and include details of the test method performed with an established sensitivity of the method (i.e., (b) (4))

Summary of Applicant's Response: To address CRL Item 12, Abeona is providing the VAL-PQ-EXT-000003 report received from (b) (4) which includes the results for the CCIT method validation for PZ drug product primary containers. Module 3, Section 3.2.P.7 has been updated to include an overview of the drug product CCIT study report.

The results of VAL-PQ-EXT-000003 confirm that the (b) (4) test is effective at (b) (4) in the P1 pouch and support the container closure integrity of the PZ DP primary packaging container. With this response, Abeona considers CRL Item 12 to be fully addressed.

[Reviewer Comment \(OM\):](#) PZ DP CCIT is acceptable and CR item 12 has been adequately addressed. Please refer to the DMPQ review memo for detailed evaluation of the firm's response.

Additional Comments:

Additional Comment 13

We reserve comment on the proposed labeling until the application is otherwise acceptable. We may have comments when we see the proposed final labeling.

Summary of Applicant's Response: Abeona acknowledges that the Agency will provide comments on the labeling during the review of the BLA resubmission. Abeona would like to take the opportunity to provide the latest proposed labeling information in the resubmission Module 1, Section 1.14.1 to include the most recent labeling updates.

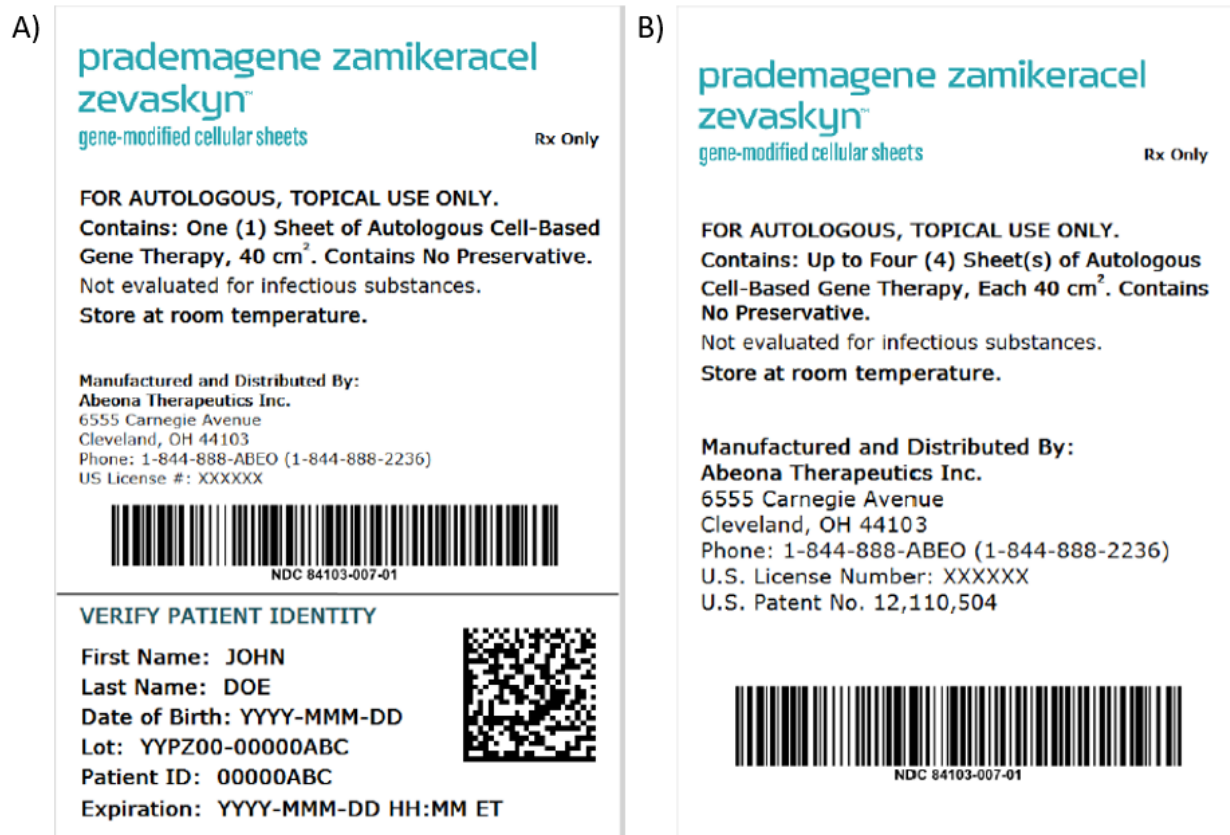
As part of the CR resubmission, the applicant provided updated draft carton and container labels in Module 1.14.1.1 "Draft Carton and Container Labels" for the 4 layers of packaging. Specifically, the 4 different packaging levels consist of the following:

- P1: Primary packaging consisting of the clamshell case (lid and tray) and (b) (4) bag.
- P2: Secondary packaging consisting of a tray and lid with an absorbing pad at the bottom.
- P3: Tertiary packaging consisting of a tray and lid with an absorbing pad at the bottom.
- P4: Transport container consisting of a single-use, one way transportation container with a calibrated data logger.

Figure 2. shows the final carton labels for P1-P3 and P4 were submitted under Amendment 69 on March 12, 2025 after several rounds of IRs (IR #47, Regulatory IR

#6, Amendment 64; IR #52, CMC IR #17, Amendment 69; CMC IR #21 Amendment 79). The applicant made necessary revisions to the carton label to follow 21 CFR 610.62 with regard to name placement, prominence, layout, and crowding. A) Depiction of Carton label for P1-P4 packaging. B) Depiction of outermost packaging carton label.

Figure 2 - Carton Label for P1-P3 and P4 Packaging



Reviewer Comment (BNN): After several IR communications, the applicant revised the carton labels adequately per 21 CFR 610.62.

During review of the USPI, additional discussions with the clinical review team resulted in an IR (IR#54, CMC IR#18), in which the applicant clarified the administration of the final DP in the operating room. Specifically, the applicant clarified that the final DP is not cut to size or altered in shape prior to administration to the wounds of patients. Therefore, the entire 40 cm² sheet is sutured to the wounds and no additional instructions are needed in the USPI to provide cutting or sizing instructions. The DP is constructed by placing the cellular sheet onto a petrolatum gauze, which are held together using titanium clips. The clarification regarding the handling and administration of the final DP was important because in the event of any sizing or cutting of the DP, the applicant would need to demonstrate additional integrity of the product after the removal of the clips as it is handled and sutured to the wounds. However, no additional testing is needed because the DP is not further manipulated prior to administration.

Additional Comment 14

In your BLA submission, you provided stability data in support of a proposed shelf-life of 84 hours for the PZ DP. However, the data you provided is insufficient to support your proposed shelf-life. Specifically, you did not provide adequate data to demonstrate robust viability and sheet integrity in a sufficient number of samples. For example, you indicated that cell viability results were not available for (b) (4) out of (b) (4) tested DP lots at the 60-hour timepoint due to equipment failure. In addition, the AC you proposed for (b) (4) is that (b) (4). However, at some timepoints you tested less than (b) (4) per (b) (4). Thus, the (b) (4) acceptance criterion is not adequate because you did not demonstrate you have enough product (b) (4) to treat a patient from a (b) (4) at all timepoints. Therefore, to demonstrate the stability of your product, we recommend the AC for the (b) (4) be revised to show that (b) (4) per (b) (4) at any given timepoint remain intact. Please also refer to comment 5 above related to the validation of (b) (4).

Summary of Applicant's Response: Abeona thanks the Agency for guidance and alignment received to date on CRL Item 14, DP stability data. For context, the related Sponsor-Agency interactions for this topic leading up to resubmission are detailed below:

- In the Type A Meeting Briefing Book submitted on July 8, 2024, Abeona provided the Agency with the justification of extending PZ shelf-life from 36-hours to 84-hours.
- In the preliminary response received from the Agency on August 6, 2024, the Agency agreed with the:
- revised (b) (4) acceptance criteria (AC) for time points where at (b) (4) were tested (i.e., 60-hour and 84-hour).
- Abeona's proposal not to apply the new AC to time points (i.e., 0-hour and 36-hour) where (b) (4) was tested.
- Abeona's justification for the lack of viability data at the 60-hour timepoint for lot (b) (4) due to equipment failure. The linear regression analysis data showing that viability does not decrease to (b) (4) over 84 hours is acceptable to support viability is maintained for the duration of the 84-hour shelf life for the DP.

The stability protocol has been updated and the AC for (b) (4) has been revised to require that at (b) (4) per (b) (4) at any given timepoint must remain intact to support the product stability at that timepoint. Please refer to Module 3, section 3.2.P.8.1. In this resubmission Abeona is also supplying revised method validation reports for (b) (4). (b) (4) visual inspection in response to CRL Item 5. Abeona believes that these validations support the testing performed as part of the stability extension study. The validations also included stability sheets that were held for a minimum of 84 hours, then tested during validation, and all sheets passed both Visual Inspection and (b) (4). (b) (4) at these time points further supporting our drug product stability shelf-life extension. With the information submitted with this response, Abeona considers the

all requests related to CRL Item 14, DP stability data, to be fully addressed and that the DP stability data support an 84-hour shelf-life.

Reviewer Comment (CP): In the resubmission, the stability protocol has been revised to include the change in the acceptance criteria (AC) for the (b) (4) from (b) (4) of the cell sheets tested to at (b) (4) tested (sufficient to treat a patient (b) (4) (b) (4) at all time points. In addition, the Applicant proposed that the linear regression analysis data (provided in the original submission) were sufficient to support the stability of the product. The analysis is based on the available stability data showing that viability does not decrease to (b) (4) over 84 hours for all (b) (4) tested. The Applicant regression analysis and justification are acceptable to support viability is maintained for the duration of the 84-hour shelf life for the DP despite the lack of viability data at the 60-hour timepoint for lot (b) (4) due to equipment failure used for viability. The revision of the (b) (4) acceptance criteria and the justification for lack of viability data for lot (b) (4) support the proposed DP shelf-life of 84 hours. Please also refer to CR Deficiency #5 above. The Applicant's response is adequate to resolve CR item #14.

Additional Comment 15

In response to information requests (IRs) during the review cycle, you proposed modified AC for (b) (4) lot release testing. Please note that AC for (b) (4) lot release testing will be finalized upon review of your complete response. We reserve comment on your proposed AC until the application is otherwise acceptable.

Summary of Applicant's Response:

Abeona acknowledges FDA's comment on acceptance criteria (AC) for (b) (4) (b) (4) lot release testing. The revised AC for (b) (4) lot release testing based on (b) (4) (b) (4) and the justification can be found in the following sections:

- *RVV Module 3, Section 3.2.S.4.1 Specifications*
- *RVV Module 3, section 3.2.S.4.5 Justification of Specification*

(b) (4)

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

(b) (4)

Additional Comment 16

In response to IRs during the review cycle, you changed criticality designations for several process parameters in (b) (4) manufacturing. Please note that criticality designations for in-process parameters and AC for in-process testing of your (b) (4) will be finalized upon review of your complete response.

Summary of Applicant's Response: Abeona acknowledges FDA's comment on finalizing criticality designations for in-process parameters and acceptance criteria (AC) for in-process testing of (b) (4) upon review of the complete response.

(b) (4) **Controls of Critical Steps and Intermediates**

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

(b) (4)

Additional Comment 17

In section 3.2.S.7 of your BLA submission, you propose a shelf-life of (b) (4) for your LZRSE-Col7A1 RVV. Your proposed LZRSE-Col7A1 RVV shelf-life is still under review, pending FDA receipt of additional stability data. In your resubmission, please include any stability data collected prior to the resubmission date, including but not limited to the following:

- a. Data collected from (b) (4) stability studies you proposed in amendment 33 received on March 1, 2024, which was expected to be submitted to the FDA by June 30, 2024.
- b. Any additional long-term stability data collected according to your stability protocols STA-DS-000001 and STA-DS-000003.

Summary of Applicant's Response: All available RVV stability data including data from (b) (4) study are provided in Module 3, Section 3.2.S.7.3, Stability Data.

(b) (4)

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

Additional Changes Reviewed

Reviewer Comment: All additional information reviewed under the resubmission of this BLA that was not in direct response to the CR Letter deficiencies is outlined below.

LZRSE-Col7A1 RVV

Updates to LZRSE-Col7A1 RVV manufacture in the CR or through IRs during the CR review cycle are documented below. These include changes to manufacture and testing sites and information regarding (b) (4)

(b) (4)

3.2.S.2.1 Manufacturer(s)

*LZRSE-Col7A1 RVV manufacturers and testing facilities were updated in the CR. Changes include the addition of the (b) (4) and the addition of CCIT at the (b) (4) site. The updated manufacturers are listed in **Table 15***

Table 15 – LZRSE-Col7A1 RVV Manufacturers

(b) (4)

Reviewer Comment (JTK): Adequate information regarding LZRSE-Col7A1 RVV manufacturers has been provided.

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

(b) (4)

Section 3.2.R Regional Information

Device Design / Quality System Requirements

The final DP contains several device constituents, making the DP a combination product. Specifically, the device components are the following:

- *Petrolatum Gauze (b) (4)*
 - *Pre-amendment exempt medical device*
 - *Vaseline petrolatum gauze is a fine mesh, absorbent gauze impregnated with white petrolatum. This product remains moist, nontoxic, and nonirritating. It is nonadherent and sterile.*
- *Petroleum Jelly (b) (4)*
 - *Pre-amendment exempt medical device*
 - *White petroleum jelly that is a soothing ointment base for many topical therapeutic agents. Latex free. Sterile.*
- *Titanium Ligating Clips (b) (4)*
 - *510k-cleared medical device: (b) (4)*
 - *Pre-formed chevron shaped titanium ligating clips (size small) used to secure skin sheet to petrolatum gauze backing.*

Additionally, to act as a visual aid and help with orientation of the product (top vs. bottom), the applicant includes a surgical suture on bottom of the petrolatum gauze (non-patient and cellular sheet contacting), which is considered an excipient.

In this CR resubmission, the applicant provided a revised and updated quality system requirements document, outlining the management responsibilities, design controls, design validation, design transfer, design change, and design history file. The document also included updated sections for purchasing controls and corrective and preventive actions (CAPA) based on discussion items provided to the firm during the final close out

of the PLI (refer to Establishment Inspection Report (EIR) Section 14 – General Discussion with Management).

Since the submission of the original BLA, the applicant completed additional design validation studies, including additional packaging assembly and transportation studies. The applicant also completed Phase 3 of the Design and Development Plan, which finalized and design of the product, requiring any additional changes to go through the design control (SOP-000306) and change control protocols (SOP-000024) that have been put in place. Lastly, the applicant provided an updated design history file (FRM-000394) to demonstrate that their system consists of a comprehensive, organized documentation of a medical device's design and development process, demonstrating compliance with design controls and regulatory requirements.

Reviewer Comment (BNN): The applicant provided updated design control documents for their device constituents of the DP. While the applicant provided adequate documents in the original BLA submission to demonstrate an adequate quality system, additional validations and updated protocols were put in place and submitted in this CR response. There are no CMC concerns and this information is adequate. DMPQ did not review these updated documents as their review focuses on abbreviated 21 CFR 820 sections, including Management Responsibilities (820.20), Purchasing Controls (820.50), and CAPAs (820.100).

Section 3.2.P.5.2 Analytical Procedure

The resubmission included an updated final lot release table (**Table 18**), including the additional assays (e.g., identity testing, rapid sterility testing) and the associated analytical procedures and acceptance criteria.

Table 18 - Final Lot Release Testing

Test Parameter (Attribute)	Analytical Procedure (Protocol)	Final Acceptance Criteria
(b) (4)	(b) (4)	(b) (4)
Cell Viability		
Endotoxin		
(b) (4)		

Test Parameter (Attribute)	Analytical Procedure (Protocol)	Final Acceptance Criteria
(b) (4)	(b) (4)	(b) (4)
Mycoplasma		(b) (4)
(b) (4)		(b) (4)
Identity - Keratinocytes		(b) (4)
(b) (4)		(b) (4)
Visual Inspection		Uniform intact sheet; Confluent, no holes, no contamination
DP Rapid Sterility using excipient media from packaging – results available (b) (4) after release		No Growth
Sterility* *The batch is administered before (b) (4) guided sterility results are available. When sterility results are available, it is evaluated and added to the PZ COA for final product release.		No growth
Vector Detection		(b) (4)

Reviewer Comment (BNN): The updated lot release testing table shows additional assays and their respective validated methods and acceptance criteria to include feedback and changes made in response to the CR Letter. Based on the review of the individual assays and the assessments outlined in this memo, this updated table is acceptable.

(b) (4)

(b) (4)

Section 3.2.P.7 Container Closure System

As part of the resubmission, the applicant provided validated (b) (4) (b) (4) and (b) (4) (b) (4) methods that were utilized as part of the extraction and leachables testing (E&L) of the drug product container closure, along with updated human toxicological risk assessments (TRA) of the E&L chemical associated with the (b) (4) DP.

Reviewer Comment: E&L review was previously performed by Andrey Sarafanov, PhD (CBER/OTP/OPPT/DH/HB2) for the original submission of the BLA (please refer to E&L memo for full details), with no concerns or issues identified. The applicant submitted additional TRA documents as part of the resubmission, although not requested by FDA (OTP). These were reviewed by Amanda Szucsik, DVM, MS (CBER/OTP/OPT/DPT1/PTB1). Please refer to the TRA memo for full details.

The applicant provided three human TRAs of E&L chemicals associated with the (b) (4) (b) (4) PZ DP container closures. The first study concerns the (b) (4) a collection device used in the production of (b) (4) and the (b) (4) (b) (4) container-closure system (CCS). The second and third studies concern a (b) (4) used in the production of the (b) (4)

(b) (4) E&L chemicals from the (b) (4) DP P1 were analyzed using unvalidated (b) (4) and (b) (4) per reports provided in the original BLA submission. Validated (b) (4) methods were utilized in E&L reports provided in the BLA re-submission (Amendment 57). Review of these TRAs did not identify any significant safety concerns with use of the (b) (4) (b) (4) DP P1 packaging.

Reviewer Comment (AS): Based on the review of human TRAs associated with the (b) (4) and ZEVASKYN P1, there were no deficiencies identified. These data provide sufficient support for the suitability of the listed materials in the manufacture and storage of ZEVASKYN.