

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

022458Orig1s000

CHEMISTRY REVIEW(S)



DEPARTMENT OF HEALTH & HUMAN SERVICES

Center for Drugs Evaluation and Research – Food and Drug Administration
Office of Biotechnology Products / Office of Pharmaceutical Science
Division of Therapeutic Proteins

Quality Review: AMENDMENT

Taliglucerase alfa

From: **Richard Ledwidge, Ph.D.**
Division of Therapeutic Proteins (DTP)
Office of Biotechnology Products (OBP)

Through: **Gibbes Johnson, PhD**
Chief, Lab of Chemistry
DTP, OBP

NDA Number: **022458**
Product: **Elelyso**
Sponsor: **Protalix Biotherapeutics, Inc.**

Date of review: **03/01/2012**
Received Date: **08/01/2011**
PDUFA Date: **05/01/2012**

This amendment documents two teleconferences with the sponsor, Post-Marketing Commitments and exemption from environmental assessment for NDA 22458.

I. TELECONFERENCES

Teleconference Call with Protalix Biotherapeutics on 3/21/12:

Several minor deficiencies were identified in the firm's responses to the Complete Response letter. These issues included the following:

- 1) Appropriate system suitability criteria were needed for the cellular uptake specification.
- 2) Appropriate acceptance criteria were needed for the moisture content specification which were supported by data.
- 3) Appropriate criteria for determining the reportable result were needed for the moisture content specification.
- 4) Appropriate acceptance criteria were needed for the capillary isoelectric focusing specification.
- 5) Appropriate acceptance criteria were needed for the mass spectrometry specification that takes into consideration both the precision of the MALDI-TOF instrument and the results from the commercial manufacturing process.
- 6) Appropriate action limits were needed for the quantity and color (b) (4)

The sponsor responded on 3/27/12 and 4/2/12.

- 1) Appropriate system suitability criteria for the cellular uptake assay.

Sponsor response: The sponsor proposed the following as additional system suitability criteria:

- a. Average OD value at highest reference standard taliglucerase alfa concentration must have $OD_{450} > (b) (4)$
- b. The difference (delta) OD at highest reference standard taliglucerase alfa concentration must be $> (b) (4)$ at OD_{450} .
- c. The ratio of the OD_{450} values at the highest concentration of reference standard and untreated sample both without mannan inhibition should be (b) (4).
- d. The EC50 value of the unconstrained curve range between (b) (4).
- e. All plates must pass all system suitability criteria to produce a meaningful potency result.

Reviewer comment: The system suitability criteria proposed by the sponsor are acceptable. However, we want the sponsor to add an additional system suitability criterion that specifies the minimally acceptable percentage of specific uptake by the reference standard. A teleconference with the sponsor will be required to address this concern.

2.3) Appropriate acceptance criteria for moisture content specification.

Sponsor response: The sponsor provided release and stability data to support a no more than (NMT) (b) (4) moisture content acceptance criterion. In addition, the sponsor clarified that (b) (4) vials are tested for moisture content testing and that all (b) (4) must pass the NMT (b) (4) to pass the specification. The reportable result is the average of (b) (4) vials.

Reviewer comment: The sponsor provided both release and stability data to support a (b) (4) moisture content acceptance criterion. Lots on stability with (b) (4) moisture content results have passed all specifications to date. The sponsor's response is acceptable.

4) Appropriate acceptance criteria for the iCE assay and characterization of the (b) (4) from the electropherograms.

Sponsor response: The sponsor provided initial characterization data on the heterogeneity observed in the iCE assay. Mass spectrometry data were consistent with the (b) (4). In addition, to confirm identity of the (b) (4) the sponsor treated taliglucerase alfa with (b) (4) and demonstrated by mass spectrometry that (b) (4)

The sponsor proposed new acceptance criteria based on limited release and stability data under different storage conditions. The sponsor requested that the (b) (4)

Reviewer Comment: The characterization data the sponsor provided is incomplete. It wasn't obvious why other assays, for instance peptide mapping, weren't employed to confirm the identity (b) (4). A thorough characterization of the (b) (4) including the relationship to potency will be addressed as a PMC. Although the sponsor has limited experience with the iCE assay the proposed acceptance criteria are too wide and would provide little assurance of product quality, and therefore the proposed acceptance criteria are unacceptable. Revising the acceptance criteria for the (b) (4) will be addressed in a follow up teleconference.

5) Appropriate system suitability criteria for the mass spectrometry specification.

Sponsor response: The sponsor used mass spectrometry release and stability results from the current manufacturing process to revise and tighten their acceptance criteria for release and stability from (b) (4)

Reviewer Comment: The sponsor did as requested. The sponsor's response is acceptable.

6) Appropriate action limits for the quantity and color (b) (4)

(b) (4)

Sponsor response: The sponsor changed the action limit for **the in-process visual inspection** (b) (4)

In addition the sponsor put in place photographic images from a (b) (4) If these action limits are not met a deviation investigation will be initiated.

Reviewer Comment: *Meaningful action limits have been put in place during (b) (4) so that observations outside these action limits will instigate an investigation. The sponsor's response is acceptable.*

Conclusions from Sponsors response to teleconference of 3/21/12: *There remains two outstanding issues that require agreement with DTP and included to (i) put in place a percentage of specific uptake as a system suitability criterion for the cellular uptake assay and (ii) tighten the release acceptance criteria for the iCE assay.*

Teleconference Call with Protalix Biotherapeutics on 4/17/12:

Based on the sponsor's responses to our teleconference call on 3/21/12 we identified two issues that require clarification:

- 1) Appropriate system suitability criteria are needed for the cellular uptake specification.
We requested that the sponsor propose a minimal percentage of specific uptake as a system suitability criterion at the highest taliglucerase alfa concentration in the cellular uptake assay.

The sponsor and DTP negotiated a (b) (4) specific uptake for reference standard at the highest concentration of taliglucerase alfa as a system suitability criterion. We also agreed to have a PMC where the sponsor will revise the minimal percentage of specific uptake upon gaining additional assay experience.

Reviewer comment: *The proposed (b) (4) specific uptake at the highest taliglucerase alfa concentration as a system suitability criterion in the cellular uptake assay is acceptable. The percentage specific uptake will be revised once further experience with the assay is gained (See PMC 3 below).*

- 2) Appropriate acceptance criteria are needed for the imaged capillary isoelectric focusing specification.

The sponsor proposed revised acceptance criteria based on release data and drug product stability data stored under the proposed conditions (b) (4) within its (b) (4) dating period. The sponsor agreed to set acceptance criteria based on release data and stability data stored under ideal conditions and less than (b) (4). The sponsor and DTP negotiated NLT (b) (4) for (b) (4) and NMT (b) (4) for (b) (4). In addition, we agreed that this assay will be used for release testing only at this time.

Reviewer comment: The proposed acceptance criteria were still too wide because stability data beyond (b) (4) skews the data. An analysis of release results and stability up to (b) (4) show consistent values. The sponsor agreed to acceptance criteria of NLT (b) (4) for the (b) (4) and NMT (b) (4) for the (b) (4) for release for both drug substance and drug product. Revising the acceptance criteria observed until further manufacturing experience is gained will be documented as a PMC (See PMC 4 below).

Conclusion from teleconference of 4/17/12: The sponsor amended the application with the percentage of specific uptake in the cellular uptake assay and acceptance criteria for the (b) (4) in the iCE assay as agreed upon in the teleconference of 4/17/12. The amendment, sequence 53, was submitted to the NDA on April 25, 2012 and I confirmed the presence of the contents of the amendment on April 25, 2012

II. POST MARKETING COMMITMENTS

- 1) To revise the cellular uptake potency assay release and stability acceptance criteria after 15 lots of drug product have been manufactured.

Final Report Submission Date: July 2015

- 2) To revise Experion automated electrophoresis release and stability acceptance criteria after 15 lots of drug product have been manufactured.

Final Report Submission Date: July 2015

- 3) To evaluate and revise as appropriate the minimal percentage of specific uptake of reference standard as a system suitability criterion in the cellular uptake potency assay after at least 80 independent assay runs of release and stability testing of drug substance and drug product lots have been completed.

Study Completion Date: December 2013

Final Report Submission Date: March 2014

- 4) To perform a thorough biochemical characterization of the (b) (4) detected in the iCE assay and to evaluate the impact of this heterogeneity on product quality, including any effects on potency (specific uptake, enzyme kinetics, and cellular uptake). The characterization should use additional analytical assays (e.g., peptide mapping and (b) (4)) to confirm the identity of the characterized peaks. Perform an assessment regarding the suitability and

the implementation of the iCE method and other analytical assays as appropriate into your stability protocol. The results of these studies should guide the revision of the release and stability specifications after at least 30 lots of drug substance and at least 15 lots of drug product have been manufactured.

Study Completion Date: April 2015

Final Report Submission Date: July 2015

III. REQUEST FOR WAIVER OF ENVIRONMENTAL ASSESSMENT

The sponsor has requested a categorical exclusion for environmental assessment.

Reviewer Comment: This application involves "biologic" substances, glucocerebrosidase enzymes, that occur naturally in the environment as describe in FDA Guidance Environmental Assessment of Human Drug and Biologics Applications. Approval of this supplement will not alter significantly the concentration or distribution of the substance or its degradation products in the environment therefore based on regulations established in part 21 CFR 25.31 (c), I recommend approval of this request.

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/s/

RICHARD LEDWIDGE
04/26/2012

GIBBES R JOHNSON
04/26/2012



DEPARTMENT OF HEALTH & HUMAN SERVICES

Center for Drugs Evaluation and Research – Food and Drug Administration
Office of Biotechnology Products / Office of Pharmaceutical Science
Division of Therapeutic Proteins

The Quality Team Leader's Executive Summary AMENDMENT

From: Gibbes Johnson, PhD
Division of Therapeutic Proteins (DTP)

Through: Amy Rosenberg, MD
Division Director, DTP

NDA Number: 22458
Product: Protalix Biotherapeutics, Inc

Date of Review: 3/31/2012
Due Date of CDTL Memo: 4/6/2012
PDUFA Date: 5/1/2012

This amendment documents the introduction of Post-Marketing Commitments in the approval letter for NDA 22458. The PMC's are stated as follows:

II. POST MARKETING COMMITMENTS

- 1) To revise the cellular uptake potency assay release and stability acceptance criteria after 15 lots of drug product have been manufactured.

Final Report Submission Date: July 2015

- 2) To revise Experion automated electrophoresis release and stability acceptance criteria after 15 lots of drug product have been manufactured.

Final Report Submission Date: July 2015

- 3) To evaluate and revise as appropriate the minimal percentage of specific uptake of reference standard as a system suitability criterion in the cellular uptake potency assay after at least 80 independent assay runs of release and stability testing of drug substance and drug product lots have been completed.

Study Completion Date: December 2013

Final Report Submission Date: March 2014

- 4) To perform a thorough biochemical characterization of the (b) (4) detected in the iCE assay and to evaluate the impact of this heterogeneity on product quality, including any effects on potency (specific uptake, enzyme kinetics, and cellular uptake). The characterization should use additional analytical assays (e.g., peptide mapping and (b) (4)) to confirm the identity of the characterized peaks. Perform an assessment regarding the suitability and the implementation of the iCE method and other analytical assays as appropriate into your stability protocol. The results of these studies should guide the revision of the release and stability specifications after at least 30 lots of drug substance and at least 15 lots of drug product have been manufactured.

Study Completion Date: April 2015

Final Report Submission Date: July 2015

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/s/

GIBBES R JOHNSON
04/26/2012

AMY S ROSENBERG
04/26/2012



DEPARTMENT OF HEALTH & HUMAN SERVICES

Center for Drugs Evaluation and Research – Food and Drug Administration
Office of Biotechnology Products / Office of Pharmaceutical Science
Division of Therapeutic Proteins

The Quality Team Leader's Executive Summary

From: Gibbes Johnson, PhD
Division of Therapeutic proteins (DTP)

Through: Barry Cherney, PhD
Deputy Division Director, DTP

NDA Number: 22458
Product: Protalix Biotherapeutics, Inc

Date of Review: 3/29/2012
Due Date of CDTL Memo: 4/6/2012
PDUFA Date: 5/1/2012

I. RECOMMENDATIONS AND CONCLUSIONS ON APPROVABILITY

The Division of Therapeutic Proteins, Office of Biotechnology Products, OPS, CDER, recommends approval of NDA 22458 for Eleyso manufactured by Protalix Biotherapeutics, Inc.. The data submitted in the application and in the responses to the Complete Response letter, pending resolution of some minor issues as described below, are adequate to support the conclusion that the manufacture of Eleyso is well controlled, and leads to a product that is potent. It is recommended that this product be approved for human use (under conditions specified in the package insert).

II. POST MARKETING COMMITMENTS/POST MARKETING REQUIREMENTS

APPEARS THIS WAY ON ORIGINAL

EXECUTIVE SUMMARY

This summary covers the responses provided by the firm to the Complete Response letter issued February 24, 2011. A detailed description of drug substance and drug product quality control and stability as well as conditions of use were covered in the Team Leader memo uploaded in DARRTS on February 24, 2011. The Team Leader memo of 2/24/11 is attached to this summary in Appendix I, for ease of reference.

The deficiencies listed in the complete response letter pertained to the following:

- 1) Specifications- multiple identity, impurity, and potency assays were revised.
- 2) Comparability- the effects of changing the components of the growth media in the commercial manufacturing process were evaluated.
- 3) Process Validation- validation data for particular aspects of manufacturing were provided.
- 4) Control of Impurities- the levels of several (b) (4) impurities were evaluated.

All other sections of NDA 22458 were previously reviewed and were deemed adequate. The remaining issues will be addressed as Post-Marketing Commitments, as outlined in the Approval/PMC section.

Resolution of the CR issues:Issues Related to Test Methods and Specifications

The specifications were revised as follows:

1. The sponsor revised release and stability testing by incorporating USP<788> particulate testing and appearance testing on reconstituted drug product. Release and stability testing results for both assays were provided.
2. The sponsor revised the potency assay for cellular uptake in macrophages by using multiple taliglucerase alfa concentrations to generate a complete dose-response curve which is used to determine a half-maximal effective concentration. The improved uptake assay was implemented for release and stability testing. In a teleconference with the sponsor on 3/21/12 the sponsor was asked to identify and implement additional system suitability controls for the cellular uptake assay. The evaluation of the firm's response will be provided in an addendum to the primary review.
3. The sponsor characterized the (b) (4) that was occasionally observed in SE-HPLC chromatographs and demonstrated that the assay is highly sensitive to detect the (b) (4).
4. The sponsor revised the RP-HPLC assay to better resolve (b) (4) and provided characterization data on the nature of the (b) (4).
5. The sponsor provided enzyme kinetic data with a physiologically relevant glucocerebroside substrate.
6. The sponsor tightened acceptance criteria for the enzyme kinetic parameters Vmax and Km with the pNP-Glc substrate.
7. The sponsor provided USP<788> testing on (b) (4) (b) (4) is under control during typical in-use applications.
8. The sponsor revised the mass spectrometry specification so that the acceptance criteria are defined by the mass to charge ratio. In a teleconference with sponsor on 3/21/12 the sponsor was asked to tighten the acceptance criteria to reflect both the precision of the

- assay and the results from the current manufacturing process. The evaluation of the firm's response will be provided in an addendum to the primary review.
9. The sponsor provided a statistical argument for a (b) (4) acceptance criteria for moisture content without providing any biochemical data to support that there is no impact on product quality. In a teleconference with sponsor on 3/21/12 we asked the sponsor to lower the moisture content to levels for which they have biochemical data to show there is no negative impact to product quality. We also stated that acceptable reportable values for moisture content can not have any vials failing the acceptance criteria. The evaluation of the firm's response will be provided in an addendum to the primary review.
 10. The sponsor provided data to demonstrate that the (b) (4) observed in the monosaccharide analysis is consistent with that observed by the orthogonal method of glycan profiling.
 11. The sponsor provided validation data, both temperature and moisture content testing, to demonstrate that all of shelves of the lyophilizer are functionally equivalent.
 12. The sponsor implemented a capillary isoelectric focusing assay for release and stability testing to monitor charge variants. In a teleconference with sponsor on 3/21/12 we asked the sponsor to put in place additional acceptance criteria than the proposed (b) (4). The evaluation of the firm's response will be provided in an addendum to the primary review.
 13. The sponsor changed the (b) (4) by reporting the result to two significant digits.
 14. The sponsor revised the SDS-PAGE assay by switching to the Experion electrophoresis system for better separation, identification and quantification of electrophoresis bands.
 15. The sponsor revised the system suitability criteria for the peptide mapping specification.

Pending resolution of the issues raised in the teleconference on 3/21/12 the DTP primary reviewer found the responses to the Complete Response letter of 2/24/11 adequate and I concur with his assessment.

Issues Related to Comparability:

1. The switch from (b) (4) caused a shift in the glycan profile. Since glycan structures are critical to the product's mechanism of action the improved potency assay was used to demonstrate that cellular uptake into macrophages was unaffected by the observed change in the glycan profile.
2. The switch from (b) (4) caused a slight increase in the levels of (b) (4). The sponsor revised the specification so that (b) (4) are reported separately. The observed levels of (b) (4) are small regardless of the growth media.
3. A one time SE-HPLC with light scattering detector study (b) (4) was performed to demonstrate that (b) (4) when grown in (b) (4).

The DTP primary reviewer found these actions adequate to address issues of comparability and I concur with his assessment.

Issues Related to Process Validation:

1. The sponsor submitted data to support the defined *in vitro* cell age as (b) (4).
[Redacted]
2. The sponsor submitted data to show that the lyophilizer can consistently perform as intended. The temperature and moisture content variation between shelves is minimal.
3. The sponsor characterized the levels and types of (b) (4).
[Redacted]. The evaluation of the firm's response will be provided in an addendum to the primary review.

Pending resolution of the issue raised in the teleconference on 3/21/12 the DTP primary reviewer found these actions adequate to address issues of process validation and I concur with his assessment.

Issues Related to Control of Impurities:

1. The sponsor submitted data with appropriate system suitability controls to demonstrate that the master and working cell banks were free of plant and carrot specific viruses.
2. The sponsor demonstrated that (b) (4) levels are below the limit of detection (b) (4) and a risk assessment was provided indicating that the dose level an adult would experience in a lifetime would not warrant a safety risk.
3. The sponsor demonstrated that the levels of (b) (4) in the drug product formulation are accurate with the label listing.

The DTP primary reviewer found these actions adequate to address issues of impurity control and I concur with his assessment.

Teleconference Call with the Sponsor on 3/21/12:

Several minor deficiencies were identified in the firm's responses to the Complete Response letter. These issues included the following:

- 1) Appropriate system suitability criteria are needed for the cellular uptake specification.
- 2) Appropriate acceptance criteria are needed for the moisture content specification for which biochemical data is available to support that there is no impact on product quality.
- 3) Appropriate criteria for determining the reportable result are needed for the moisture content specification.
- 4) Appropriate acceptance criteria are needed for the capillary isoelectric focusing specification.
- 5) Appropriate acceptance criteria are needed for the mass spectrometry specification that takes into consideration both the precision of the MALDI-TOF instrument and the results from the commercial manufacturing process.

- 6) Appropriate action limits are needed for the quantity and color

(b) (4)

The firm stated in the teleconference that they will respond to the Agency in writing. Our evaluation of the sponsor's responses and the impact on PMC's will be addressed in an addendum to this memo. I anticipate that all remaining issues will be addressed as Post-Marketing Commitments.

Appendix I: TL memorandum dated February 24, 2011

Center for Drug Evaluation and Research
Office of Pharmaceutical Science
Office of Biotechnology Products
Division of Therapeutic Proteins
HFD-122

The Quality Team Leader's Executive Summary

FROM: Gibbes Johnson, PhD
Division of Therapeutic Proteins

THROUGH: Amy Rosenberg, MD
Division of Therapeutic Proteins

NDA NUMBER: 022458
PRODUCT: ELELYSO
SPONSOR: THE SPONSOR, Inc

DATE: 2/23/2011
PDUFA DATE: 2/24/2011

Page 8 to 23 (16 pgs) has been withheld as a duplicate copy of the "Quality Team Leader's Executive Summary" electronically dated 2/24/12 located further in this Chemistry Review section.

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/s/

GIBBES R JOHNSON
03/29/2012

BARRY W CHERNEY
03/30/2012



Quality Review: Taliglucerase alfa

From: Richard Ledwidge, Ph.D.
Division of Therapeutic Proteins (DTP)
Office of Biotechnology Products (OBP)

Through: Gibbes Johnson, PhD
Chief, Lab of Chemistry
DTP, OBP

NDA Number: 022458
Product: Elelyso
Sponsor: Protalix Biotherapeutics, Inc.

Date of review: 03/01/2012
Received Date: 08/01/2011
PDUFA Date: 05/01/2012

Recommendation: I, Richard Ledwidge, recommend approval of NDA 22458 for taliglucerase alfa manufactured by Protalix Biotherapeutics, Inc pending resolution of several minor issues. With the exception of these issues, the data submitted to address the deficiencies listed in the complete response of 02/24/2011 are resolved satisfactorily. I anticipate that the outstanding issues will be addressed by post-marketing commitments.

SUMMARY

This review covers the responses provided by the firm to the Complete Response letter issued February 24, 2011. The detailed description of drug substance and drug product quality control and stability as well as conditions of use were covered in the original quality review uploaded in DARRTS on February 24, 2011.

The deficiencies listed in the complete response letter pertained to the following:

- 1) Specifications- multiple identity, impurity, and potency assays were revised.
- 2) Comparability- the effects of changing the components of the growth media in the commercial manufacturing process were evaluated.
- 3) Process Validation- validation data for particular aspects of manufacturing were provided.
- 4) Control of Impurities- the levels of several process-related impurities were evaluated.

Teleconference Call with Protalix Biotherapeutics on 3/21/12:

Several minor deficiencies were identified in the firm's responses to the Complete Response letter. These issues included the following:

- 1) Appropriate system suitability criteria are needed for the cellular uptake specification.
- 2) Appropriate acceptance criteria are needed for the moisture content specification for which biochemical data is available to support that there is no impact on product quality.
- 3) Appropriate criteria for determining the reportable result are needed for the moisture content specification.
- 4) Appropriate acceptance criteria are needed for the capillary isoelectric focusing specification.
- 5) Appropriate acceptance criteria are needed for the mass spectrometry specification that takes into consideration both the precision of the MALDI-TOF instrument and the results from the commercial manufacturing process.
- 6) Appropriate action limits are needed for the quantity and color of visible protein particulates that form during the thawing of the drug substance.

The firm stated in the teleconference that they will respond to the Agency in writing. Our evaluation of the sponsor's responses and the impact on PMC's will be addressed in an addendum to the review. I anticipate that all remaining issues will be addressed as Post-

Marketing Commitments and the final language will be included in the Team Leader Memo.

(b) (4)



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/s/

RICHARD LEDWIDGE
03/29/2012

GIBBES R JOHNSON
03/29/2012



DEPARTMENT OF HEALTH & HUMAN SERVICES

Center for Drugs Evaluation and Research – Food and Drug Administration
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Division of Therapeutic Proteins

Quality Review: AMENDMENT

Taliglucerase alfa

From: **Richard Ledwidge, Ph.D.**
Division of Therapeutic Proteins (DTP)
Office of Biotechnology Products (OBP)

Through: **Gibbes Johnson, PhD**
Chief, Lab of Chemistry
DTP, OBP

NDA Number: **022458**
Product: **Elelyso**
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Date of review: **03/01/2012**
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The sponsor responded on 3/27/12 and 4/2/12.

- 1) Appropriate system suitability criteria for the cellular uptake assay.

Sponsor response: The sponsor proposed the following as additional system suitability criteria:

- a. Average OD value at highest reference standard taliglucerase alfa concentration must have OD₄₅₀ (b) (4).
- b. The difference (delta) OD at highest reference standard taliglucerase alfa concentration must be (b) (4) at OD₄₅₀.
- c. The ratio of the OD₄₅₀ values at the highest concentration of reference standard and untreated sample both without mannan inhibition should be (b) (4).
- d. The EC50 value of the unconstrained curve range between (b) (4)
- e. All plates must pass all system suitability criteria to produce a meaningful potency result.

Reviewer comment: The system suitability criteria proposed by the sponsor are acceptable. However, we want the sponsor to add an additional system suitability criterion that specifies the minimally acceptable percentage of specific uptake by the reference standard. A teleconference with the sponsor will be required to address this concern.

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The sponsor proposed new acceptance criteria based on limited release and stability data under different storage conditions. The sponsor requested that the (b) (4) and the (b) (4)

Reviewer Comment: *The characterization data the sponsor provided is incomplete. It wasn't obvious why other assays, for instance peptide mapping, weren't employed to confirm the identity of the (b) (4). A thorough characterization of the (b) (4) including the relationship to potency will be addressed as a PMC. Although the sponsor has limited experience with the iCE assay the proposed acceptance criteria are too wide and would provide little assurance of product quality, and therefore the proposed acceptance criteria are unacceptable. Revising the acceptance criteria for the (b) (4) will be addressed in a follow up teleconference.*

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Teleconference Call with Protalix Biotherapeutics on 4/17/12:

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The sponsor and DTP negotiated a (b) (4) specific uptake for reference standard at the highest concentration of taliglucerase alfa as a system suitability criterion. We also agreed to have a PMC where the sponsor will revise the minimal percentage of specific uptake upon gaining additional assay experience.

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- 2) Appropriate acceptance criteria are needed for the imaged capillary isoelectric focusing specification.

The sponsor proposed revised acceptance criteria based on release data and drug product stability data stored under the proposed conditions (b) (4) within its (b) (4). The sponsor agreed to set acceptance criteria based on release data and stability data stored under ideal conditions and less than (b) (4). The sponsor and DTP negotiated (b) (4) and (b) (4). In addition, we agreed that this assay will be used for release testing only at this time.

Reviewer comment: The proposed acceptance criteria were still too wide because stability data beyond (b) (4) skews the data. An analysis of release results and stability up to (b) (4) show consistent values. The sponsor agreed to acceptance criteria of NLT (b) (4) and NMT (b) (4) for release for both drug substance and drug product. Revising the acceptance criteria observed until further manufacturing experience is gained will be documented as a PMC (See PMC 4 below).

Conclusion from teleconference of 4/17/12: The sponsor amended the application with the percentage of specific uptake in the cellular uptake assay and acceptance criteria for the (b) (4) in the iCE assay as agreed upon in the teleconference of 4/17/12. The amendment, sequence 53, was submitted to the NDA on April 25, 2012 and I confirmed the presence of the contents of the amendment on April 25, 2012

II. POST MARKETING COMMITMENTS

- 1) To revise the cellular uptake potency assay release and stability acceptance criteria after 15 lots of drug product have been manufactured.

Final Report Submission Date: July 2015

- 2) To revise Experion automated electrophoresis release and stability acceptance criteria after 15 lots of drug product have been manufactured.

Final Report Submission Date: July 2015

- 3) To evaluate and revise as appropriate the minimal percentage of specific uptake of reference standard as a system suitability criterion in the cellular uptake potency assay after at least 80 independent assay runs of release and stability testing of drug substance and drug product lots have been completed.

Study Completion Date: December 2013

Final Report Submission Date: March 2014

- 4) To perform a thorough biochemical characterization of the (b) (4) detected in the iCE assay and to evaluate the impact of this heterogeneity on product quality, including any effects on potency (specific uptake, enzyme kinetics, and cellular uptake). The characterization should use additional analytical assays (e.g., peptide mapping and (b) (4)) to confirm the identity of the characterized peaks. Perform an assessment regarding the suitability and

the implementation of the iCE method and other analytical assays as appropriate into your stability protocol. The results of these studies should guide the revision of the release and stability specifications after at least 30 lots of drug substance and at least 15 lots of drug product have been manufactured.

Study Completion Date: April 2015

Final Report Submission Date: July 2015

III. REQUEST FOR WAIVER OF ENVIRONMENTAL ASSESSMENT

The sponsor has requested a categorical exclusion for environmental assessment.

Reviewer Comment: This application involves "biologic" substances, glucocerebrosidase enzymes, that occur naturally in the environment as describe in FDA Guidance Environmental Assessment of Human Drug and Biologics Applications. Approval of this supplement will not alter significantly the concentration or distribution of the substance or its degradation products in the environment therefore based on regulations established in part 21 CFR 25.31 (c), I recommend approval of this request.

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/s/

RICHARD LEDWIDGE
04/26/2012

GIBBES R JOHNSON
04/26/2012

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

BLA/NDA Number: 22458 **Applicant:** Protalix, Inc. **Stamp Date:** April 26, 2010

Established/Proper Name: Taliglucerase alfa (b) (4) **BLA/NDA Type:** NDA

On initial overview of the BLA/NDA application for filing:

CTD Module 1 Contents	Present?	If not, justification, action & status
Cover Letter	Y	
Form 356h completed	Y	
<input type="checkbox"/> including list of all establishment sites and their registration numbers	Y	
Comprehensive Table of Contents	Y	
Environmental assessment or request for categorical exclusion (21 CFR Part 25)	Y	
Labeling:	Y	
<input type="checkbox"/> PI –non-annotated	Y	
<input type="checkbox"/> PI –annotated	Y	
<input type="checkbox"/> PI (electronic)	Y	
<input type="checkbox"/> Medication Guide	Y	
<input type="checkbox"/> Patient Insert	Y	
<input type="checkbox"/> package and container	Y	
<input type="checkbox"/> diluent	Y	
<input type="checkbox"/> other components	Y	
<input type="checkbox"/> established name (e.g. USAN)	Y	
<input type="checkbox"/> proprietary name (for review)	Y	

Examples of Filing Issues	Yes?	If not, justification, action & status
Content, presentation, and organization of paper and electronic components sufficient to permit substantive review?: Examples include:	Y	
<input type="checkbox"/> legible	Y	
<input type="checkbox"/> English (or translated into English)	Y	
<input type="checkbox"/> compatible file formats	Y	
<input type="checkbox"/> navigable hyper-links	Y	
<input type="checkbox"/> interpretable data tabulations (line listings) & graphical displays	Y	
<input type="checkbox"/> summary reports reference the location of individual data and records	Y	
<input type="checkbox"/> all electronic submission components usable (e.g. conforms to published guidance)	Y	
Companion application received if a shared or divided manufacturing arrangement	N/A	

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

CTD Module 2 Contents	Present?	If not, justification, action & status
Overall CTD Table of Contents [2.1]	Y	
Introduction to the summary documents (1 page) [2.2]	Y	
Quality overall summary [2.3]	Y	Excipient list provided in 3.2.P.4
<input type="checkbox"/> Drug Substance	Y	
<input type="checkbox"/> Drug Product	Y	
<input type="checkbox"/> Facilities and Equipment	Y	
<input type="checkbox"/> Adventitious Agents Safety Evaluation	Y	
<input type="checkbox"/> Novel Excipients	N	
<input type="checkbox"/> Executed Batch Records	Y	
<input type="checkbox"/> Method Validation Package	Y	
<input type="checkbox"/> Comparability Protocols	Y	

CTD Module 3 Contents	Present?	If not, justification, action & status
Module Table of Contents [3.1]	N	Section 3.1 not provided
Drug Substance [3.2.S]		
<input type="checkbox"/> general info	Y	
<input type="checkbox"/> nomenclature		
<input type="checkbox"/> structure (e.g. sequence, glycosylation sites)		
<input type="checkbox"/> properties		
<input type="checkbox"/> manufacturers (names, locations, and responsibilities of all sites involved)	Y	
<input type="checkbox"/> description of manufacturing process and process control	Y	
<input type="checkbox"/> batch numbering and pooling scheme		
<input type="checkbox"/> cell culture and harvest		
<input type="checkbox"/> purification		
<input type="checkbox"/> filling, storage and shipping		
<input type="checkbox"/> control of materials	Y	
<input type="checkbox"/> raw materials and reagents		
<input type="checkbox"/> biological source and starting materials		
<input type="checkbox"/> cell substrate: source, history, and generation		
<input type="checkbox"/> cell banking system, characterization, and testing		
<input type="checkbox"/> control of critical steps and intermediates	Y	
<input type="checkbox"/> justification of specifications		
<input type="checkbox"/> stability		

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
<ul style="list-style-type: none"> and sterilization validation <ul style="list-style-type: none"> ○ Validation of aseptic processing (media simulations) ○ Environmental Monitoring Program ○ Lyophilizer validation ○ Other needed validation data (hold times) <input type="checkbox"/> control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin) <input type="checkbox"/> control of drug product (justification of specifications; analytical method validation; batch analyses, characterization of impurities) <input type="checkbox"/> reference standards or materials <input type="checkbox"/> container closure system [3.2.P.7] <ul style="list-style-type: none"> ○ specifications (vial, elastomer, drawings) ○ availability of DMF & LOAs ○ administration device(s) <input type="checkbox"/> stability <ul style="list-style-type: none"> <input type="checkbox"/> summary <input type="checkbox"/> post-approval protocol and commitment <input type="checkbox"/> pre-approval <ul style="list-style-type: none"> ○ protocol ○ results ○ method validation 	<p align="center">Y</p> <p align="center">Y</p> <p align="center">Y</p> <p align="center">Y</p> <p align="center">Y</p>	
<p>Diluent (vials or filled syringes) [3.2P']</p> <ul style="list-style-type: none"> <input type="checkbox"/> description and composition of diluent <input type="checkbox"/> pharmaceutical development <ul style="list-style-type: none"> ○ preservative effectiveness ○ container-closure integrity <input type="checkbox"/> manufacturers (names, locations, and responsibilities of all sites involved) <input type="checkbox"/> batch formula <input type="checkbox"/> description of manufacturing process for production through 	<p align="center">N/A</p>	

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities) <ul style="list-style-type: none"> <input type="checkbox"/> controls of critical steps and intermediates <input type="checkbox"/> process validation including aseptic processing & sterility assurance: <ul style="list-style-type: none"> <input type="checkbox"/> Filter validation <input type="checkbox"/> Component, container, closure depyrogenation and sterilization validation <input type="checkbox"/> Validation of aseptic processing (media simulations) <input type="checkbox"/> Environmental Monitoring Program <input type="checkbox"/> Lyophilizer sterilization validation <input type="checkbox"/> Other needed validation data (hold times) <input type="checkbox"/> control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin, other novel excipients) <input type="checkbox"/> control of diluent (justification of specifications; analytical method validation, batch analysis, characterization of impurities) <input type="checkbox"/> reference standards <input type="checkbox"/> container closure system <ul style="list-style-type: none"> <input type="checkbox"/> specifications (vial, elastomer, drawings) <input type="checkbox"/> availability of DMF & LOAs <input type="checkbox"/> stability <ul style="list-style-type: none"> <input type="checkbox"/> summary <input type="checkbox"/> post-approval protocol and commitment <input type="checkbox"/> pre-approval <ul style="list-style-type: none"> <input type="checkbox"/> protocol <input type="checkbox"/> results 		
Other components to be marketed (full description and supporting data, as listed above): <ul style="list-style-type: none"> <input type="checkbox"/> other devices <input type="checkbox"/> other marketed chemicals (e.g. part 	N/A	

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

Examples of Filing Issues	Yes?	If not, justification, action & status
trial to commercial production lots		
Data demonstrating comparability of product to be marketed to that used in clinical trials (when significant changes in manufacturing processes or facilities have occurred)	Y	
Certification that all facilities are ready for inspection	Y	
Data establishing stability of the product through the proposed dating period and a stability protocol describing the test methods used and time intervals for product assessment.	Y	
If not using a test or process specified by regulation, data is provided to show the alternate is equivalent (21 CFR 610.9) to that specified by regulation. List: <input type="checkbox"/> LAL instead of rabbit pyrogen <input type="checkbox"/> mycoplasma <input type="checkbox"/> sterility	N/A	
Identification by lot number, and submission upon request, of sample(s) representative of the product to be marketed; summaries of test results for those samples	Y	
Floor diagrams that address the flow of the manufacturing process for the drug substance and drug product	Y	
Description of precautions taken to prevent product contamination and cross-contamination, including identification of other products utilizing the same manufacturing areas and equipment	Y	

**PRODUCT QUALITY (Biotechnology)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

IS THE PRODUCT QUALITY SECTION OF THE APPLICATION FILEABLE? Yes

If the application is not fileable from product quality perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Richard Ledwidge, PhD

July 1, 2010

Product Quality Reviewer(s)

Date

Branch Chief/Team Leader/Supervisor

Date

Division Director

Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RICHARD LEDWIDGE
02/23/2011

GIBBES R JOHNSON
02/24/2011

BARRY W CHERNEY
02/24/2011



Center for Drug Evaluation and Research
Office of Pharmaceutical Science
Office of Biotechnology Products
Division of Therapeutic Proteins
HFD-122

The Quality Team Leader's Executive Summary

FROM: Gibbes Johnson, PhD
Division of Therapeutic Proteins

THROUGH: Amy Rosenberg, MD
Division of Therapeutic Proteins

NDA NUMBER: 022458
PRODUCT: ELELYSO
SPONSOR: PROTALIX, Inc

DATE: 2/23/2011
PDUFA DATE: 2/24/2011

I. RECOMMENDATIONS ON APPROVABILITY

The Division of Therapeutic Proteins, Office of Biotechnology Products, OPS, CDER recommends a complete response for NDA 022458 for ELELYSO manufactured by Protalix, Inc. The data in the application are inadequate to support the conclusion that the manufacture of ELELYSO is well controlled and leads to a product that is pure and potent.

II. APPROVAL LETTER INFORMATION

Not Applicable

III. POST MARKETING COMMITMENTS/POST MARKETING REQUIREMENTS

Not Applicable

IV. LIST OF CHEMISTRY, MANUFACTURING AND CONTROLS DEFICIENCIES TO BE COMMUNICATED

1) Please respond to the following deficiencies regarding specifications and assay validation:

a) Results of USP<788> particulate testing and appearance testing on reconstituted drug product have not been submitted to the NDA. Both tests provide a useful measure of product quality that is not monitored by other tests you have proposed. Add these tests to the release and stability specifications and provide available results for release and stability testing of the three conformance lots and any additional results you may have.

b) A potency assay that quantitatively measures specific receptor binding and/or high affinity internalization into cells is required since internalization is a critical component of taliglucerase alfa's mechanism-of-action and it is not fully assessed in your current potency assay. The assay should use multiple taliglucerase alfa concentrations to generate a complete dose-response curve in order to calculate the half-maximal effective concentration (EC_{50app}). Develop and implement this assay for use in release and stability testing.

c) Some SE-HPLC chromatographs exhibit a (b) (4). Because this (b) (4) may reflect variability in a product-related variant, it should be identified and, if necessary, controlled. Characterize the protein in the (b) (4) and determine whether a control strategy that better monitors this product attribute(s) should be implemented. Provide the results of your analyses and any proposed changes to your specifications.

d) RP-HPLC chromatograms suggest that taliglucerase alfa variants are (b) (4). The risk to product quality is expected to vary depending on the nature of the variant. Thus, in order to establish an appropriate control strategy, you should identify and control for the quantity of these variants, if present. It may be useful to alter assay conditions or gradients (b) (4). Provide information on the presence of unresolved

variants and, if present, provide a revised specification that more accurately quantitates and controls these variants together with supporting data.

e) Enzyme kinetic parameters and specific activity are measured using synthetic p-nitrophenyl-glucopyranoside (pNP-Glc) substrate. pNP-Glc (b) (4) may be less sensitive in detecting changes to product quality. Provide enzyme kinetic data to determine the enzyme kinetic parameters, K_m and k_{cat} , (b) (4).

Include a detailed description of the assay, supporting assay qualification data, as well as a justification for why this test should not be added to the release and stability specifications.

f) Stability testing of diluted drug product in infusion bags did not include USP<788> particulate testing or information on the impact of dilution on subvisible particulates that are between (b) (4). USP<788> testing results are critical to mitigate the risk associated with occlusion of small blood vessels and small subvisible particles may pose an immunogenicity risk. Provide USP<788> particulate testing data for in-use stability studies and an analysis of particulates between (b) (4).

g) The mannose content specification is based on a MALDI-TOF analysis of taliglucerase alfa. However, the property that is being measured in the MALDI-TOF analysis is mass to charge ratio, not mannose content. Thus, the acceptance criterion should be set around the mass to charge ratio and the mannose content acceptance criterion should be removed from the MALDI-TOF specification. Provide the new specification together with supporting data.

h) The acceptance criterion for moisture content in drug product is (b) (4) for both release and stability testing. Release and stability testing results consistently show moisture content to be below (b) (4) and no data were submitted indicating that a (b) (4) moisture content would not have an adverse impact on product stability throughout the product's dating period. Amend the moisture content acceptance criterion to reflect your manufacturing capability and consideration of any additional knowledge you may have concerning the impact of moisture on product stability and provide the new specification, if appropriate, together with supporting data.

i) Monosaccharide content and glycan structure analysis submitted in the characterization section of the NDA contained inconsistent results. Monosaccharide content analysis on two batches indicated that the (b) (4) whereas the glycan analysis data determined that (b) (4) of the glycan structures have a (b) (4). Provide an explanation for these results or submit data that identify the more accurate analysis using batches made in (b) (4).

j) The acceptance criteria for the enzyme kinetic parameters K_m and V_{max} are (b) (4) respectively. An analysis of 40 drug substance batches resulted in mean and standard deviations for K_m and V_{max} equal to (b) (4), respectively. Consequently, the acceptance criteria appear too wide and should be amended to reflect process capability and clinical experience. Provide the revised specification for enzyme kinetic

parameters or your justification as to why your proposal ensures reproducible product potency.

k) In a (b) (4) vial drug product fill, the sampling plan calls for (b) (4) (b) (4) to be collected for moisture content testing. (b) (4) vials are tested and the mean value is reported on the certificate of analysis. Because the moisture content in an individual vial will vary within any given lot, the proposed sampling plan should provide a reasonable assessment of the variability of the results within a lot. While data from a robust validation study will provide a basis for establishing the sampling plan for the moisture specification, your current sample size and the mean value set as the reportable result are insufficient to assess the moisture content of the final drug product. Please submit the revised specification for moisture content with these considerations in mind and provide a justification for your proposal.

l) Chromatograms for drug substance and drug product RP-HPLC analyses contain data from (b) (4). Perform the RP-HPLC analysis such that data from (b) (4) is included so that all potential impurities and contaminants can be detected and controlled if necessary. Provide chromatograms where all data are shown (b) (4) on lots evaluated in the (b) (4)

m) The isoelectric focusing (IEF) assay has acceptance criteria of (b) (4) in a pI range of (b) (4) reportedly because of assay variation. This level of assay variability is not consistent with the expected validation characteristics for this type of assay. Develop, implement, and provide data on a validated IEF method in which the reference standard always produces the same number of bands in a consistent pI range. In addition, each gel should have a quantity of reference standard loaded near the limits of detection to verify the sensitivity of the analysis.

n) The (b) (4) results are rounded off to the nearest integer which can mask significant differences in (b) (4) between lots. Report all (b) (4) results to two significant digits without rounding off to the nearest integer, revise the acceptance criterion accordingly and submit the revised specification.

o) (b) (4)

p) The peptide map specification calls for (b) (4) peptide peaks where a countable peak is defined as (b) (4). Justify the use of this acceptance criterion in light of the potential amounts of impurities and contaminants that would be acceptable, or revise the criteria for countable peaks. Also, include a revision of

the acceptance criteria such that relative peak areas on several selected peptides are specified. Provide the new specification together with supporting data.

q) A host cell protein standard curve is used to determine the levels of host cell proteins in drug substance. The data from the standard curve is fit to a four parameter logistic regression model even though the data doesn't reach a plateau and the fitted curve is not fully determined. However, there is a simple linear relationship between host cell protein and assay response. Provide a justification for the use of a four parameter logistic regression model or use a linear regression model to generate a host cell protein standard curve. Submit the revised specification along with the supporting analytical method validation data.

2) Please respond to the following deficiencies regarding comparability:

a) The relative amounts of the individual glycans in the glycan profile shifted upon the switch to (b) (4). Since the glycan structures are critical to taliglucerase alfa's mechanism-of-action, a change in the concentration of the glycan structures has the potential to adversely impact clinical performance. Using a potency assay that quantitatively measures specific receptor binding and/or high affinity internalization into cells (see previous comment), perform a head-to-head comparison of three drug substance lots of taliglucerase alfa manufactured in (b) (4)

b) Results for SE-HPLC data provided in the NDA are reported as (b) (4). As (b) (4) may represent a different risk to product quality, they should be independently monitored and controlled. To support your revised acceptance criteria, provide all SE-HPLC data available to date in the application with (b) (4) reported separately. For comparison purposes, provide tabulated drug product stability SE-HPLC data separating drug product lots that were manufactured with drug substance made exclusively in (b) (4)

c) Your SE-HPLC test method employed a UV detector. However, use of a light scattering detector may allow (b) (4) that migrate in the void volume to be observed following SE-HPLC. This provides a much more sensitive qualitative method for monitoring this product attribute. Perform a head-to-head comparison of three drug product lots manufactured exclusively from drug substance made in (b) (4) using light scatter detection and submit the results to your application.

3) Please respond to the following deficiencies regarding process validation:

a) The time limits for individual manufacturing steps and for the complete manufacturing process are not clearly defined in the NDA. For example, strict limits for (b) (4)

Provide this information and relate it to the processes used to manufacture clinical study lot PB-06-001, commercial validation lots and the genomic stability sequencing study.

b) Process validation reports indicate that vials containing drug product were put on (b) (4) Validation of the lyophilization process should include assessment of vials placed on (b) (4) in different positions within a shelf to confirm consistency of the lyophilization process. Provide a revised validation protocol and report including the results for moisture content testing.

c) (b) (4)

4) Please respond to the following deficiencies regarding control of impurities:

a) The testing to demonstrate that the master cell bank was free of plant specific viruses tabulated the results without providing data on the suitability of the PCR methods to detect viruses. In order to interpret the results you provided, we need to assess whether the methods are suitable for their intended purpose. Provide the assay qualification data and a description of the system suitability controls for each PCR method used to detect plant specific viruses.

b) The compound (b) (4) is a component of the (b) (4) and levels in drug substance or drug product were not determined. (b) (4) and may exhibit toxicity to humans (b) (4) and is therefore viewed as a (b) (4) impurity that should be well controlled. Provide a control strategy to either include a limit on (b) (4) to a level that will not impact product quality as it may relate to safety or efficacy, or validate that the process can clear (b) (4) to an appropriate level.

c) (b) (4) but its final concentration in drug product has not been determined. The label should accurately describe the final concentration of all excipients which should be confirmed at release. Provide the results on the (b) (4) concentration for three drug product lots and provide your justification for not implementing the determination of (b) (4) as a drug product release test.

V. EXECUTIVE SUMMARY

A. Description of the Product

Taliglucerase alfa is a parenteral single use lyophilized drug consisting of human glucocerebrosidase. Taliglucerase alfa is produced in a carrot cell line and is purified to homogeneity using standard chromatographic techniques. Taliglucerase alfa is a single polypeptide chain containing the exact amino acid sequence of human glucocerebrosidase (b) (4)

The molecular weight is ~ 60,800 Da (b) (4)

Glucocerebrosidases are enzymes that catalyze the hydrolysis of long chain fatty acid glucocerebrosides to glucose and ceramide. The hydrolysis reaction occurs via a double displacement reaction where sugar-enzyme intermediates are formed and hydrolyzed. No cofactors or metal ions are required for catalysis. Due to assay difficulties with glucocerebrosides, enzyme activity is measured by monitoring the hydrolysis of p-nitrophenyl- β -glucopyranoside (pNP-Glc) which contains the critical glycosidic bond.

Glucocerebrosidases are used as standard of care enzyme replacement therapies for patients with Gaucher's Disease. Gaucher's Disease is a lysosomal storage disorder that is marked by the inability to clear cellular glucocerebrosides resulting in their accumulation in the lysosomes of tissue macrophages primarily in the spleen, liver and bone marrow. The lyophilized drug product, containing the excipients mannitol, polysorbate 80, and sodium citrate, is reconstituted in sterile water. The dosing regimen is 60 U/kg once every two weeks by IV infusion over 1-2 hours.

Manufacturing taliglucerase alfa using the carrot cell substrate has several advantages. The growth media is devoid of any mammalian derived products (including serum) thus avoiding issues with mammalian adventitious viral agents. In addition, the (b) (4) fashion by which glycan structures are synthesized in (b) (4)

Taliglucerase alfa with terminal mannose residues is critical to its mechanism-of-action (and glucocerebrosidases as an enzyme replacement therapy) since it is through the mannose receptor that taliglucerase alfa is internalized into the macrophages that have accumulated high levels of glucocerebrosides.

B. Clinical Trial Information

Gaucher's Disease is a lysosomal storage disorder that is marked by the inability to clear cellular glucocerebrosides resulting in their accumulation in the lysosomes of tissue macrophages primarily in the spleen, liver and bone marrow. Enzyme replacement therapies with glucocerebrosidase are the current standard of care for patients with Gaucher's Disease.

Exogenous glucocerebrosidases are administered intravenously over several hours once every two weeks. Glucocebrosidases are endocytosed by macrophage cells via binding to macrophage mannose receptors. Once inside macrophage cells, glucocebrosidases pass through the endosomal compartments until they are delivered to the lysosome. In the lysosome, glucocebrosidases cleave the accumulated glucocerebrosides to generate glucose and ceramide.

The safety and efficacy data provided to support taliglucerase alfa for the treatment of Gaucher's Disease were from a single Phase III trial enrolling 31 patients naïve to enzyme replacement therapy (PB-06-001). Patients were broken down into two groups of 30 or 60 units/Kg. Efficacy was primarily determined by reduction in spleen volume by MRI, but also supported by changes in hemoglobin levels, platelet counts and liver volume. The duration of the study was nine months.

Other trials to support safety and efficacy include a Phase I non-randomized open label safety trial (P-01-2005), a switch-over trial for patients that were stable on imiglucerase (Cerezyme) enzyme replacement therapy (PB-06-002), and a trial (PB-06-003) open to patients who have completed either PB-06-001 and PB-06-002.

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/s/

GIBBES R JOHNSON
02/24/2011

AMY S ROSENBERG
02/24/2011



Quality Review: Taliglucerase alfa

From: **Richard Ledwidge, Ph.D.**
Division of Therapeutic Proteins (DTP)
Office of Biotechnology Products (OBP)

Through: **Gibbes Johnson, PhD**
Team Leader
DTP, OBP
Barry Cherney, PhD
Deputy Division Director
DTP, OBP

NDA Number: **022458**
Product: **Elelyso**
Sponsor: **Protalix, Inc.**

Date of review: **2/22/2011**
Received Date: **4/26/2010**
PDUFA Date: **2/26/2011**

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RECOMMENDATION: Complete Response

DEFICIENCIES CMC COMMENTS

1) Please respond to the following deficiencies regarding specifications and assay validation:

a) Results of USP<788> particulate testing and appearance testing on reconstituted drug product have not been submitted to the NDA. Both tests provide a useful measure of product quality that is not monitored by other tests you have proposed. Add these tests to the release and stability specifications and provide available results for release and stability testing of the three conformance lots and any additional results you may have.

b) A potency assay that quantitatively measures specific receptor binding and/or high affinity internalization into cells is required since internalization is a critical component of taliglucerase alfa's mechanism-of-action and it is not fully assessed in your current potency assay. The assay should use multiple taliglucerase alfa concentrations to generate a complete dose-response curve in order to calculate the half-maximal effective concentration (EC_{50app}). Develop and implement this assay for use in release and stability testing.

c) Some SE-HPLC chromatographs exhibit a (b) (4). Because this (b) (4) may reflect variability in a product-related variant, it should be identified and, if necessary, controlled. Characterize the protein in the (b) (4) and determine whether a control strategy that better monitors this product attribute(s) should be implemented. Provide the results of your analyses and any proposed changes to your specifications.

d) RP-HPLC chromatograms suggest that taliglucerase alfa variants (b) (4). The risk to product quality is expected to vary depending on the nature of the variant. Thus, in order to establish an appropriate control strategy, you should identify and control for the quantity of these variants, if present. It may be useful to alter assay conditions or gradients (b) (4). Provide information on the presence of unresolved variants and, if present, provide a revised specification that more accurately quantitates and controls these variants together with supporting data.

e) Enzyme kinetic parameters and specific activity are measured using synthetic p-nitrophenyl-gluco-pyranoside (pNP-Glc) substrate. pNP-Glc (b) (4). Provide enzyme kinetic data to determine the enzyme kinetic parameters, K_m and k_{cat} , (b) (4) glucocerebroside substrate on three lots manufactured in (b) (4). Include a detailed description of the assay, supporting

assay qualification data, as well as a justification for why this test should not be added to the release and stability specifications.

f) Stability testing of diluted drug product in infusion bags did not include USP<788> particulate testing or information on the impact of dilution on subvisible particulates that are between (b) (4). USP<788> testing results are critical to mitigate the risk associated with occlusion of small blood vessels and small subvisible particles may pose an immunogenicity risk. Provide USP<788> particulate testing data for in-use stability studies and an analysis of particulates between (b) (4).

g) The mannose content specification is based on a MALDI-TOF analysis of taliglucerase alfa. However, the property that is being measured in the MALDI-TOF analysis is mass to charge ratio, not mannose content. Thus, the acceptance criterion should be set around the mass to charge ratio and the mannose content acceptance criterion should be removed from the MALDI-TOF specification. Provide the new specification together with supporting data.

h) The acceptance criterion for moisture content in drug product is (b) (4) for both release and stability testing. Release and stability testing results consistently show moisture content to be below (b) (4) and no data were submitted indicating that (b) (4) moisture content would not have an adverse impact on product stability throughout the product's dating period. Amend the moisture content acceptance criterion to reflect your manufacturing capability and consideration of any additional knowledge you may have concerning the impact of moisture on product stability and provide the new specification, if appropriate, together with supporting data.

i) Monosaccharide content and glycan structure analysis submitted in the characterization section of the NDA contained inconsistent results. Monosaccharide content analysis on two batches indicated that the (b) (4) (b) (4) whereas the glycan analysis data determined that (b) (4) of the glycan structures have a (b) (4). Provide an explanation for these results or submit data that identify the more accurate analysis using batches made in (b) (4).

j) The acceptance criteria for the enzyme kinetic parameters K_m and V_{max} are (b) (4), respectively. An analysis of 40 drug substance batches resulted in mean and standard deviations for K_m and V_{max} equal to (b) (4), respectively. Consequently, the acceptance criteria appear too wide and should be amended to reflect process capability and clinical experience. Provide the revised specification for enzyme kinetic parameters or your justification as to why your proposal ensures reproducible product potency.

k) In a (b) (4) vial drug product fill, the sampling plan calls for (b) (4) (b) (4) to be collected for moisture content testing. (b) (4) vials are tested and the mean value is reported on the certificate of analysis. Because the moisture content in an individual vial will vary within any given

lot, the proposed sampling plan should provide a reasonable assessment of the variability of the results within a lot. While data from a robust validation study will provide a basis for establishing the sampling plan for the moisture specification, your current sample size and the mean value set as the reportable result are insufficient to assess the moisture content of the final drug product. Please submit the revised specification for moisture content with these considerations in mind and provide a justification for your proposal.

l) Chromatograms for drug substance and drug product RP-HPLC analyses contain data from (b) (4). Perform the RP-HPLC analysis such that data from (b) (4) and from (b) (4) is included so that all potential impurities and contaminants can be detected and controlled if necessary. Provide chromatograms where all data are shown (b) (4) on lots evaluated in the (b) (4).

m) The isoelectric focusing (IEF) assay has acceptance criteria of (b) (4) in a pI range of (b) (4) reportedly because of assay variation. This level of assay variability is not consistent with the expected validation characteristics for this type of assay. Develop, implement, and provide data on a validated IEF method in which the reference standard always produces the same number of bands in a consistent pI range. In addition, each gel should have a quantity of reference standard loaded near the limits of detection to verify the sensitivity of the analysis.

n) The (b) (4) assay results are rounded off to the nearest integer which can mask significant differences in (b) (4) between lots. Report all (b) (4) assay results to two significant digits without rounding off to the nearest integer, revise the acceptance criterion accordingly and submit the revised specification.

o) (b) (4)

p) The peptide map specification calls for (b) (4) peptide peaks where a countable peak is defined as (b) (4). Justify the use of this acceptance criterion in light of the potential amounts of impurities and contaminants that would be acceptable, or revise the criteria for countable peaks. Also, include a revision of the acceptance criteria such that relative peak areas on several selected peptides are specified. Provide the new specification together with supporting data.

q) A host cell protein standard curve is used to determine the levels of host cell proteins in drug substance. The data from the standard curve is fit to a four parameter logistic regression model even though the data doesn't reach a plateau and the fitted curve is not fully determined. However, there is a simple linear relationship between host cell protein and assay response. Provide a justification for the use of a four parameter logistic regression model or use a linear regression model to generate a host cell protein standard curve. Submit the revised specification along with the supporting analytical method validation data.

2) Please respond to the following deficiencies regarding comparability:

a) The relative amounts of the individual glycans in the glycan profile shifted upon the switch (b) (4). Since the glycan structures are critical to taliglucerase alfa's mechanism-of-action, a change in the concentration of the glycan structures has the potential to adversely impact clinical performance. Using a potency assay that quantitatively measures specific receptor binding and/or high affinity internalization into cells (see previous comment), perform a head-to-head comparison of three drug substance lots of taliglucerase alfa manufactured (b) (4).

b) Results for SE-HPLC data provided in the NDA are reported as (b) (4). As (b) (4) may represent a different risk to product quality, they should be independently monitored and controlled. To support your revised acceptance criteria, provide all SE-HPLC data available to date in the application with (b) (4) reported separately. For comparison purposes, provide tabulated drug product stability SE-HPLC data separating drug product lots that were manufactured with drug substance made exclusively in (b) (4).

c) Your SE-HPLC test method employed a UV detector. However, use of a light scattering detector may allow (b) (4) following SE-HPLC. This provides a much more sensitive qualitative method for monitoring this product attribute. Perform a head-to-head comparison of three drug product lots manufactured exclusively from drug substance made in (b) (4) using light scatter detection and submit the results to your application.

3) Please respond to the following deficiencies regarding process validation:

a) The time limits for individual manufacturing steps and for the complete manufacturing process are not clearly defined in the NDA. For example, strict limits for (b) (4). Provide this information and relate it to the processes used to

manufacture clinical study lot PB-06-001, commercial validation lots and the genomic stability sequencing study.

b) Process validation reports indicate that vials containing drug product were put on [REDACTED] (b) (4). Validation of the lyophilization process should include assessment of vials [REDACTED] (b) (4) and in different positions within a shelf to confirm consistency of the lyophilization process. Provide a revised validation protocol and report including the results for moisture content testing.

c) [REDACTED] (b) (4)

4) Please respond to the following deficiencies regarding control of impurities:

a) The testing to demonstrate that the master cell bank was free of plant specific viruses tabulated the results without providing data on the suitability of the PCR methods to detect viruses. In order to interpret the results you provided, we need to assess whether the methods are suitable for their intended purpose. Provide the assay qualification data and a description of the system suitability controls for each PCR method used to detect plant specific viruses.

b) The compound [REDACTED] (b) (4) is a component [REDACTED] (b) (4) and levels in drug substance or drug product were not determined. [REDACTED] (b) (4) may exhibit toxicity to humans [REDACTED] (b) (4) and is therefore viewed as a [REDACTED] (b) (4) impurity that should be well controlled. Provide a control strategy to either include a limit on [REDACTED] (b) (4) to a level that will not impact product quality as it may relate to safety or efficacy, or validate that the process can clear [REDACTED] (b) (4) to an appropriate level.

c) [REDACTED] (b) (4) but its final concentration in drug product has not been determined. The label should accurately

describe the final concentration of all excipients which should be confirmed at release. Provide the results on the (b) (4) concentration for three drug product lots and provide your justification for not implementing the determination of (b) (4) as a drug product release test.

SUMMARY OF QUALITY ASSESSMENTS

1. General Information

Drug substance and drug product manufacturers and their responsibilities are shown in the table below.

Table 1 – Manufacturer

Name and Address	Responsibilities
Protalix Ltd. 2 Snunit St. Science Park Carmiel 20100 Israel Facility registration number: 600721419	(b) (4)
(b) (4)	

3.2.P.3.1 Manufacturer(s)

Table 1 - Manufacturers for the Drug Product

Name and Address	Responsibilities
(b) (4)	

(b) (4)

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RICHARD LEDWIDGE
02/23/2011

GIBBES R JOHNSON
02/24/2011