CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125359Orig1s000

<u>OTHER REVIEW(S)</u>

Product Quality (CMC) PMR/PMC Development Template
TO BE USED FOR PMCS NOT REPORTABLE UNDER 506(B)

This template should be completed by the review chemist (ONDQA) or review biologist/microbiologist (OBP/BMAB) and included for ***each*** type of CMC PMR/PMC in the Action Package. See #4 below for list of CMC PMR/PMC types

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PMC #1 Description: To conduct a container closure integrity study and determine the sensitivity of the test methods. Submit the final study report with validation data for the container closure integrity tests by December 31, 2011.

PMC Schedule Milestones:

Final protocol Submission Date:	MM/YYYY
Study Completion Date:	MM/YYYY
Final Report Submission Date:	12/31/2011
Other: _____	MM/YYYY

PMC #2 Description: To conduct performance qualification of the Erwinaze lyophilization process and submit the qualification data by March 31, 2012.

PMC Schedule Milestones:

Final protocol Submission Date:	MM/YYYY
Study Completion Date:	MM/YYYY
Final Report Submission Date:	03/31/2012
Other: _____	MM/YYYY

PMC #3 Description: To provide validation data from the executed protocol for shipping Erwinaze drug product from the (b) (4) o the US market by December 31, 2011.

PMC Schedule Milestones:

Final protocol Submission Date:	MM/YYYY
Study Completion Date:	MM/YYYY
Final Report Submission Date:	12/31/2011
Other: _____	MM/YYYY

PMC #4 Description: To conduct a study to substantiate the use of (b) (4) as the (b) (4) (b) (4) and submit the validation study report by December 31, 2011.

PMC Schedule Milestones:

Final protocol Submission Date:	MM/YYYY
Study Completion Date:	MM/YYYY
Final Report Submission Date:	12/31/2011
Other: _____	MM/YYYY

PMC #5 Description: To collect data from rabbit pyrogen testing on three lots of thawed and diluted drug substance solution prior to (b) (4). Submit a

final report containing a description of the method, the rabbit pyrogen test results, and an assessment of the impact of [redacted] (b) (4) on drug product quality and the drug product manufacturing process by June 30, 2012.

PMC Schedule Milestones:		
	Final protocol Submission Date:	MM/YYYY
	Study Completion Date:	MM/YYYY
	Final Report Submission Date:	06/30/2012
	Other: _____	MM/YYYY

- **ADD MORE AS NEEDED USING THE SAME TABULAR FORMAT FOR EACH PMC.**
- **INCLUDE DESCRIPTIONS AND MILESTONES IN THE TABLE ABOVE FOR ALL CMC/OBP NON-REPORTABLE PMCS *FOR WHICH THE FOLLOWING ANSWERS WILL BE IDENTICAL.* USE A SEPARATE TEMPLATE FOR EACH PMR/PMC FOR WHICH THE ANSWERS TO THE FOLLOWING QUESTIONS DIFFER.**
- ***DO NOT USE THIS FORM* IF ANY STUDIES WILL BE REQUIRED UNDER FDAAA OR WILL BE PUBLICLY REPORTABLE**

1. During application review, explain why this issue is appropriate for a PMC instead of a pre-approval requirement. Check the reason below and describe.

   - ☒ Need for drug (Unmet need/ Life-threatening condition)
   - ☐ Long-term data needed (e.g., stability data)
   - ☐ Only feasible to conduct post-approval
   - ☐ Improvements to methods
   - ☐ Theoretical concern
   - ☐ Manufacturing process analysis
   - ☒ Other

For PMC#1, some container closure integrity test data were submitted in the BLA. However, sensitivity of the test methods and validation data for use in stability studies was not provided. A revised protocol for container closure integrity study was provided. Data from the execution of this protocol will be available by December 31, 2011.

For PMC#2, the qualification data provided for lyophilization process is incomplete with respect to load configuration, temperature uniformity, and worst case shelf location for moisture. A performance qualification study will be performed and this data will be available by March 31, 2012.

Foe PMC#3, a protocol for commercial shipping of Erwinaze drug product from (b) (4) to US market was submitted in the BLA. Data from the executed protocol will be available by December 31, 2011.

For PMC#4, data to support (b) (4) was provided but data to substantiate the (b) (4) and its continued effectiveness was not provided. A study will be conducted to substantiate the (b) (4) and the study report will be available by December 31, 2011.

For PMC#5, the expectation is that pyrogenic substances are significantly reduced and/or removed during the drug substance purification process and not during the drug product manufacturing process. The use o (b) (4) in the drug product manufacturing process to remove pyrogens seems to be a legacy part of the manufacturing process and not needed. Data from rabbit pyrogen testing on three lots of thawed and diluted drug substance solution prior to (b) (4) will be collected and the final study report containing procedure, test results and assessment of impact on drug product quality and manufacturing will be submitted by June 30, 2012.

2. Describe the particular review issue and the goal of the study.

For PMC#1, the goal of the study is to validate the container closure integrity test for the stability program and determine the sensitivity of the test method. Information included in the BLA was insufficient.

For PMC#2, the goal of the study is to obtain performance qualification data for the Erwinaze lyophilization process. There was insufficient information in the BLA. The lyophilization process will be monitored for moisture in the interim.

For PMC#3, the goal of the study is to obtain commercial shipping validation data. The BLA included a shipping validation protocol that has not been executed. Supportive shipping data was included in the submission.

For PMC#4, the goal of the study is obtain validation data to substantiate the (b) (4) and its continued effectiveness. (b) (4) study and information on the bioburden content of (b) (4) were included in the submission but the validation data was incomplete.

For PMC#5, the goal of the study is to understand the impact of (b) (4) on drug product quality and the drug product manufacturing process. The manufacturing process uses (b) (4) drug product manufacture. The current Agency expectation is to (b) (4) during drug substance manufacturing and (b) (4) (b) (4) (b) (4) ns.

3. [OMIT—for PMRs only]

4. What type of study is agreed upon (describe and check the type below)?

Select only one. Fill out a new sheet for each type of PMR/PMC study.

- ☐ Dissolution testing
- ☐ Assay
- ☐ Sterility
- ☐ Potency
- ☐ Product delivery
- ☐ Drug substance characterization
- ☐ Intermediates characterization
- ☐ Impurity characterization
- ☐ Reformulation
- ☐ Manufacturing process issues
- ☒ Other

Describe the Agreed upon study:

PMC#1: To conduct a container closure integrity study and determine the sensitivity of the test methods. Submit the final study report with validation data for the container closure integrity tests by December 31, 2011.

PMC#2: To conduct performance qualification of the Erwinaze lyophilization process and submit the qualification data by March 31, 2012.

PMC#3: To provide validation data from the executed protocol for shipping Erwinaze drug product from the (b) (4) to the US market by December 31, 2011.

PMC#4: To conduct a study to substantiate the use of (b) (4) (b) (4): and submit the validation study report by December 31, 2011.

PMC#5: To collect data from rabbit pyrogen testing on three lots of thawed and diluted drug substance solution prior to (b) (4)t. Submit a final report containing a description of the method, the rabbit pyrogen test results, and an assessment of the impact of (b) (4) on drug product quality and the drug product manufacturing process by June 30, 2012.

5. To be completed by ONDQA/OBP Manager:

CMC Reviewer
Suva
Kalavati Suvarna

- ☐ Does the study meet criteria for PMCs?
- ☐ Are the objectives clear from the description of the PMC?
- ☐ Has the applicant adequately justified the choice of schedule milestone dates?
- ☐ Has the applicant had sufficient time to review the PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

(signature line for BLAs only) Summers 11/9/11

Product Quality (CMC) PMR/PMC Development Template
TO BE USED FOR PMCS NOT REPORTABLE UNDER 506(B)

This template should be completed by the review chemist (ONDQA) or review biologist (OBP) and included for ***each*** type of CMC PMR/PMC in the Action Package. See #4 below for list of CMC PMR/PMC types

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PMC #1 Description: Implement the proposed process improvements described in BLA amendment # 11, dated March 4, 2011, and re-assess the bioburden and endotoxin limits. Submit bioburden and endotoxin data from three extraction batches by July 31, 2012.

PMC Schedule Milestones:

Final protocol Submission Date:	n/a
Study Completion Date:	n/a
Final Report Submission Date:	07/2012
Other:	n/a

1. During application review, explain why this issue is appropriate for a PMC instead of a pre-approval requirement. Check the reason below and describe.

- ☐ Need for drug (Unmet need/ Life-threatening condition)
- ☒ Long-term data needed (e.g., stability data)
- ☐ Only feasible to conduct post-approval
- ☐ Improvements to methods
- ☐ Theoretical concern
- ☐ Manufacturing process analysis
- ☐ Other

The firm's proposed process improvements are designed to reduce bioburden during the extraction and purification steps. Implementation of these improvements is not a pre-approval requirement because (b) (4) purification steps have consistently met established limits for bioburden and endotoxin.

2. Describe the particular review issue and the goal of the study.

Multiple (b) (4) bioburden samples for the batches used to validate the extraction and purification process exceeded the limits established by the applicant. The applicant has proposed several improvements to the drug substance manufacturing process that are designed to reduce bioburden. The effectiveness of these improvements will be demonstrated with bioburden and endotoxin data (b) (4)

3. [OMIT—for PMRs only]

4. What type of study is agreed upon (describe and check the type below)?

Select only one. Fill out a new sheet for each type of PMR/PMC study.

- ☐ Dissolution testing
- ☐ Assay
- ☐ Sterility
- ☐ Potency
- ☐ Product delivery
- ☐ Drug substance characterization
- ☐ Intermediates characterization
- ☐ Impurity characterization
- ☐ Reformulation
- ☒ Manufacturing process issues

Implement the proposed process improvements described in BLA amendment # 11, dated March 4, 2011, and re-assess the bioburden and endotoxin limits. Submit bioburden and endotoxin data from three extraction batches by July 31, 2012.

☐

Other

Describe the Agreed upon study:

5. To be completed by ONDQA/OBP Manager:

- ☒ Does the study meet criteria for PMCs?
- ☒ Are the objectives clear from the description of the PMC?
- ☒ Has the applicant adequately justified the choice of schedule milestone dates?
- ☒ Has the applicant had sufficient time to review the PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

Summer

(signature line for BLAs only) 11/8/11

Mary E. Fanbrua

Product Quality (CMC) PMR/PMC Development Template
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This template should be completed by the review chemist (ONDQA) or review biologist (OBP) and included for ***each*** type of CMC PMR/PMC in the Action Package. See #4 below for list of CMC PMR/PMC types

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PMC #3 Description: Qualification of bioburden and endotoxin in-process test methods:

a. Complete the qualification of the endotoxin assay using two additional batches of drug substance and submit the report by July 31, 2012.
b. Complete the qualification of the bioburden assay using two additional batches of drug substance and submit the report by July 31, 2012.

PMC Schedule Milestones:

Final protocol Submission Date:	n/a
Study Completion Date:	n/a
Final Report Submission Date:	07/2012
Other: _____	n/a

1. During application review, explain why this issue is appropriate for a PMC instead of a pre-approval requirement. Check the reason below and describe.

- ☐ Need for drug (Unmet need/ Life-threatening condition)
- ☐ Long-term data needed (e.g., stability data)
- ☐ Only feasible to conduct post-approval
- ☒ Improvements to methods
- ☐ Theoretical concern
- ☐ Manufacturing process analysis
- ☐ Other

These are not pre-approval requirements because the assays in question are compendial and do not require full validation. The applicant has qualified the assays with a single batch of drug substance but is being requested to complete the qualification using additional drug substance batches.

2. Describe the particular review issue and the goal of the study.

The bioburden and endotoxin assays are compendial assays and have been qualified using one batch of drug substance. Confirmation using two additional batches should be completed.

3. [OMIT—for PMRs only]

4. What type of study is agreed upon (describe and check the type below)?

Select only one. Fill out a new sheet for each type of PMR/PMC study.

- ☐ Dissolution testing
- ☐ Assay
- ☐ Sterility
- ☐ Potency
- ☐ Product delivery
- ☒ Drug substance characterization
- ☐ Intermediates characterization
- ☐ Impurity characterization
- ☐ Reformulation
- ☐ Manufacturing process issues

Qualification of bioburden and endotoxin in-process test methods: a. Complete the qualification of the endotoxin assay using two additional batches of drug substance and submit the report by July 31, 2012. b. Complete the qualification of the bioburden assay using two additional batches of drug substance and submit the report by July 31, 2012.	☐

Other

Describe the Agreed upon study:

5. To be completed by ONDQA/OBP Manager:

- ☒ Does the study meet criteria for PMCs?
- ☒ Are the objectives clear from the description of the PMC?
- ☒ Has the applicant adequately justified the choice of schedule milestone dates?
- ☒ Has the applicant had sufficient time to review the PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

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(signature line for BLAs only) 11/8/11

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PMC #2 Description: Monitor bioburden and endotoxin levels in CM6 pooled fractions, CM8 pooled fractions, and DEAE pooled fractions held for more than 24 hours at scale. (b) (4) (b) (4)

PMC Schedule Milestones:

Final protocol Submission Date:	n/a
Study Completion Date:	n/a
Final Report Submission Date:	11/2012
Other:	n/a

1. During application review, explain why this issue is appropriate for a PMC instead of a pre-approval requirement. Check the reason below and describe.

- ☐ Need for drug (Unmet need/ Life-threatening condition)
- ☒ Long-term data needed (e.g., stability data)
- ☐ Only feasible to conduct post-approval
- ☐ Improvements to methods
- ☐ Theoretical concern
- ☐ Manufacturing process analysis
- ☐ Other

The applicant needs to conduct hold time validation studies of process intermediates at scale. Completion of these studies will increase microbial control in the process. This request is appropriate for a PMC rather than a pre-approval requirement because the risk of microbial contamination is mitigated by other microbial controls in place during the manufacturing process.

2. Describe the particular review issue and the goal of the study.

The process intermediate hold times need to be validated at scale for microbial control. The hold times have been validated using samples held at laboratory scale only.

3. [OMIT—for PMRs only]

4. What type of study is agreed upon (describe and check the type below)?

Select only one. Fill out a new sheet for each type of PMR/PMC study.

- ☐ Dissolution testing
- ☐ Assay
- ☐ Sterility
- ☐ Potency
- ☐ Product delivery
- ☐ Drug substance characterization
- ☐ Intermediates characterization
- ☐ Impurity characterization
- ☐ Reformulation
- ☒ Manufacturing process issues

Monitor bioburden and endotoxin levels in CM6 pooled fractions, CM8 pooled fractions, and DEAE pooled fractions held for more than 24 hours at scale. (b) (4) (b) (4) (b) (4)

☐

Other

Describe the Agreed upon study:

5. To be completed by ONDQA/OBP Manager:

- ☒ Does the study meet criteria for PMCs?
- ☒ Are the objectives clear from the description of the PMC?
- ☒ Has the applicant adequately justified the choice of schedule milestone dates?
- ☒ Has the applicant had sufficient time to review the PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

Summer

(signature line for BLAs only) 11/8/11

Mary E. Gardner

Product Quality (CMC) PMR/PMC Development Template
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This template should be completed by the review chemist (ONDQA) or review biologist/microbiologist (OBP/BMAB) and included for ***each*** type of CMC PMR/PMC in the Action Package. See #4 below for list of CMC PMR/PMC types

---

PMC #1 Description: To conduct a container closure integrity study and determine the sensitivity of the test methods. Submit the final study report with validation data for the container closure integrity tests by December 31, 2011.

PMC Schedule Milestones:

Final protocol Submission Date:	MM/YYYY
Study Completion Date:	MM/YYYY
Final Report Submission Date:	12/31/2011
Other:	MM/YYYY

PMC #2 Description: To conduct performance qualification of the Erwinaze lyophilization process and submit the qualification data by March 31, 2012.

PMC Schedule Milestones:

Final protocol Submission Date:	MM/YYYY
Study Completion Date:	MM/YYYY
Final Report Submission Date:	03/31/2012
Other:	MM/YYYY

PMC #3 Description: To provide validation data from the executed protocol for shipping Erwinaze drug product from the (b) (4) to the US market by December 31, 2011.

PMC Schedule Milestones:

Final protocol Submission Date:	MM/YYYY
Study Completion Date:	MM/YYYY
Final Report Submission Date:	12/31/2011
Other:	MM/YYYY

PMC #4 Description: To conduct a study to substantiate the use (b) (4) (b) (4) and submit the validation study report by December 31, 2011.

PMC Schedule Milestones:

Final protocol Submission Date:	MM/YYYY
Study Completion Date:	MM/YYYY
Final Report Submission Date:	12/31/2011
Other:	MM/YYYY

PMC #5 Description: To collect data from rabbit pyrogen testing on three lots of thawed and diluted drug substance solution prior to (b) (4). Submit a

final report containing a description of the method, the rabbit pyrogen test results, and an assessment of the impact of [redacted] (b) (4) on drug product quality and the drug product manufacturing process by June 30, 2012.

PMC Schedule Milestones:		
	Final protocol Submission Date:	MM/YYYY
	Study Completion Date:	MM/YYYY
	Final Report Submission Date:	06/30/2012
	Other: _____	MM/YYYY

- **ADD MORE AS NEEDED USING THE SAME TABULAR FORMAT FOR EACH PMC.**
- **INCLUDE DESCRIPTIONS AND MILESTONES IN THE TABLE ABOVE FOR ALL CMC/OBP NON-REPORTABLE PMCS *FOR WHICH THE FOLLOWING ANSWERS WILL BE IDENTICAL.* USE A SEPARATE TEMPLATE FOR EACH PMR/PMC FOR WHICH THE ANSWERS TO THE FOLLOWING QUESTIONS DIFFER.**
- ***DO NOT USE THIS FORM* IF ANY STUDIES WILL BE REQUIRED UNDER FDAAA OR WILL BE PUBLICLY REPORTABLE**

1. During application review, explain why this issue is appropriate for a PMC instead of a pre-approval requirement. Check the reason below and describe.

   ☒ Need for drug (Unmet need/ Life-threatening condition)
   ☐ Long-term data needed (e.g., stability data)
   ☐ Only feasible to conduct post-approval
   ☐ Improvements to methods
   ☐ Theoretical concern
   ☐ Manufacturing process analysis
   ☒ Other

For PMC#1, some container closure integrity test data were submitted in the BLA. However, sensitivity of the test methods and validation data for use in stability studies was not provided. A revised protocol for container closure integrity study was provided. Data from the execution of this protocol will be available by December 31, 2011.

For PMC#2, the qualification data provided for lyophilization process is incomplete with respect to load configuration, temperature uniformity, and worst case shelf location for moisture. A performance qualification study will be performed and this data will be available by March 31, 2012.

Foe PMC#3, a protocol for commercial shipping of Erwinaze drug product from (b) (4) o US market was submitted in the BLA. Data from the executed protocol will be available by December 31, 2011.

For PMC#4, data to support (b) (4) was provided but data to substantiate the (b) (4) and its continued effectiveness was not provided. A study will be conducted to substantiate the (b) (4) and the study report will be available by December 31, 2011.

For PMC#5, the expectation is that pyrogenic substances are significantly reduced and/or removed (b) (4) (b) (4) (b) (4) Data from rabbit pyrogen testing on three lots of thawed and diluted drug substance solution prior to (b) (4) will be collected and the final study report containing procedure, test results and assessment of impact on drug product quality and manufacturing will be submitted by June 30, 2012.

2. Describe the particular review issue and the goal of the study.

For PMC#1, the goal of the study is to validate the container closure integrity test for the stability program and determine the sensitivity of the test method. Information included in the BLA was insufficient.

For PMC#2, the goal of the study is to obtain performance qualification data for the Erwinaze lyophilization process. There was insufficient information in the BLA. The lyophilization process will be monitored for moisture in the interim.

For PMC#3, the goal of the study is to obtain commercial shipping validation data. The BLA included a shipping validation protocol that has not been executed. Supportive shipping data was included in the submission.

For PMC#4, the goal of the study is obtain validation data to substantiate the (b) (4) and its continued effectiveness. (b) (4) study and information on the bioburden content of (b) (4) were included in the submission but the validation data was incomplete.

For PMC#5, the goal of the study is to understand the impact of (b) (4) on drug product quality and the drug product manufacturing process. (b) (4) (b) (4) (b) (4)

3. [OMIT—for PMRs only]

4. What type of study is agreed upon (describe and check the type below)?

Select only one. Fill out a new sheet for each type of PMR/PMC study.

- ☐ Dissolution testing
- ☐ Assay
- ☐ Sterility
- ☐ Potency
- ☐ Product delivery
- ☐ Drug substance characterization
- ☐ Intermediates characterization
- ☐ Impurity characterization
- ☐ Reformulation
- ☐ Manufacturing process issues
- ☒ Other

Describe the Agreed upon study:

PMC#1: To conduct a container closure integrity study and determine the sensitivity of the test methods. Submit the final study report with validation data for the container closure integrity tests by December 31, 2011.

PMC#2: To conduct performance qualification of the Erwinaze lyophilization process and submit the qualification data by March 31, 2012.

PMC#3: To provide validation data from the executed protocol for shipping Erwinaze drug product from the (b) (4) to the US market by December 31, 2011.

PMC#4: To conduct a study to substantiate the use of (b) (4) (b) (4) and submit the validation study report by December 31, 2011.

PMC#5: To collect data from rabbit pyrogen testing on three lots of thawed and diluted drug substance solution prior to (b) (4) Submit a final report containing a description of the method, the rabbit pyrogen test results, and an assessment of the impact of (b) (4) on drug product quality and the drug product manufacturing process by June 30, 2012.

5. To be completed by ONDQA/OBP Manager:

- ☐ Does the study meet criteria for PMCs?
- ☐ Are the objectives clear from the description of the PMC?
- ☐ Has the applicant adequately justified the choice of schedule milestone dates?
- ☐ Has the applicant had sufficient time to review the PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

Summer 11/9/11

(signature line for BLAs only)

Product Quality (CMC) PMR/PMC Development Template
TO BE USED FOR PMCS NOT REPORTABLE UNDER 506(B)

This template should be completed by the review chemist (ONDQA) or review biologist (OBP) and included for ***each*** type of CMC PMR/PMC in the Action Package. See #4 below for list of CMC PMR/PMC types

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PMC #16 Description: To validate hold times for each DS process intermediate, where applicable, in order to demonstrate that the quality of Erwinaze DS is not affected. This study should include a worst case hold scenario, defined by the cumulative maximal time for each hold step along with an evaluation of the purity and potency of process intermediates and of the resulting DS. The complete hold times validation report and supporting test results together with any revisions in the established hold times will be submitted by December 31, 2013 in accordance with 21 CFR 601.12.

PMC Schedule Milestones:		
	Final protocol Submission Date:	MM/YYYY
	Study Completion Date:	MM/YYYY
	Final Report Submission Date:	12/2013
	Other: _____	MM/YYYY

- **ADD MORE AS NEEDED USING THE SAME TABULAR FORMAT FOR EACH PMC.**
- **INCLUDE DESCRIPTIONS AND MILESTONES IN THE TABLE ABOVE FOR ALL CMC/OBP NON-REPORTABLE PMCS *FOR WHICH THE FOLLOWING ANSWERS WILL BE IDENTICAL.* USE A SEPARATE TEMPLATE FOR EACH PMR/PMC FOR WHICH THE ANSWERS TO THE FOLLOWING QUESTIONS DIFFER.**
- ***DO NOT USE THIS FORM* IF ANY STUDIES WILL BE REQUIRED UNDER FDAAA OR WILL BE PUBLICLY REPORTABLE**

1. During application review, explain why this issue is appropriate for a PMC instead of a pre-approval requirement. Check the reason below and describe.

   - ☐ Need for drug (Unmet need/ Life-threatening condition)
   - ☐ Long-term data needed (e.g., stability data)
   - ☐ Only feasible to conduct post-approval
   - ☐ Improvements to methods
   - ☐ Theoretical concern
   - ☒ Manufacturing process analysis
   - ☐ Other

In order to perform a validation of the hold times, EUSA will have to manufacture additional lots of Erwinaze. Although the current lack of validation is an important product quality issue, it should not hold up approvability because EUSA has provided data that provisionally support the hold times. Additionally, due to the [redacted] (b) (4), the length of specific hold times (e.g. 6 and 12 months), and the limited demand for product, EUSA will not be able to provide the required data prior to December 2013. The lack of complete validation of hold times does not justify postponing BLA approval an additional two years.

2. Describe the particular review issue and the goal of the study.

EUSA has reported individual studies for the hold times of different intermediates, however, the requested worst case cumulative hold scenario for the Erwinaze DS manufacturing process has not been validated. The goal of the study is to validate the acceptable hold times of process intermediates to ensure that they do not affect the quality attributes of Erwinaze DS.

3. [OMIT—for PMRs only]

4. What type of study is agreed upon (describe and check the type below)?

Select only one. Fill out a new sheet for each type of PMR/PMC study.

- ☐ Dissolution testing
- ☐ Assay
- ☐ Sterility
- ☐ Potency
- ☐ Product delivery
- ☐ Drug substance characterization
- ☐ Intermediates characterization
- ☐ Impurity characterization
- ☐ Reformulation
- ☒ Manufacturing process issues
- ☐ Other

Describe the Agreed upon study:

EUSA will validate the hold times for each DS process intermediate through manufacturing Erwinaze DS and holding each process intermediate over the maximal period of time allowed under their respective in-process controls (worst case hold scenario). The purity and potency of Erwinaze DS manufactured under a worst case hold time scenario will allow EUSA to determine whether the hold times do not affect the quality attributes of the DS.

5. To be completed by ONDQA/OBP Manager:

- ☐ Does the study meet criteria for PMCs?
- ☐ Are the objectives clear from the description of the PMC?
- ☐ Has the applicant adequately justified the choice of schedule milestone dates?
- ☐ Has the applicant had sufficient time to review the PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

(signature line for BLAs only) Summer 11/9/11

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This template should be completed by the review chemist (ONDQA) or review biologist (OBP) and included for ***each*** type of CMC PMR/PMC in the Action Package. See #4 below for list of CMC PMR/PMC types

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PMC #21 Description: To provide an experimental plan to assess the types and amounts of smaller sub-visible particulates [redacted] (b) (4) in the final drug product under real-time and stress stability conditions along with a timetable for this work. The plan and a timescale for the subsequent assessment of the impact sub-visible particles may have on the quality, clinical safety and efficacy of Erwinaze Drug Product (DP) along with a control strategy will be provided by December 31, 2011 in accordance with 21 CFR 601.12.

PMC Schedule Milestones:		
	Final protocol Submission Date:	MM/YYYY
	Study Completion Date:	MM/YYYY
	Final Report Submission Date:	12/2011
	Other:	MM/YYYY

- **ADD MORE AS NEEDED USING THE SAME TABULAR FORMAT FOR EACH PMC.**
- **INCLUDE DESCRIPTIONS AND MILESTONES IN THE TABLE ABOVE FOR ALL CMC/OBP NON-REPORTABLE PMCS *FOR WHICH THE FOLLOWING ANSWERS WILL BE IDENTICAL.* USE A SEPARATE TEMPLATE FOR EACH PMR/PMC FOR WHICH THE ANSWERS TO THE FOLLOWING QUESTIONS DIFFER.**
- ***DO NOT USE THIS FORM* IF ANY STUDIES WILL BE REQUIRED UNDER FDAAA OR WILL BE PUBLICLY REPORTABLE**

1. During application review, explain why this issue is appropriate for a PMC instead of a pre-approval requirement. Check the reason below and describe.

- ☐ Need for drug (Unmet need/ Life-threatening condition)
- ☐ Long-term data needed (e.g., stability data)
- ☐ Only feasible to conduct post-approval
- ☐ Improvements to methods
- ☐ Theoretical concern
- ☒ Manufacturing process analysis
- ☐ Other

Regarding the presence of particulates in Erwinaze DP, EUSA should confirm the results obtained for particles smaller than [redacted] (b) (4) using an orthogonal method and perform a risk assessment of the impact of the particles on the quality, clinical safety and efficacy of Erwinaze DP. This issue should not hold up approvability because EUSA has developed and is currently using an assay to monitor the subvisible particulates content of Erwinaze DP at release and on stability. The studies requested in this PMC will provide some added assurance of product quality and safety.

2. Describe the particular review issue and the goal of the study.

EUSA is planning on using the compendial USP <788> light obscuration method to evaluate the presence of particulates that are smaller than [redacted] (b) (4) To confirm the results obtained it is necessary to evaluate their presence using a sensitive orthogonal method. EUSA will also evaluate the risk that these particulates may pose to the product quality.

3. [OMIT—for PMRs only]

4. What type of study is agreed upon (describe and check the type below)?

Select only one. Fill out a new sheet for each type of PMR/PMC study.

- ☐ Dissolution testing
- ☐ Assay
- ☐ Sterility
- ☐ Potency
- ☐ Product delivery
- ☒ Drug substance characterization
- ☐ Intermediates characterization
- ☐ Impurity characterization
- ☐ Reformulation
- ☐ Manufacturing process issues
- ☐ Other

Describe the Agreed upon study:

EUSA will provide an experimental plan for the analytical studies necessary to generate information on the types and amounts of sub-visible particulates in the range of [redacted] (b) (4) in the final Erwinaze DP under real-time and stress stability conditions and determine the relative accuracy of the light obscuration method through the use of a sensitive orthogonal technique. Following the generation of the data EUSA will submit an assessment of the risk that these particulates may pose to product quality, clinical safety and efficacy, and their strategy for controlling this risk.

5. To be completed by ONDQA/OBP Manager:

- ☐ Does the study meet criteria for PMCs?
- ☐ Are the objectives clear from the description of the PMC?
- ☐ Has the applicant adequately justified the choice of schedule milestone dates?
- ☐ Has the applicant had sufficient time to review the PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

Barry Chang

(signature line for BLAs only)

Summer 11/9/11

Product Quality (CMC) PMR/PMC Development Template
TO BE USED FOR PMCS NOT REPORTABLE UNDER 506(B)

This template should be completed by the review chemist (ONDQA) or review biologist (OBP) and included for ***each*** type of CMC PMR/PMC in the Action Package. See #4 below for list of CMC PMR/PMC types

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PMC #19 Description: To provide a revised protocol for qualification of the current and future Erwinaze reference standards as a prior approval supplement by December 31, 2011.

PMC Schedule Milestones:		
	Final protocol Submission Date:	MM/YYYY
	Study Completion Date:	MM/YYYY
	Final Report Submission Date:	12/2011
	Other:	MM/YYYY

- **ADD MORE AS NEEDED USING THE SAME TABULAR FORMAT FOR EACH PMC.**
- **INCLUDE DESCRIPTIONS AND MILESTONES IN THE TABLE ABOVE FOR ALL CMC/OBP NON-REPORTABLE PMCS *FOR WHICH THE FOLLOWING ANSWERS WILL BE IDENTICAL.* USE A SEPARATE TEMPLATE FOR EACH PMR/PMC FOR WHICH THE ANSWERS TO THE FOLLOWING QUESTIONS DIFFER.**
- ***DO NOT USE THIS FORM* IF ANY STUDIES WILL BE REQUIRED UNDER FDAAA OR WILL BE PUBLICLY REPORTABLE**

1. During application review, explain why this issue is appropriate for a PMC instead of a pre-approval requirement. Check the reason below and describe.

☐ Need for drug (Unmet need/ Life-threatening condition)
☐ Long-term data needed (e.g., stability data)
☐ Only feasible to conduct post-approval
☐ Improvements to methods
☐ Theoretical concern
☐ Manufacturing process analysis
X Other

The acceptance criteria of EUSA's current protocol for qualification of Erwinaze reference standard are only slightly tighter than those used for the release of Erwinaze drug product. In order to improve the qualification protocol for future reference standards and prevent drifting of their quality attributes, EUSA should further tighten acceptance criteria for release testing. The revised qualification protocol will have to be approved before a new Erwinaze reference standard is established. It is however not necessary to revise the qualification protocol prior to approval.

2. Describe the particular review issue and the goal of the study.

The protocol for qualification of the current and future Erwinaze reference standards should detail the assays used for release under appropriate acceptance criteria. Adequate testing should be performed to fully establish the identity and purity of the Erwinaze reference standard, as per ICH Q7.

3. [OMIT—for PMRs only]

4. What type of study is agreed upon (describe and check the type below)?

Select only one. Fill out a new sheet for each type of PMR/PMC study.

- ☐ Dissolution testing
- ☐ Assay
- ☐ Sterility
- ☐ Potency
- ☐ Product delivery
- ☐ Drug substance characterization
- ☐ Intermediates characterization
- ☐ Impurity characterization
- ☐ Reformulation
- ☐ Manufacturing process issues
- X Other

Describe the Agreed upon study:

EUSA should revise the qualification protocol for Erwinaze reference standard so that it includes a description of the tests being used (e.g. MALDI-TOF MS) along with their intended purposes (e.g. oligosaccharide quantification), their requirements (e.g. single vs. multiple reference standard aliquots to be tested), and their adequate acceptance criteria as per ICH Q6B.

5. To be completed by ONDQA/OBP Manager:

- ☐ Does the study meet criteria for PMCs?
- ☐ Are the objectives clear from the description of the PMC?
- ☐ Has the applicant adequately justified the choice of schedule milestone dates?
- ☐ Has the applicant had sufficient time to review the PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

(signature line for BLAs only) Simnc5 11/9/11

Product Quality (CMC) PMR/PMC Development Template
TO BE USED FOR PMCS NOT REPORTABLE UNDER 506(B)

This template should be completed by the review chemist (ONDQA) or review biologist (OBP) and included for ***each*** type of CMC PMR/PMC in the Action Package. See #4 below for list of CMC PMR/PMC types

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PMC #15 Description: To review the specifications for all release and stability test methods when the manufacture of a statistically significant number of Erwinaze Drug Substance (DS) and Drug Product (DP) lots is completed. The final report of this analysis together with any revised release and stability specifications will be submitted in accordance with 21 CFR 601.12 by October 31, 2013.

PMC Schedule Milestones:

Final protocol Submission Date:	MM/YYYY
Study Completion Date:	MM/YYYY
Final Report Submission Date:	10/2013
Other: _____	MM/YYYY

PMC #17 Description: To increase the assay sensitivity for SDS-PAGE. The revised assay together with the validation report and supporting test results will be submitted as a CBE-30 supplement by December 31, 2011.

PMC Schedule Milestones:

Final protocol Submission Date:	
Study Completion Date:	
Final Report Submission Date:	12/2011
Other: _____	

PMC #18 Description: To perform size exclusion chromatography (SEC) and analytical ultracentrifugation (AUC) testing in a side-by-side analysis of Erwinaze DS samples that have been subjected to stress conditions. Results of these studies together with any revisions to your control strategy will be submitted by March 31, 2012 in accordance with 21 CFR 601.12.

PMC Schedule Milestones:

Final protocol Submission Date:	
Study Completion Date:	
Final Report Submission Date:	03/2012
Other: _____	

PMC #20 Description: To submit an experimental plan for evaluating and if appropriate, implementing L-asparagine, as the substrate for measuring the K_m and k_{cat} of Erwinaze DS and DP. The plan will be submitted by December 31, 2011.

PMC Schedule Milestones:

Final protocol Submission Date:	
Study Completion Date:	
Final Report Submission Date:	

Other: Experimental Plan Submission Date 12/2011

PMC #22 Description: To revise the peptide mapping method used for Erwinaze DS and DP release testing in order to enable chromatographic base-line resolution of most peptide fragments while accounting for ~98% of the protein sequence. The revised assay together with the validation report will be submitted as a prior approval supplement by September 30, 2012.

PMC Schedule Milestones:

Final protocol Submission Date:	
Study Completion Date:	
Final Report Submission Date:	09/2012
Other:	

- **ADD MORE AS NEEDED USING THE SAME TABULAR FORMAT FOR EACH PMC.**
- **INCLUDE DESCRIPTIONS AND MILESTONES IN THE TABLE ABOVE FOR ALL CMC/OBP NON-REPORTABLE PMCS *FOR WHICH THE FOLLOWING ANSWERS WILL BE IDENTICAL.* USE A SEPARATE TEMPLATE FOR EACH PMR/PMC FOR WHICH THE ANSWERS TO THE FOLLOWING QUESTIONS DIFFER.**
- ***DO NOT USE THIS FORM* IF ANY STUDIES WILL BE REQUIRED UNDER FDAAA OR WILL BE PUBLICLY REPORTABLE**

1. During application review, explain why this issue is appropriate for a PMC instead of a pre-approval requirement. Check the reason below and describe.

☐ Need for drug (Unmet need/ Life-threatening condition)
☐ Long-term data needed (e.g., stability data)
☐ Only feasible to conduct post-approval
X Improvements to methods`
☐ Theoretical concern
☐ Manufacturing process analysis
☐ Other

The analytical methods and their corresponding acceptance criteria are already in place for BLA approval; however, due to the recent development of new release and stability test methods, optimization/improvement is necessary for a number of them.

2. Describe the particular review issue and the goal of the study.

The sponsor should: (i) improve the assay sensitivity of SDS-PAGE and peptide mapping, (ii) confirm the appropriateness of the results reported by SEC using an orthogonal method (AUC), (iii) implement the use of a biologically relevant substrate to determine kinetic parameters, and (iv) revise the acceptance criteria of all the analytical methods used in the release and stability protocols when a statistically significant number of DS and DP lots have been manufactured. These developments will allow EUSA to confirm that appropriate controls of the manufacturing process have been achieved.

3. [OMIT—for PMRs only]

4. What type of study is agreed upon (describe and check the type below)?

Select only one. Fill out a new sheet for each type of PMR/PMC study.

- ☐ Dissolution testing
- X Assay
- ☐ Sterility
- ☐ Potency
- ☐ Product delivery
- ☐ Drug substance characterization
- ☐ Intermediates characterization
- ☐ Impurity characterization
- ☐ Reformulation
- ☐ Manufacturing process issues

Due to the recent development of new analytical test methods used for release and stability testing of Erwinaze DS and DP, it is necessary for EUSA to improve the assay capabilities of SDS-PAGE and peptide mapping. The results obtained by SEC testing of Erwinaze DS samples that have been subjected to stress conditions will have to be confirmed by AUC analyses. Kinetic parameters should be determined using a biologically relevant substrate. EUSA will develop an experimental plan for evaluating L-asparagine, as the substrate. Finally, once a statistically significant number of Erwinaze DS and DP lots have been manufactured, EUSA will evaluate and revise where appropriate the acceptance criteria for the release and stability test methods.

☐

Other

Describe the Agreed upon study:

5. To be completed by ONDQA/OBP Manager:

- ☐ Does the study meet criteria for PMCs?
- ☐ Are the objectives clear from the description of the PMC?
- ☐ Has the applicant adequately justified the choice of schedule milestone dates?
- ☐ Has the applicant had sufficient time to review the PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

Barry Cherny

(signature line for BLAs only)

PMR/PMC Development Template

This template should be completed by the PMR/PMC Development Coordinator and included for ***each*** PMR/PMC in the Action Package.

PMR/PMC Description: EUSA Pharma will submit the final report for the non-clinical fertility and early pregnancy (Segment I; ICH S5(R2) Harmonized Segment A-B) studies of ERWINAZE in rats.

PMR/PMC Schedule Milestones:		
	Final Protocol Submission:	04/30/2012
	Study/Trial Completion:	07/31/2012
	Final Report Submission:	02/10/2013
	Other: _____	MM/DD/YYYY

1. During application review, explain why this issue is appropriate for a PMR/PMC instead of a pre-approval requirement. Check type below and describe.

   X Unmet need
   X Life-threatening condition
   ☐ Long-term data needed
   ☐ Only feasible to conduct post-approval
   ☐ Prior clinical experience indicates safety
   X Small subpopulation affected
   X Theoretical concern
   ☐ Other

FDA is asking for is study to be conducted as a post-marketing requirement (PMR), based on the small numbers of patients with acute lymphoblastic leukemia (ALL) that may potentially become pregnant while receiving Erwinaze treatment. Since Erwinaze will be used to treat a subset of ALL patients who have developed hypersensitivity to other asparaginases and have no other treatment options, the condition being treated is advanced cancer and therefore life-threatening, and Erwinaze will be used to treat only a subset of ALL patients, data to demonstrate the effects of Erwinase on fertility and early pregnancy outcome were not required before approval of the BLA.

2. Describe the particular review issue and the goal of the study/clinical trial. If the study/clinical trial is a FDAAA PMR, describe the risk. If the FDAAA PMR is created post-approval, describe the "new safety information."

Data from nonclinical studies showing the effects of Erwinaze treatment on fertility, mating behavior and conception and early pregnancy is necessary to support the safety of Erwinaze use in young adult male and female ALL patients of reproductive potential, and to convey this information in the product labeling.

3. If the study/clinical trial is a **PMR**, check the applicable regulation.
   If not a PMR, skip to 4.

   - **Which regulation?**
     - ☐ Accelerated Approval (subpart H/E)
     - ☐ Animal Efficacy Rule
     - ☐ Pediatric Research Equity Act
     - X FDAAA required safety study/clinical trial

   - **If the PMR is a FDAAA safety study/clinical trial, does it: (check all that apply)**
     - ☐ Assess a known serious risk related to the use of the drug?
     - X Assess signals of serious risk related to the use of the drug?
     - X Identify an unexpected serious risk when available data indicate the potential for a serious risk?

   - **If the PMR is a FDAAA safety study/clinical trial, will it be conducted as:**
     - ☐ Analysis of spontaneous postmarketing adverse events?
       Do not select the above study/clinical trial type if: such an analysis will not be sufficient to assess or identify a serious risk

     - ☐ Analysis using pharmacovigilance system?
       Do not select the above study/clinical trial type if: the new pharmacovigilance system that the FDA is required to establish under section 505(k)(3) has not yet been established and is thus not sufficient to assess this known serious risk, or has been established but is nevertheless not sufficient to assess or identify a serious risk

     - X Study: all other investigations, such as investigations in humans that are not clinical trials as defined below (e.g., observational epidemiologic studies), animal studies, and laboratory experiments?
       Do not select the above study type if: a study will not be sufficient to identify or assess a serious risk

     - ☐ Clinical trial: any prospective investigation in which the sponsor or investigator determines the method of assigning investigational product or other interventions to one or more human subjects?

4. What type of study or clinical trial is required or agreed upon (describe and check type below)? If the study or trial will be performed in a subpopulation, list here.

FDA is requiring and the applicant has agreed to conduct nonclinical fertility, mating and early pregnancy studies treating male and female rats with Erwinaze prior to and during mating, and through early pregnancy up to the time of embryo implantation. [Segment I, also referred to as ICH S5(R2) Harmonized Segment A-B studies], and evaluate the effects of Erwinaze on male and female fertility endpoints, conception rates, early embryo losses, and fertility indexes. Once completed, the resulting data will be added to Section 13.1 of the labeling, to convey the outcomes to patients and prescribing physicians.

Required

- ☐ Observational pharmacoepidemiologic study
- ☐ Registry studies
- ☐ Primary safety study or clinical trial
- ☐ Pharmacogenetic or pharmacogenomic study or clinical trial if required to further assess safety

☐ Thorough Q-T clinical trial
X Nonclinical (animal) safety study (e.g., carcinogenicity, reproductive toxicology)
Continuation of Question 4

☐ Nonclinical study (laboratory resistance, receptor affinity, quality study related to safety)
☐ Pharmacokinetic studies or clinical trials
☐ Drug interaction or bioavailability studies or clinical trials
☐ Dosing trials
☐ Additional data or analysis required for a previously submitted or expected study/clinical trial (provide explanation)

☐ Meta-analysis or pooled analysis of previous studies/clinical trials
☐ Immunogenicity as a marker of safety
☐ Other (provide explanation)

Agreed upon:

☐ Quality study without a safety endpoint (e.g., manufacturing, stability)
☐ Pharmacoepidemiologic study not related to safe drug use (e.g., natural history of disease, background rates of adverse events)
☐ Clinical trials primarily designed to further define efficacy (e.g., in another condition, different disease severity, or subgroup) that are NOT required under Subpart H/E
☐ Dose-response study or clinical trial performed for effectiveness
☐ Nonclinical study, not safety-related (specify)

☐ Other

5. Is the PMR/PMC clear, feasible, and appropriate?

X Does the study/clinical trial meet criteria for PMRs or PMCs?
X Are the objectives clear from the description of the PMR/PMC?
X Has the applicant adequately justified the choice of schedule milestone dates?
X Has the applicant had sufficient time to review the PMRs/PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

4/8/2011 D S Summer 11/8/11
(signature line for BLAs)

PMR/PMC Development Template

This template should be completed by the PMR/PMC Development Coordinator and included for ***each*** PMR/PMC in the Action Package.

PMR/PMC Description: EUSA Pharma will submit the final report for the embryo-fetal development and toxicity (EFT; ICH S5(R2) Harmonized Segment C) studies of ERWINAZE in rats and rabbits.

PMR/PMC Schedule Milestones:		
	Final Protocol Submission:	02/28/2012
	Study/Trial Completion:	05/15/2012
	Final Report Submission:	12/17/2012
	Other: _____	MM/DD/YYYY

1. During application review, explain why this issue is appropriate for a PMR/PMC instead of a pre-approval requirement. Check type below and describe.

   X Unmet need
   X Life-threatening condition
   ☐ Long-term data needed
   ☐ Only feasible to conduct post-approval
   ☐ Prior clinical experience indicates safety
   X Small subpopulation affected
   X Theoretical concern
   ☐ Other

   FDA is asking for these studies to be conducted as a post-marketing requirement (PMR), based on the small numbers of patients with acute lymphoblastic leukemia (ALL) that may potentially become pregnant while receiving Erwinaze treatment. Since Erwinaze will be used to treat a subset of ALL patients who have developed hypersensitivity to other asparaginases and have no other treatment options, the condition being treated is advanced cancer and therefore life-threatening, and Erwinaze will be used to treat only a subset of ALL patients, studies to demonstrate the effects of Erwinase on reproductive and developmental toxicity were not required before approval of the BLA.

2. Describe the particular review issue and the goal of the study/clinical trial. If the study/clinical trial is a FDAAA PMR, describe the risk. If the FDAAA PMR is created post-approval, describe the "new safety information."

   Data from nonclinical studies showing the effects of Erwinaze treatment of pregnant animals during the critical period of organogenesis, i.e. embryo-fetal development and toxicity (EFT) is necessary to support the safety of Erwinaze use during pregnancy, and to convey this information in the product labeling. The risk of Erwinaze on EFT has not been characterized; however, animal studies with other asparaginases including one derived from the same bacteria as Erwinase have shown adverse effects on embryofetal lethality, teratogenesis, and pregnancy outcomes.

3. If the study/clinical trial is a **PMR**, check the applicable regulation.
   If not a PMR, skip to 4.

   - **Which regulation?**
     - ☐ Accelerated Approval (subpart H/E)
     - ☐ Animal Efficacy Rule
     - ☐ Pediatric Research Equity Act
     - X FDAAA required safety study/clinical trial

   - **If the PMR is a FDAAA safety study/clinical trial, does it: (check all that apply)**
     - ☐ Assess a known serious risk related to the use of the drug?
     - X Assess signals of serious risk related to the use of the drug?
     - X Identify an unexpected serious risk when available data indicate the potential for a serious risk?

   - **If the PMR is a FDAAA safety study/clinical trial, will it be conducted as:**
     - ☐ Analysis of spontaneous postmarketing adverse events?
       Do not select the above study/clinical trial type if: such an analysis will not be sufficient to assess or identify a serious risk

     - ☐ Analysis using pharmacovigilance system?
       Do not select the above study/clinical trial type if: the new pharmacovigilance system that the FDA is required to establish under section 505(k)(3) has not yet been established and is thus not sufficient to assess this known serious risk, or has been established but is nevertheless not sufficient to assess or identify a serious risk

     - X Study: all other investigations, such as investigations in humans that are not clinical trials as defined below (e.g., observational epidemiologic studies), animal studies, and laboratory experiments?
       Do not select the above study type if: a study will not be sufficient to identify or assess a serious risk

     - ☐ Clinical trial: any prospective investigation in which the sponsor or investigator determines the method of assigning investigational product or other interventions to one or more human subjects?

4. What type of study or clinical trial is required or agreed upon (describe and check type below)? If the study or trial will be performed in a subpopulation, list here.

FDA is requiring and the applicant has agreed to conduct nonclinical reproductive toxicology studies treating pregnant rats and rabbits with Erwinaze during the critical period of organogenesis [EFT, also referred to as ICH S5(R2) Harmonized Segment C studies], and evaluate the effects of Erwinaze on embryofetal mortality, malformations, fetal outcome, and maternal toxicity. Once completed, the resulting data will be added to Section 8.1 of the labeling, to convey the outcomes to patients and prescribing physicians.

Required

- ☐ Observational pharmacoepidemiologic study
- ☐ Registry studies
- ☐ Primary safety study or clinical trial
- ☐ Pharmacogenetic or pharmacogenomic study or clinical trial if required to further assess safety
- ☐ Thorough Q-T clinical trial

X Nonclinical (animal) safety study (e.g., carcinogenicity, reproductive toxicology)
Continuation of Question 4

☐ Nonclinical study (laboratory resistance, receptor affinity, quality study related to safety)
☐ Pharmacokinetic studies or clinical trials
☐ Drug interaction or bioavailability studies or clinical trials
☐ Dosing trials
☐ Additional data or analysis required for a previously submitted or expected study/clinical trial (provide explanation)

_____

☐ Meta-analysis or pooled analysis of previous studies/clinical trials
☐ Immunogenicity as a marker of safety
☐ Other (provide explanation)

_____

Agreed upon:

☐ Quality study without a safety endpoint (e.g., manufacturing, stability)
☐ Pharmacoepidemiologic study not related to safe drug use (e.g., natural history of disease, background rates of adverse events)
☐ Clinical trials primarily designed to further define efficacy (e.g., in another condition, different disease severity, or subgroup) that are NOT required under Subpart H/E
☐ Dose-response study or clinical trial performed for effectiveness
☐ Nonclinical study, not safety-related (specify)

_____

☐ Other

_____

5. Is the PMR/PMC clear, feasible, and appropriate?

X Does the study/clinical trial meet criteria for PMRs or PMCs?
X Are the objectives clear from the description of the PMR/PMC?
X Has the applicant adequately justified the choice of schedule milestone dates?
X Has the applicant had sufficient time to review the PMRs/PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

11/8/2011 11/8/11
(signature line for BLAs)

PMR/PMC Development Template

This template should be completed by the PMR/PMC Development Coordinator and included for ***each*** PMR/PMC in the Action Package.

PMR/PMC Description: EUSA will conduct nonclinical peri-postnatal developmental (PPND, Segment III; ICH S5(R2) Harmonized Segment D-F) studies of Erwinaze in rats, and submit the final study report for FDA review.

PMR/PMC Schedule Milestones:		
	Final Protocol Submission:	09/27/2012
	Study/Trial Completion:	02/15/2013
	Final Report Submission:	11/15/2013
	Other: _____	MM/DD/YYYY

1. During application review, explain why this issue is appropriate for a PMR/PMC instead of a pre-approval requirement. Check type below and describe.

   X Unmet need
   X Life-threatening condition
   ☐ Long-term data needed
   ☐ Only feasible to conduct post-approval
   ☐ Prior clinical experience indicates safety
   X Small subpopulation affected
   X Theoretical concern
   ☐ Other

   FDA is asking for this study to be conducted as a post-marketing requirement, based on the small numbers of patients with acute lymphoblastic leukemia (ALL) that may potentially become pregnant while receiving Erwinaze treatment. An assessment of effects of Erwinaze on late stage pregnancy, labor and delivery, and early neo-natal development is warranted based on the indicated population, i.e. ALL patients up to 35 years of age, which may include females of childbearing potential. Since Erwinaze will be used to treat a subset of ALL patients who have developed hypersensitivity to other asparaginases and have no other treatment options, the condition being treated is advanced cancer and therefore life-threatening, and Erwinaze will be used to treat only a subset of ALL patients, data to demonstrate the effects of Erwinase on fertility and early pregnancy outcome were not required before approval of the BLA.

2. Describe the particular review issue and the goal of the study/clinical trial. If the study/clinical trial is a FDAAA PMR, describe the risk. If the FDAAA PMR is created post-approval, describe the "new safety information."

   Data from nonclinical studies showing the effects of Erwinaze treatment on late fetal toxicity, labor, partuition, pregnancy outcome, early neonatal growth and development, and weaning are necessary to support the safety of Erwinaze use in female ALL patients of reproductive potential who may become pregnant while receiving Erwinaze treatment, and to convey this information in the product labeling.

3. If the study/clinical trial are a **PMR**, check the applicable regulation.
If not a PMR, skip to 4.

- **Which regulation?**
  - ☐ Accelerated Approval (subpart H/E)
  - ☐ Animal Efficacy Rule
  - ☐ Pediatric Research Equity Act
  - ☐ FDAAA required safety study/clinical trial

- **If the PMR is a FDAAA safety study/clinical trial, does it: (check all that apply)**
  - ☐ Assess a known serious risk related to the use of the drug?
  - ☐ Assess signals of serious risk related to the use of the drug?
  - ☐ Identify an unexpected serious risk when available data indicate the potential for a serious risk?

- **If the PMR is a FDAAA safety study/clinical trial, will it be conducted as:**
  - ☐ Analysis of spontaneous postmarketing adverse events?
    Do not select the above study/clinical trial type if: such an analysis will not be sufficient to assess or identify a serious risk

  - ☐ Analysis using pharmacovigilance system?
    Do not select the above study/clinical trial type if: the new pharmacovigilance system that the FDA is required to establish under section 505(k)(3) has not yet been established and is thus not sufficient to assess this known serious risk, or has been established but is nevertheless not sufficient to assess or identify a serious risk

  - X Study: all other investigations, such as investigations in humans that are not clinical trials as defined below (e.g., observational epidemiologic studies), animal studies, and laboratory experiments?
    Do not select the above study type if: a study will not be sufficient to identify or assess a serious risk

  - ☐ Clinical trial: any prospective investigation in which the sponsor or investigator determines the method of assigning investigational product or other interventions to one or more human subjects?

4. What type of study or clinical trial is required or agreed upon (describe and check type below)? If the study or trial will be performed in a subpopulation, list here.

FDA is requiring and the applicant has agreed to conduct nonclinical peri-postnatal developmental toxicity studies of Erwinaze in rats [Segment III, or PPND studies also referred to as ICH S5(R2) Harmonized Segment E-F studies], and evaluate the effects of Erwinaze treatment on late stage pregnancy, labor and delivery, and early neo-natal development. Once completed, the resulting data will be added to Section 13.2 of the labeling, to convey the outcomes to patients and prescribing physicians.

Required

- ☐ Observational pharmacoepidemiologic study
- ☐ Registry studies
- ☐ Primary safety study or clinical trial
- ☐ Pharmacokinetic or pharmacogenomic study or clinical trial if required to further assess safety
- ☐ Thorough Q-T clinical trial

X Nonclinical (animal) safety study (e.g., carcinogenicity, reproductive toxicology)
Continuation of Question 4

- ☐ Nonclinical study (laboratory resistance, receptor affinity, quality study related to safety)
- ☐ Pharmacokinetic studies or clinical trials
- ☐ Drug interaction or bioavailability studies or clinical trials
- ☐ Dosing trials
- ☐ Additional data or analysis required for a previously submitted or expected study/clinical trial (provide explanation)

_____

- ☐ Meta-analysis or pooled analysis of previous studies/clinical trials
- ☐ Immunogenicity as a marker of safety
- ☐ Other (provide explanation)

_____

Agreed upon:

- ☐ Quality study without a safety endpoint (e.g., manufacturing, stability)
- ☐ Pharmacoepidemiologic study not related to safe drug use (e.g., natural history of disease, background rates of adverse events)
- ☐ Clinical trials primarily designed to further define efficacy (e.g., in another condition, different disease severity, or subgroup) that are NOT required under Subpart H/E
- ☐ Dose-response study or clinical trial performed for effectiveness
- ☐ Nonclinical study, not safety-related (specify)

_____

- ☐ Other

_____

5. Is the PMR/PMC clear, feasible, and appropriate?

- ☐ Does the study/clinical trial meet criteria for PMRs or PMCs?
- ☐ Are the objectives clear from the description of the PMR/PMC?
- ☐ Has the applicant adequately justified the choice of schedule milestone dates?
- ☐ Has the applicant had sufficient time to review the PMRs/PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

- ☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

11/8/2011 Summer 11/9/11
(signature line for BLAs)

PMR/PMC Development Template

This template should be completed by the PMR/PMC Development Coordinator and included for ***each*** PMR/PMC in the Action Package.

NDA #/Product Name: **BLA STN 125359/0-ERWINAZE**

PMR/PMC Description: PMR#4: To develop a validated, sensitive, and accurate assay for the detection of binding antibodies to ERWINAZE, including procedures for accurate detection of antibodies to ERWINAZE in the presence of ERWINAZE levels that are expected to be present in the serum at the time of patient sampling. A summary of the validation exercise including supporting data, a summary of the development data supporting assay suitability for parameters not assessed in the validation exercise, and the assay SOP will be provided to FDA.

PMR/PMC Schedule Milestones:		
	Final Protocol Submission:	NA
	Study/Trial Completion:	NA
	Final Report Submission:	10/30/2012
	Other: _____	MM/DD/YYYY

1. During application review, explain why this issue is appropriate for a PMR/PMC instead of a pre-approval requirement. Check type below and describe.

   - ☒ Unmet need
   - ☒ Life-threatening condition
   - ☐ Long-term data needed
   - ☐ Only feasible to conduct post-approval
   - ☐ Prior clinical experience indicates safety
   - ☐ Small subpopulation affected
   - ☐ Theoretical concern
   - ☐ Other

Erwinia derived aspariginase meets an unmet medical need for a life threatening condition. Therefore data from the assessment of anti-erwinase antibodies in serum samples from participants in the clinical trials described in the BLA can be obtained post-marketing. E coli-derived asparaginases (Elspar and Oncaspar, a PEGylated asparaginase) are currently used as a component of multi-agent chemotherapy for the treatment of childhood acute lymphoblastic leukemia (ALL). Therapeutic use of these asparaginases has been shown to elicit immune responses in human resulting in the development of anti-drug antibodies and loss of efficacy. The sequences of E. coli and Erwinia derived aspariginases are highly divergent. Therefore antibodies against E. coli derived aspariginase are not expected to efficiently cross-react against Erwinia derived aspariginase. Because of this Erwinia derived aspariginase can be used to treat patients who lose efficacy with E.coli derived aspariginase due to anti-drug antibody formation.

2. Describe the particular review issue and the goal of the study/clinical trial. If the study/clinical trial is a FDAAA PMR, describe the risk. If the FDAAA PMR is created post-approval, describe the "new safety information."

Issue and goal of the study:
The goal of this PMR is to validate an assay that is suitable for the assessment of anti-drug binding antibodies. This assay will be used to assess serum samples collected during the clinical trials submitted in the BLA (see PMR 3).

Protein therapeutics, are known to elicit anti-drug antibody responses in treated patients. Such antibodies can impact drug efficacy and safety (e.g. hypersensitivity responses). The Sponsor did not develop suitable assays for anti-drug antibody assessment. The Sponsor does not have validated immunogenicity assays (anti-Erwinase antibody screening assay, Erwinase-specific neutralizing antibody assay) to assess such immunological reactions to Erwinaze in treated patients.

Risk:
Erwinaze has potential to cause immunological reactions in treated subjects that can result in loss of efficacy or hypersensitivity.

3. If the study/clinical trial is a **PMR**, check the applicable regulation.
If not a PMR, skip to 4.

- **Which regulation?**
  - ☐ Accelerated Approval (subpart H/E)
  - ☐ Animal Efficacy Rule
  - ☐ Pediatric Research Equity Act
  - ☒ FDAAA required safety study/clinical trial

- **If the PMR is a FDAAA safety study/clinical trial, does it: (check all that apply)**
  - ☐ Assess a known serious risk related to the use of the drug?
  - ☐ Assess signals of serious risk related to the use of the drug?
  - ☒ Identify an unexpected serious risk when available data indicate the potential for a serious risk?

- **If the PMR is a FDAAA safety study/clinical trial, will it be conducted as:**
  - ☐ Analysis of spontaneous postmarketing adverse events?
    Do not select the above study/clinical trial type if: such an analysis will not be sufficient to assess or identify a serious risk

  - ☐ Analysis using pharmacovigilance system?
    Do not select the above study/clinical trial type if: the new pharmacovigilance system that the FDA is required to establish under section 505(k)(3) has not yet been established and is thus not sufficient to assess this known serious risk, or has been established but is nevertheless not sufficient to assess or identify a serious risk

  - ☒ Study: all other investigations, such as investigations in humans that are not clinical trials as defined below (e.g., observational epidemiologic studies), animal studies, and laboratory experiments?
    Do not select the above study type if: a study will not be sufficient to identify or assess a serious risk

☐ Clinical trial: any prospective investigation in which the sponsor or investigator determines the method of assigning investigational product or other interventions to one or more human subjects?

4. What type of study or clinical trial is required or agreed upon (describe and check type below)? If the study or trial will be performed in a subpopulation, list here.

The purpose of this PMR is to develop the tools for anti-drug antibody assessment. Upon development of sensitive, accurate and validated immunogenicity assays (anti-Erwinaze antibody binding assay (PMR 1) and anti-Erwinaze-specific neutralizing antibody assay (PMR 2)) the Sponsor is required to study the patient samples archived from clinical studies and submit data to the FDA (PMR 3).

Required

☐ Observational pharmacoepidemiologic study
☐ Registry studies
☒ Primary safety study or clinical trial
☐ Pharmacogenetic or pharmacogenomic study or clinical trial if required to further assess safety
☐ Thorough Q-T clinical trial
☐ Nonclinical (animal) safety study (e.g., carcinogenicity, reproductive toxicology)

Continuation of Question 4

☐ Nonclinical study (laboratory resistance, receptor affinity, quality study related to safety)
☐ Pharmacokinetic studies or clinical trials
☐ Drug interaction or bioavailability studies or clinical trials
☐ Dosing trials
☐ Additional data or analysis required for a previously submitted or expected study/clinical trial (provide explanation)

☐ Meta-analysis or pooled analysis of previous studies/clinical trials
☒ Immunogenicity as a marker of safety
☐ Other (provide explanation)

Agreed upon:

☐ Quality study without a safety endpoint (e.g., manufacturing, stability)
☐ Pharmacoepidemiologic study not related to safe drug use (e.g., natural history of disease, background rates of adverse events)
☐ Clinical trials primarily designed to further define efficacy (e.g., in another condition, different disease severity, or subgroup) that are NOT required under Subpart H/E
☐ Dose-response study or clinical trial performed for effectiveness
☐ Nonclinical study, not safety-related (specify)

☒ Other
To develop validated, sensitive assay for the detection of anti-Erwinase antibodies, neutralizing antibodies and to perform immunogenicity assessment on clinical trial samples.

5. Is the PMR/PMC clear, feasible, and appropriate?

☒ Does the study/clinical trial meet criteria for PMRs or PMCs?

PMR/PMC Development Template

This template should be completed by the PMR/PMC Development Coordinator and included for ***each*** PMR/PMC in the Action Package.

NDA #/Product Name: **BLA STN 125359/0-ERWINAZE**

PMR/PMC Description: PMR#5: To develop a validated, sensitive, and accurate assay for the detection of neutralizing antibodies to ERWINAZE, including procedures for accurate detection of neutralizing antibodies to ERWINAZE in the presence of ERWINAZE levels that are expected to be present in the serum at the time of patient sampling. A summary of the validation exercise including supporting data, a summary of the development data supporting assay suitability for parameters not assessed in the validation exercise, and the assay SOP will be provided to FDA.

PMR/PMC Schedule Milestones:		
	Final Protocol Submission:	NA
	Study/Trial Completion:	NA
	Final Report Submission:	10/30/2012
	Other: _____	MM/DD/YYYY

1. During application review, explain why this issue is appropriate for a PMR/PMC instead of a pre-approval requirement. Check type below and describe.

   - ☒ Unmet need
   - ☒ Life-threatening condition
   - ☐ Long-term data needed
   - ☐ Only feasible to conduct post-approval
   - ☐ Prior clinical experience indicates safety
   - ☐ Small subpopulation affected
   - ☐ Theoretical concern
   - ☐ Other

Erwinia derived aspariginase meets an unmet medical need for a life threatening condition. Therefore data from the assessment of anti-erwinase antibodies in serum samples from participants in the clinical trials described in the BLA can be obtained post-marketing. E coli-derived asparaginases (Elspar and Oncaspar, a PEGylated asparaginase) are currently used as a component of multi-agent chemotherapy for the treatment of childhood acute lymphoblastic leukemia (ALL). Therapeutic use of these asparaginases has been shown to elicit immune responses in human resulting in the development of anti-drug antibodies and loss of efficacy. The sequences of E. coli and Erwinia derived aspariginases are highly divergent. Therefore antibodies against E. coli derived aspariginase are not expected to efficiently cross-react against Erwinia derived aspariginase. Because of this Erwinia derived aspariginase can be used to treat patients who lose efficacy with E.coli derived aspariginase due to anti-drug antibody formation.

2. Describe the particular review issue and the goal of the study/clinical trial. If the study/clinical trial is a FDAAA PMR, describe the risk. If the FDAAA PMR is created post-approval, describe the "new safety information."

Issue and goal of the study:
The goal of this PMR is to validate an assay that is suitable for the assessment of anti-drug neutralizing antibodies. This assay will be used to assess serum samples collected during the clinical trials submitted in the BLA (see PMR 3). Neutralizing antibodies are a subset of binding antibodies that specifically interfere with receptor ligand interactions and may thereby interfere with efficacy.

Protein therapeutics, are known to elicit anti-drug antibody responses in treated patients. The Sponsor does not have validated immunogenicity assays (anti-Erwinase antibody screening assay, Erwinase-specific neutralizing antibody assay) to assess such immunological reactions to Erwinaze in treated patients.

Risk:
Erwinaze has potential to cause immunological reactions in treated subjects that can result in loss of efficacy or hypersensitivity.

3. If the study/clinical trial is a **PMR**, check the applicable regulation.
If not a PMR, skip to 4.

- **Which regulation?**
  - ☐ Accelerated Approval (subpart H/E)
  - ☐ Animal Efficacy Rule
  - ☐ Pediatric Research Equity Act
  - ☒ FDAAA required safety study/clinical trial

- **If the PMR is a FDAAA safety study/clinical trial, does it: (check all that apply)**
  - ☐ Assess a known serious risk related to the use of the drug?
  - ☐ Assess signals of serious risk related to the use of the drug?
  - ☒ Identify an unexpected serious risk when available data indicate the potential for a serious risk?

- **If the PMR is a FDAAA safety study/clinical trial, will it be conducted as:**
  - ☐ Analysis of spontaneous postmarketing adverse events?
    Do not select the above study/clinical trial type if: such an analysis will not be sufficient to assess or identify a serious risk

  - ☐ Analysis using pharmacovigilance system?
    Do not select the above study/clinical trial type if: the new pharmacovigilance system that the FDA is required to establish under section 505(k)(3) has not yet been established and is thus not sufficient to assess this known serious risk, or has been established but is nevertheless not sufficient to assess or identify a serious risk

☒ Study: all other investigations, such as investigations in humans that are not clinical trials as defined below (e.g., observational epidemiologic studies), animal studies, and laboratory experiments?
Do not select the above study type if: a study will not be sufficient to identify or assess a serious risk

☐ Clinical trial: any prospective investigation in which the sponsor or investigator determines the method of assigning investigational product or other interventions to one or more human subjects?

4. What type of study or clinical trial is required or agreed upon (describe and check type below)? If the study or trial will be performed in a subpopulation, list here.

The purpose of this PMR is to develop the tools for anti-drug antibody assessment. Upon development of sensitive, accurate and validated immunogenicity assays (anti-Erwinaze antibody binding assay (PMR 1) and anti-Erwinaze-specific neutralizing antibody assay (PMR 2)) the Sponsor is required to study the patient samples archived from clinical studies and submit data to the FDA (PMR 3).

Required

☐ Observational pharmacoepidemiologic study
☐ Registry studies
☒ Primary safety study or clinical trial
☐ Pharmacogenetic or pharmacogenomic study or clinical trial if required to further assess safety
☐ Thorough Q-T clinical trial
☐ Nonclinical (animal) safety study (e.g., carcinogenicity, reproductive toxicology)

Continuation of Question 4

☐ Nonclinical study (laboratory resistance, receptor affinity, quality study related to safety)
☐ Pharmacokinetic studies or clinical trials
☐ Drug interaction or bioavailability studies or clinical trials
☐ Dosing trials
☐ Additional data or analysis required for a previously submitted or expected study/clinical trial (provide explanation)

☐ Meta-analysis or pooled analysis of previous studies/clinical trials
☒ Immunogenicity as a marker of safety
☐ Other (provide explanation)

Agreed upon:

☐ Quality study without a safety endpoint (e.g., manufacturing, stability)
☐ Pharmacoepidemiologic study not related to safe drug use (e.g., natural history of disease, background rates of adverse events)
☐ Clinical trials primarily designed to further define efficacy (e.g., in another condition, different disease severity, or subgroup) that are NOT required under Subpart H/E
☐ Dose-response study or clinical trial performed for effectiveness
☐ Nonclinical study, not safety-related (specify)

☒ Other

To develop validated, sensitive assay for the detection of anti-Erwinase antibodies, neutralizing antibodies and to perform immunogenicity assessment on clinical trial samples.

5. Is the PMR/PMC clear, feasible, and appropriate?

☒ Does the study/clinical trial meet criteria for PMRs or PMCs?
☒ Are the objectives clear from the description of the PMR/PMC?
☒ Has the applicant adequately justified the choice of schedule milestone dates?
☒ Has the applicant had sufficient time to review the PMRs/PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

Susan L. Kusiner / [signature] SMMC 11/8/11
(signature line for BLAs)

PMR/PMC Development Template

This template should be completed by the PMR/PMC Development Coordinator and included for ***each*** PMR/PMC in the Action Package.

NDA #/Product Name: **BLA STN 125359/0-ERWINAZE**

PMR/PMC Description: PMR#6: To conduct an assessment of anti-drug antibody (ADA) binding response and neutralizing ADA response to ERWINAZE with validated assays (required under PMR 4 and 5) capable of sensitively detecting ADA responses in the presence of ERWINAZE levels that are expected to be present at the time of patient sampling. The ADA response will be evaluated in all archived sampling time points available from all patients in the COG Study AALL07P2.

PMR/PMC Schedule Milestones:		
	Final Protocol Submission:	NA
	Study/Trial Completion:	NA
	Final Report Submission:	4/30/2013
	Other: _____	MM/DD/YYYY

1. During application review, explain why this issue is appropriate for a PMR/PMC instead of a pre-approval requirement. Check type below and describe.

   - ☒ Unmet need
   - ☒ Life-threatening condition
   - ☐ Long-term data needed
   - ☐ Only feasible to conduct post-approval
   - ☐ Prior clinical experience indicates safety
   - ☐ Small subpopulation affected
   - ☐ Theoretical concern
   - ☐ Other

Erwinia derived aspariginase meets an unmet medical need for a life threatening condition. Therefore data from the assessment of anti-erwinase antibodies in serum samples from participants in the clinical trials described in the BLA can be obtained post-marketing. E coli-derived asparaginases (Elspar and Oncaspar, a PEGylated asparaginase) are currently used as a component of multi-agent chemotherapy for the treatment of childhood acute lymphoblastic leukemia (ALL). Therapeutic use of these asparaginases has been shown to elicit immune responses in human resulting in the development of anti-drug antibodies and loss of efficacy. The sequences of E. coli and Erwinia derived aspariginases are highly divergent. Therefore antibodies against E. coli derived aspariginase are not expected to efficiently cross-react against Erwinia derived aspariginase. Because of this Erwinia derived aspariginase can be used to treat patients who lose efficacy with E.coli derived aspariginase due to anti-drug antibody formation.

2. Describe the particular review issue and the goal of the study/clinical trial. If the study/clinical trial is a FDAAA PMR, describe the risk. If the FDAAA PMR is created post-approval, describe the "new safety information."

Issue and goal of the study:
The goal of this PMR is to assess serum samples collected during the clinical trials submitted in the BLA (see PMR 3) using the assays developed under PMRs 4 and 5.

Protein therapeutics, are known to elicit anti-drug antibody responses in treated patients. Such antibodies can impact drug efficacy and safety (e.g. hypersensitivity responses). The Sponsor did not develop suitable assays for anti-drug antibody assessment. The Sponsor does not have validated immunogenicity assays (anti-Erwinase antibody screening assay, Erwinase-specific neutralizing antibody assay) to assess such immunological reactions to Erwinaze in treated patients.

Risk:
Erwinaze has potential to cause immunological reactions in treated subjects that can result in loss of efficacy or hypersensitivity.

3. If the study/clinical trial is a **PMR**, check the applicable regulation.
If not a PMR, skip to 4.

- **Which regulation?**
  - ☐ Accelerated Approval (subpart H/E)
  - ☐ Animal Efficacy Rule
  - ☐ Pediatric Research Equity Act
  - ☒ FDAAA required safety study/clinical trial

- **If the PMR is a FDAAA safety study/clinical trial, does it: (check all that apply)**
  - ☐ Assess a known serious risk related to the use of the drug?
  - ☐ Assess signals of serious risk related to the use of the drug?
  - ☒ Identify an unexpected serious risk when available data indicate the potential for a serious risk?

- **If the PMR is a FDAAA safety study/clinical trial, will it be conducted as:**
  - ☐ Analysis of spontaneous postmarketing adverse events?
    Do not select the above study/clinical trial type if: such an analysis will not be sufficient to assess or identify a serious risk

  - ☐ Analysis using pharmacovigilance system?
    Do not select the above study/clinical trial type if: the new pharmacovigilance system that the FDA is required to establish under section 505(k)(3) has not yet been established and is thus not sufficient to assess this known serious risk, or has been established but is nevertheless not sufficient to assess or identify a serious risk

  - ☒ Study: all other investigations, such as investigations in humans that are not clinical trials as defined below (e.g., observational epidemiologic studies), animal studies, and laboratory experiments?
    Do not select the above study type if: a study will not be sufficient to identify or assess a serious risk

☐ Clinical trial: any prospective investigation in which the sponsor or investigator determines the method of assigning investigational product or other interventions to one or more human subjects?

4. What type of study or clinical trial is required or agreed upon (describe and check type below)? If the study or trial will be performed in a subpopulation, list here.

The purpose of this PMR is to assess patient serum samples for the presence of anti-drug binding and neutralizing antibodies using assays developed under PMRs 4 and 5

Required

☐ Observational pharmacoepidemiologic study
☐ Registry studies
☒ Primary safety study or clinical trial
☐ Pharmacogenetic or pharmacogenomic study or clinical trial if required to further assess safety
☐ Thorough Q-T clinical trial
☐ Nonclinical (animal) safety study (e.g., carcinogenicity, reproductive toxicology)

Continuation of Question 4

☐ Nonclinical study (laboratory resistance, receptor affinity, quality study related to safety)
☐ Pharmacokinetic studies or clinical trials
☐ Drug interaction or bioavailability studies or clinical trials
☐ Dosing trials
☐ Additional data or analysis required for a previously submitted or expected study/clinical trial (provide explanation)

☐ Meta-analysis or pooled analysis of previous studies/clinical trials
☒ Immunogenicity as a marker of safety
☐ Other (provide explanation)

Agreed upon:

☐ Quality study without a safety endpoint (e.g., manufacturing, stability)
☐ Pharmacoepidemiologic study not related to safe drug use (e.g., natural history of disease, background rates of adverse events)
☐ Clinical trials primarily designed to further define efficacy (e.g., in another condition, different disease severity, or subgroup) that are NOT required under Subpart H/E
☐ Dose-response study or clinical trial performed for effectiveness
☐ Nonclinical study, not safety-related (specify)

☒ Other
To develop validated, sensitive assay for the detection of anti-Erwinase antibodies, neutralizing antibodies and to perform immunogenicity assessment on clinical trial samples.

5. Is the PMR/PMC clear, feasible, and appropriate?

☒ Does the study/clinical trial meet criteria for PMRs or PMCs?
☒ Are the objectives clear from the description of the PMR/PMC?

☒ Has the applicant adequately justified the choice of schedule milestone dates?
☒ Has the applicant had sufficient time to review the PMRs/PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

Susan L. Kushner
(signature line for BLAs)

11/8/11

☒ Are the objectives clear from the description of the PMR/PMC?
☒ Has the applicant adequately justified the choice of schedule milestone dates?
☒ Has the applicant had sufficient time to review the PMRs/PMCs, ask questions, determine feasibility, and contribute to the development process?

PMR/PMC Development Coordinator:

☐ *This PMR/PMC has been reviewed for clarity and consistency, and is necessary to further refine the safety, efficacy, or optimal use of a drug, or to ensure consistency and reliability of drug quality.*

Susan L. Kirshner / DG Summer 11/8/11
(signature line for BLAs)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Memorandum

Date: 10/28/11 ESL 10/28/11

From: Erik Laughner, RPM DOP2/OHOP/CDER/FDA

Subject: EUSA Pharma; BLA STN 125359; TB-EER completed

From: Ramanadham, Mahesh
Sent: Friday, October 28, 2011 8:42 AM
To: Laughner, Erik; CDER-TB-EER
Subject: RE: Request for EER(Compliance) Check; Pending BLA STN 125359 (EUSA Pharma); Erwinaze

Hi Erik

NDMAB has reviewed this TB-EER. There are no ongoing or pending compliance actions that prevent approval of the BLA.

TB-EER_125359(1).
doc (36 KB)

Sincerely,

Mahesh Ramanadham, PharmD/M.B.A.
LT., USPHS
Regulatory Compliance Officer
CDER, Office of Compliance
Office of Manufacturing and Product Quality,
Division of Good Manufacturing Practice Assessment
New Drug Manufacturing Assessment Branch
(301)796-3272

3Therapeutic Biological Establishment Evaluation Request (TB-EER) Form

Version 1.0

Instructions:
The review team should email this form to the email account "CDER-TB-EER" to submit:

1) an initial TB-EER within 10 business days of the application filing date
2) a final TB-EER 15-30 days prior to the action date

Note: All manufacturing[1] locations named in the pending submission, whether contract facilities or facilities owned by the applicant, should be listed on this form. For bundled supplements, one TB-EER to include all STNs should be submitted.

APPLICATION INFORMATION

PDUFA Action Date: 8/2/11

Applicant Name: EUSA Pharma (USA) Inc.
U.S. License #: 1829
STN(s): 125359/0
Product(s): Erwinia L-asparaginase
Short summary of application: New BLA

FACILITY INFORMATION

Manufacturing Location:
Firm Name (b) (4)

FEI: (b) (4)
Short summary of manufacturing activities performed: production and testing for drug substance and drug product

Inspected by CDER-DMPQ from 3/14/11-3/22/11 and classified VAI. This was a comprehensive, five system pre-approval inspection. This site is acceptable for the purposes of this supplement.

---

[1]The regulations at 21 C.F.R. § 207.3(a)(8) defines "manufacturing or processing" as "the manufacture, preparation, propagation, compounding, or processing of a drug or drugs as used in section 510 of the act [21 U.S.C. § 360] and is the making by chemical, physical, biological, or other procedures of any articles that meet the definition of drugs in section 201(g) of the act. The term includes manipulation, sampling, testing, or control procedures applied to the final product or to any part of the process. The term also includes repackaging or otherwise changing the container, wrapper, or labeling of any drug package to further the distribution of the drug from the original place of manufacture to the person who makes final delivery or sale to the ultimate consumer."

Manufacturing Location:
Firm Name (b) (4)
Address: (b) (4)
(b) (4)
FEI: (b) (4)
Short summary of manufacturing activities performed: Endotoxin testing and sterility testing

Inspected by (b) (4) and classified VAI. This was a pre-approval inspection to cover the testing responsibilities associated with this BLA. The CTL profile was updated and acceptable.

Manufacturing Location:
Firm Name: (b) (4)..
Address: (b) (4)
(b) (4)
FEI: (b) (4)
Short summary of manufacturing activities performed: assembly (labeling and packaging) and distribution site for drug product

Inspected by (b) (4) and classified VAI. This was a GMP/pre-approval inspection that found the packaging and labeling operations acceptable.

Manufacturing Location:
Firm Name: (b) (4)
Address: (b) (4)
(b) (4)
FEI: (b) (4)
Short summary of manufacturing activities performed: sub-visible particle testing for drug product
.
Inspected by (b) (4) and classified VAI. The CTL profile was covered and is acceptable.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Memorandum

Date: 10/26/11 EL 10/26/11

From: Erik Laughner, RPM DOP2/OHOP/CDER/FDA

Subject: EUSA Pharma; BLA STN 125359; TB-EER request

From: Laughner, Erik
Sent: Wednesday, October 26, 2011 1:24 PM
To: CDER-TB-EER
Subject: Request for EER(Compliance) Check; Pending BLA STN 125359 (EUSA Pharma); Erwinaze
Importance: High

Good Afternoon,

DOP2 is preparing to take an approval action on BLA STN 125359 and wishes to request the final EER check. Please see attached form which contains the list of sites.

If you have any questions or need additional information, please contact me.

Sincerely,

Erik

125359 TB- EER
EUSA Pharma doc...

Erik S. Laughner, M.S., RAC (US)
Senior Regulatory Health Project Manager
Division of Oncology Products 2 (DOP2)
Office of Hematology & Oncology Products (OHOP)
CDER/FDA
301-796-1393
erik.laughner@fda.hhs.gov
http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm091745.htm

If you have received this message in error, do not use, disclose, reproduce, or distribute this message (including any attachments) and notify me immediately. Thank you.

Therapeutic Biological Establishment Evaluation Request (TB-EER) Form

Version 1.0

Instructions:
The review team should email this form to the email account "CDER-TB-EER" to submit:

1) an initial TB-EER within 10 business days of the application filing date
2) a final TB-EER 15-30 days prior to the action date

Note: All manufacturing[1] locations named in the pending submission, whether contract facilities or facilities owned by the applicant, should be listed on this form. For bundled supplements, one TB-EER to include all STNs should be submitted.

APPLICATION INFORMATION

Action Date: November 21, 2011

Applicant Name: EUSA Pharma (USA), Inc.
U.S. License #: 1829
STN(s): 125359
Product(s): ERWINAZE
Short summary of application: DOP2 is completing the review of new original BLA STN 125359 for ERWINAZE. Indicated as a component of a multi-agent chemotherapeutic regimen for the treatment of patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to E. coli derived asparaginase

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FACILITY INFORMATION

SEE ATTACHED.

[1]The regulations at 21 C.F.R. § 207.3(a)(8) defines "manufacturing or processing" as "the manufacture, preparation, propagation, compounding, or processing of a drug or drugs as used in section 510 of the act [21 U.S.C. § 360] and is the making by chemical, physical, biological, or other procedures of any articles that meet the definition of drugs in section 201(g) of the act. The term includes manipulation, sampling, testing, or control procedures applied to the final product or to any part of the process. The term also includes repackaging or otherwise changing the container, wrapper, or labeling of any drug package to further the distribution of the drug from the original place of manufacture to the person who makes final delivery or sale to the ultimate consumer."

Drug Substance

The sites for the manufacture, batch release, quality control and storage of Erwinaze Drug Substance are provided below:

Company name: (b) (4)

Address:

FEI number:

Function: Production and test site for drug substance

Responsibility:

- Manufacture of the drug substance
- Storage of the drug substance
- Raw material testing and approval
- Quality Control testing of the drug substance

Company name: (b) (4)

Address:

FEI number:

Function: Test site for drug substance

Responsibility: Quality control testing of the drug substance (endotoxin only)

Drug Product

The sites for the manufacture, batch release, storage, quality control and assembly and distribution of Erwinaze Drug Product are provided below:

Company name: (b) (4)

Function: Production and test site for drug product

Responsibility:

- Manufacture of the drug product
- Storage of the drug product
- Raw material testing of the drug product
- Quality control testing of the drug product

Company name: (b) (4)

Address:

FEI number: Application has been submitted

Function: Assembly and distribution site for drug product

Responsibility:
- Storage of the drug product
- Assembly of the drug product
- Release of the drug product
- Distributor of the drug product

Company name:

Address:

FEI number:

(b) (4)

Function: Test site for drug product

Responsibility: Quality control testing of the drug product (sterility only)

Company name:

Address:

FEI number:

(b) (4)

Function: Test site for drug product

Responsibility: Quality control testing of the drug product (sub-visible particle test only)

Manufacturing Schedule

All systems within the (b) (4) manufacturing site will be available for inspection during the third month of the review cycle, as previously discussed with the FDA (see 1.6.3 Correspondence Regarding Meetings).

The HPA manufacturing is schedule depicted in Figure 1 below.

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: September 30, 2011

TO: Patricia Keegan, M.D.
Director, Division of Oncology Products 2 (DOP2)

FROM: Michael F. Skelly, Ph.D.
Pharmacologist, Division of Bioequivalence and GLP Compliance, Office of Scientific Investigations

THROUGH: Sam H. Haidar, R.Ph., Ph.D.
Chief, Bioequivalence Investigations Branch
Division of Bioequivalence and GLP Compliance
Office of Scientific Investigations

SUBJECT: Review of EIR Covering BLA 125359, *Erwinia* L-Asparaginase, sponsored by EUSA Pharma (USA), Inc.

At the request of the Division of Oncology Products 2, the Division of Bioequivalence and GLP Compliance (DBGC) audited bioanalytical portions of the following study:

> ***COG Study AALL07P2:*** *"Pharmacology and Toxicity of Erwinia* Asparaginase (Erwinaze; Crisantaspase: IND 290) Following Allergy to Pegaspargase in Treatment of Children with Acute Lymphoblastic Leukemia (ALL)"

The original bioanalytical portions of the study conducted at the [redacted] (b)(4) and at the [redacted] (b)(4) [redacted] (b)(4), were audited previously and recommended to be unreliable. The sponsor EUSA then arranged to retrieve serum samples from storage, intended for immunologic testing, and to have these samples assayed for asparaginase activity. This subsequent testing was conducted at [redacted] (b)(4) [redacted] (b)(4) [redacted] (b)(4) following a cross-validation of an existing assay to use *E. coli* asparaginase for the calibrators, and both *E coli* and *Erwinia* asparaginase for quality control samples.

Following the inspection at [redacted] (b)(4) there were no objectionable findings, and no Form FDA 483 was issued. Note that [redacted] (b)(4) has not evaluated stability of asparaginase activities during long-term storage. However, the

measurable asparaginase activities were unlikely to have exceeded the activities at the times of blood collection. Therefore, the original activities were probably the same or greater than those measured.

Conclusions:

Following the above inspection, the Division of Bioequivalence and GLP Compliance recommends the following:

- The asparaginase data from [redacted] (b) (4) are acceptable for your review.

After you have reviewed this transmittal memo, please append it to the original BLA submissions.

Michael F. Skelly, Ph.D.

Final Classification:
NAI [redacted] (b) (4)

cc:
OSI/DBGC/Salewski/Haidar/Lee/Dejernett/CF
DOP2/Dinndorf/Demko/Laughner
DOP2/Jun Yang/Hong Zhao
OPS/OBP/DTP/Beaucage
ORA/HFR-CE2545/Milazzo
OC/OSI/Ball/Moreno
OC/Cooper
Draft: MFS 9/30/11
Edit: SHH 9/30/11
DSI: 6260
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FACTS: 1322005

Email:
CDER DSI PM TRACK

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: July 13, 2011

TO: Patricia Keegan, M.D.
Director
Division of Biologic Oncology Products (DBOP)

FROM: Charles R. Bonapace, Pharm.D.
Pharmacologist, Division of Bioequivalence and GLP Compliance, Office of Scientific Investigations

Michael F. Skelly, Ph.D.
Pharmacologist, Division of Bioequivalence and GLP Compliance, Office of Scientific Investigations

THROUGH: Martin K. Yau, Ph.D. Martin K. Yau 7/15/11
Acting Team Leader, Bioequivalence Team, Division of Bioequivalence and GLP Compliance, Office of Scientific Investigations

SUBJECT: Addendum to the Review of EIR Covering BLA 125359, *Erwinia* L-Asparaginase for BLA 125359, sponsored by EUSA Pharma (USA), Inc.

At the request of DBOP, DSI (now OSI) audited the bioanalytical portions of AALL07P2 on [redacted] (b)(4) during an inspection at the laboratory of [redacted] (b)(4) at the [redacted] (b)(4) DSI provided inspection summary memos to DBOP on March 16 and March 23. Consequently on April 7, DBOP issued an Information Request (IR) letter to EUSA, outlining the inspectional findings. On April 24, DBOP received EUSA's response to the IR letter, discussing the inspectional observations at MGH. OSI has not received a direct response from [redacted] (b)(4)

This addendum discusses EUSA's response to the IR letter.

FINDINGS SPECIFIC TO THE ASPARAGINASE ASSAY

Finding 1 - Failure to reject analytical run #480 on 4/8/10 when one of the three quality control (QC) samples failed the acceptance criterion. The

following samples were not re-assayed, or required dilution:
Subject # Course # Sample #
794013 Course #1 PK-01, PK-02
794013 Course #2 PK-01
794013 Course #3 PK-01, PK-03
794165 Course #2 PK-01, PK-03
794669 Course #4 PK-01
794813 Course #1 PK-01 to PK-03, PK-05
794813 Course #1 PK-07, PK-08, PK-012

EUSA agrees that run #480 should have been rejected, and the study samples should have been reassayed. However, EUSA noted that only sample PK-07 for patient 794813 was used for the secondary study endpoint at 72 hours. EUSA has excluded this value from the study analyses.

OSI agrees that the value for patient 794813 should be excluded. Furthermore, OSI believes that adequate acceptance criteria were not in place at (b)(4) to assure data validity. Specifically, run #480 was accepted despite the failing results of QC determinations.

Finding #2 - Failure to exclude serum samples from clinical sites received in the thawed state

EUSA acknowledges that eleven samples for asparaginase determinations were thawed at the time when they arrived at (b)(4) EUSA notes that the eleven samples were not used for the primary study endpoint. However, they argue, without explicit data, that thawing accidents and storage at unknown temperatures were unlikely to have increased the measurable asparaginase activities, and that including data from the thawed samples probably would not have given inappropriate advantage.

OSI recommends that in the absence of data demonstrating stability during possible extreme conditions, the values from the thawed samples, which were subjected to unknown temperatures, should be excluded from the pharmacokinetic evaluations. OSI believes that adequate procedures were not in place at (b)(4) to assure data validity. Specifically, the questionable assay results from thawed samples were reported to the BLA without reservation. Furthermore, it is not appropriate to speculate on the effects of accidents and unknown conditions in storage and handling.

Finding #3 - Records of freezer temperatures for storage of asparaginase samples were not retrievable in an auditable form.

EUSA acknowledges that the freezer records at both [redacted] (b) (4) were unavailable for inspection, and offers to try to retrieve them upon an additional request from FDA. EUSA suggests that alarms at [redacted] (b) (4) would have revealed freezer accidents, and that unrecognized thawing accidents were unlikely to increase measurable asparaginase activity.

OSI notes that the alarm records collected during the inspection revealed that the alarm records did not reflect the duration of the outage and the actual temperatures observed by an analyst when she responded to the alarm in one failure incident at [redacted] (b) (4) Therefore, OSI recommends that the limited alarm records at [redacted] (b) (4) and [redacted] (b) (4) do not confirm the storage conditions of serum samples for asparaginase determinations while they were stored at [redacted] (b) (4) and [redacted] (b) (4) Furthermore, because the records at [redacted] (b) (4) in a retrievable format have not been provided in the 15 weeks since the original request during the inspection, it is unlikely that they will be retrieved in the future. Again, it is not appropriate to speculate on the effects of accidents, or stability during unknown conditions of storage.

Finding #4 - Failure to document the times when samples were removed from frozen storage for analysis

EUSA agrees that the analyst at [redacted] (b) (4) did not record the times serum samples were thawed for analysis or reanalysis. EUSA remarks, that they were kept on ice during these times and then returned to the freezer, and that any samples damaged during handling would likely have decreased asparaginase activities rather than the high proportion of samples with apparent activities >100 mU/mL.

OSI notes that the actual conditions of temporary storage (*viz.*, supposedly on ice) and whether samples were returned to the freezer and subsequently thawed, if reanalysis was necessary were not recorded. Again, it is not appropriate to speculate on stability during unknown times and conditions of storage.

Finding #5 - Failure to adequately document preparation and storage of asparaginase stock solutions

EUSA acknowledges that complete records for solution preparation and storage were not provided during the inspection. However, EUSA claims that other records were assembled since the inspection to document proper preparation and storage, specifically purchase and shipment records of nine vials of asparaginase from [redacted] (b) (4) which were used within one year of reconstitution.

EUSA's claims agree with the records observed, and the exhibit copies of them collected, during the inspection. The inspection team repeatedly asked whether additional records (purchase, receipt, reconstitution, storage, or handling) could be retrieved, to show that other vials of asparaginase were available. [redacted] (b) (4) retrieved independent purchase and receipt records to confirm that only the nine vials (8 of 100 IU/vial, and 1 of 1000 IU/vial) were opened for use. The validation report and records show that solutions of asparaginase, reconstituted as the entire contents of these vials, were stable for only 72 hours at refrigerator temperatures. Note that the claimed stabilities of the vials before reconstitution are not germaine to this discussion. Thus, there were insufficient amounts of asparaginase for preparing stock solutions to make calibrators and QC solutions for all of the claimed assay runs if the stock solutions were used within the established times of stability.

In addition, the available records were insufficient to reconstruct the concentrations claimed for finding #6 (below).

OSI continues to believe that the records for preparation and storage of asparaginase stock solutions are insufficient to assess the asparaginase determinations reported in the BLA.

Finding #6 - Failure to adjust nominal asparaginase concentrations in calibrator and quality control solutions for the actual content of L-asparaginase commercial vials

EUSA claims that actual vial contents were used to calculate the concentrations of stock solutions after reconstitution. The records examined at (b) (4) do not support this contention.

OSI notes that the exhibits collected during the inspection show that one 1,000 unit vial (lot 078K4130) and eight 100 unit vials (lot 048K4058) were used to prepare stock solutions. Both lots have the same enzymatic activity (222.9 units/mg protein) and same protein content by biuret method (61.5%). The lab notebook

states that the activities of the stock solutions were 560 U/mL for runs on 3/18/09 and 548.5 U/mL for all later runs. If the stock solutions were prepared from vials with the same activity, the activities of the resulting solutions should be the same. Furthermore, if the 100 unit vial was reconstituted with 0.2 mL of vehicle per the SOP, the resulting concentration would have been less than either of the reported activities of the stock solutions.

Finding #7 - Reported serum asparaginase activities less than the lower limit of quantitation. Specifically, the LLOQ of the asparaginase assay was 0.025 IU/mL. However, asparaginase concentrations ranging from 0.009 to 0.024 IU/mL were reported.

EUSA claims that the asparaginase activities below the Lower Limit of Quantitation (BLQ; values below the lowest calibrator activity), were used only for calculation of means and medians, and have no impact on primary or secondary endpoints.

OSI suggests that BLQ values should have been treated as zeroes for calculating descriptive means and medians. It is unlikely that the BLQ values would affect medians or alter counts versus the 0.1 U/mL criterion, as the LLOQ was 0.025 U/mL. However, the observation reflects that (b)(4) did not follow the established SOP in conduct and reporting of study activities.

FINDINGS SPECIFIC TO THE ASPARAGINE ASSAY

Sponsor Summary Comment:
The Sponsor has now reviewed every asparagine assay performed either pre-dose or prior to dose 6 to determine whether or not the samples met the following criteria for rejection:

- Sample time unknown.
- Sample not collected or received.
- Dose unknown or administered in a manner inconsistent with the protocol.
- Sample was inadequately acidified (pH > 2).
- Chromatographic peaks could not be accurately integrated.
- Internal standard peak not observed.
- Standard curve failed acceptance criteria.
- Sample processing was not completed within 5 minutes for centrifugation and 10 minutes for acidification.

> Samples were declared not evaluable if any of these conditions were met. A total of 11 patients had at least one evaluable sample.

OSI agrees that any of these conditions individually should preclude use of the asparagine concentration datum. In addition, OSI believes (see finding #8, below) that runs without at least one passing quality control result at each concentration should be rejected. Thus, the following subjects' samples in EUSA's Table 3 were assayed in runs with failing QCs:

Subject	Date
787221	3/15/10
787538	3/15/10
787968	3/25/10
791030	4/8/10
791109	4/2/10
791194	4/8/10
791637	4/8/10
791653	4/8/10
795925	12/8/10

These nine values should also be rejected. Only two pre-dose 6 values in Table 3 were derived from runs with passing singlet quality control results at three concentrations:

794813	4/19/10
796945	4/27/10

[See also finding #11: The stability of these two samples during several months of storage cannot be assured.] The fact that these impediments were not declared in the original BLA's tables is disconcerting. However, during the inspection, [redacted] (b)(4) stated that he believed that the draft data, without his revealing all of these conditions, would not be reported to the BLA. He further stated his intention to complete a final report for both asparaginase and asparaginase assays with appropriate exclusions and revisions, and to submit them to both EUSA and DSI (now OSI). To date, FDA has not received the final report. OSI believes that [redacted] (b)(4) should be responsible for completing the final report, and for assuring validity of data in them (only if he is able to do so), and that EUSA evaluators do not have intimate knowledge of all circumstances in [redacted] (b)(4) laboratory. OSI recommends that none of the asparagine concentrations reported in the BLA for the protocol-defined endpoint is credible.

Finding #8 - Failure to reject analytical runs on 3/9/10, 3/11/10, 3/15/10, 3/25/10, 3/30/10, 4/2/10, 4/8/10, 4/15/10, and 12/8/10 when the quality control (QC) samples failed the acceptance criterion at one or two of the three QC concentrations

EUSA responds that, "The results of the QC samples were not used for evaluating run acceptability," because they were prepared in transfusion plasma rather than water like the calibrators, and the nominal QCs concentrations needed to be adjusted for endogenous asparagine concentrations.

OSI believes that this rationale is incorrect, and that QCs in the same matrix as the study samples (heparinized plasma) should have been used for the method validation and run acceptance during the study. OSI notes that the endogenous asparagine concentrations were the results of replicate determinations, using the isotopic asparagine internal standard method, and in the absence of residual asparaginase. For these reasons, the measurements of endogenous asparagine were arguably more confident than any others at (b)(4) Other strategies (e.g., selecting lots of blank plasma with low endogenous asparagine concentrations, preparing QCs in another surrogate matrix) were not employed, and there is no other reason apparent for using the QCs, except to assess acceptability of the validation results and the study analytical runs. When only one or two of the singlet QC results passed the acceptance criterion (±15% of nominal) for calibrators, and the deviations greatly exceeded the performance claims in the partial validation, the runs should have been rejected.

Finding #9 - Failure to reject chromatograms Failure to reject chromatograms when no asparagine internal standard was detected or when peaks could not be accurately integrated

EUSA acknowledges that they requested all data from (b)(4) for completeness, but that they do not now intend to use the flawed data.

The records examined during the inspection, and confirmed by photocopies in the Establishment Inspection Report, demonstrated that (b)(4) and his associates deliberately replaced the unquantifiable chromatographic peak areas in multiple records with areas from other chromatograms. Although EUSA may not have been aware of this deliberate falsification at the time, it impugns the integrity of all results from this laboratory.

Finding #10 -Failure to exclude plasma samples from clinical sites which were unacidified or were received in the thawed state

EUSA acknowledges in the overall summary comment that unacidified or thawed samples would yield questionable results, and reported them only to demonstrate their intention to collect these data, despite recognizing that they could not be valid. EUSA retracted specific samples with these features in the summary comment.

OSI believes that [redacted] (b) (4) also was at fault, in that he did not declare that unacidified or thawed samples were not useful for determination of asparagine in the presence of asparaginase. Neither [redacted] (b) (4) nor EUSA should have forwarded the questionable results.

Finding #11 - Failure to demonstrate stability of samples under the conditions of the study. Examples under each subpoint

Finding #11a - The blank plasma used in method development, validation, and QC samples for the asparagine assay was citrate-phosphate-dextrose transfusion plasma, not heparin plasma as in study samples.

EUSA relays [redacted] (b) (4) claim that he was unable to obtain blank human plasma from heparin-anticoagulated blood. Instead he obtained expired transfusion plasma from the [redacted] (b) (4), collected with citrate-phosphate-dextrose anticoagulant.

Transfusion plasma is diluted (i.e., less viscous) by approximately 25% from native blood plasma, and contains additional pH and redox buffers, all of which would affect evaluations of accuracy, precision, stability, and preservation by hydrochloric acid. [redacted] (b) (4) claim that heparinized human plasma was not available from other sources is patently untrue. His claim that he attempted on multiple occasions to find a commercial source is not credible. His 2/18/09 "Final Study Report" entitled "Determination of Asparagine in Human Plasma in the Presence of Asparaginase at Therapeutic Activities," was erroneous on p. 1 when he listed, "Plasma, human donor, heparinized, frozen [redacted] (b) (4)

Finding #11b - There was no evaluation of freeze/thaw or long-term frozen stability of samples for the asparagine assay. Most plasma samples were stored frozen for 3 to 11 months before assay for asparagine.

EUSA replies that if the experiments had been conducted, and if they found no measurable concentrations of asparagine, they could not have distinguished whether the samples had degraded through residual asparaginase or other storage conditions.

OSI believes that in the absence of a demonstration that asparagine concentrations would have been stable under the conditions of handling and storage, the failure to detect or quantify asparagine in many study samples cannot be ascribed solely to *in vivo* action of asparaginase. EUSA's argument has no merit.

Finding #11c - Records of freezer temperatures for storage of asparagine samples were not retrievable in an auditable form. The alarm system for temperatures outside -70°C to -90°C did not record the extreme excursions of temperature and durations of the excursions when the alarm triggered, including the event on 3/4/10 when the majority of study asparagine samples were in this freezer.

EUSA refers to their discussion of Finding #3 (above).

OSI believes that the conditions of storage for the majority of the study's asparagine samples cannot be established by the alarm records alone, for the reasons given above. (b)(4) has not provided the detailed temperature records since the inspection. However, it is unlikely that the temperature records could overcome the multiple other impediments in the asparagine data.

Finding #11d - Some samples were received thawed (7 shipments), or without acid preservative for asparagine (61 samples), or with documented delays between sample collection and plasma acidification (multiple examples longer than 10 minutes).

EUSA refers to the response to Finding #10, and claims that selective exclusion of data is possible.

OSI believes that none of the asparagine data can be used, for one or more of the reasons given above, including failure to

acidify, thawing accidents, undocumented conditions of storage, failure to demonstrate stability for the known times and conditions of handling and storage, and inappropriate run acceptance/rejection.

Finding #11e - The effectiveness of hydrochloric acid in preserving asparagine in plasma was tested only for *E. coli* asparaginase, not for *Erwinia* asparaginase.

EUSA acknowledges that the finding is correct, and cites literature about pH dependency.

OSI notes that enzymatic activity under other conditions, especially with partial acidification, does not necessarily predict stability of asparagine for the prolonged duration of storage under undocumented conditions. See EUSA's response to finding #11f, acknowledging the same.

Finding #11f - The times when samples were removed from frozen storage for analysis were not recorded.

EUSA responds that, "... it is recognized that a lengthy delay in sample analysis could have permitted continued degradation of asparagine by residual asparaginase, despite acidification, and result in an artifactually low level of asparagine."

OSI considers the times of removal from frozen storage to be among the several undocumented conditions, which prevent a full assessment of sample stability.

Finding #12 - Between-run accuracy and precision for the asparagine assay were not evaluated.

EUSA repeats the claim that between-run accuracy and precision were evaluated, and offers to provide the calculation sheets already examined during the inspection.

OSI commented during the inspection and at the closing teleconference that (b)(4) had portrayed multiple sets of within-run accuracy and precision as between-run accuracy and precision. However, (b)(4)s data had no bridge to justify calling these between-run. Without a demonstration of between-run accuracy and precision, it is difficult to justify combining the results of individual patients when their samples were assayed in different runs.

Finding #13 - Failure to evaluate the variability in recovery of asparagine in more than one plasma sample in a run.

EUSA responds that there was no need to evaluate variability in recovery of asparagine, in that the variability was reflected in the recovery of the isotopic asparagine internal standard.

OSI is well-acquainted with the theory, and further aware that many laboratories find that the nearly simultaneously eluting peaks for analytes and isotopic internal standards can be differently detected in a time-multiplexed mass spectrometer when "matrix effects" cause ion suppression or ion enhancement. The OSI experience is that measured recoveries of analytes and isotopic internal standards are not necessarily identical, especially when there is considerable inter-sample variability in the internal standard. However, the principal focus at (b)(4) (b)(4) laboratory is that analyte asparagine (at some finite concentration) was present in the samples for several months, and the isotopic internal standard was added only on the day of analysis. It is unclear at (b)(4) laboratory whether the variabilities are due to ordinary matrix effects, differential effects on detection of analyte and internal standard, or differential recovery of the analyte and internal standard due to the different times they were exposed to plasma. Therefore, finding #13 has been addressed only to agree that this aspect of variability and accuracy was not evaluated.

Finding #14 - Failure to evaluate the stability of asparagine in stock solutions or extracts.

EUSA claims that stability of asparagine in a variety of solutions and biological fluids has been shown in the literature.

(b)(4) did not evaluate or demonstrate the stability under the conditions encountered in his laboratory. OSI believes that this would have been an essential component of a proper method validation.

Finding #15 -Only a single stock solution of asparagine was used for both calibrators and QC samples, rather than independently-prepared stock solutions, in both pre-study validation and within-study conduct.

EUSA cites FDA's Guidance on Bioanalytical Method Validation, which is applicable to certain parts of NDAs and ANDAs, and claims that separate solutions are not required by the Guidance if stock solution stability has been established elsewhere.

During routine NDA and ANDA inspections, OSI has expected to see at least one demonstration of accuracy, using calibrators and QC samples made with independently-prepared stock solutions, according to the requirement of 21 CFR 320.29, because errors in weighing and diluting a single solution can be propagated. Comparing subsequent dilutions of an incorrectly-prepared stock solution does not serve to confirm its concentration. Although this regulation does not apply to the pharmacodynamic endpoint of this biologic study, the principle calls for demonstration that measurement of concentrations in QC samples are accurate when references to independent calibrators. Thus, demonstrating that two aliquots of the same stock solution have the same concentration is not meaningful. (b) (4) and EUSA have not addressed the finding.

Finding #16 - Failure to verify (by balance printer or witness) the weights of asparagine used for calibrator and QC stock solutions

EUSA acknowledges that these significant operations were not verified. Therefore, the true concentrations of the stock solutions were not completely assured. This impacts the interpretation of Finding #15.

OSI recognizes that the asparagine stock solutions used at various times during this study were nearly the same concentration, relative to the concentration of the isotopic internal standard. This is reflected in the mean slope of the calibration curves, 0.0458 mL/µg, although the theoretical slope would have been 0.0256, calculated from the nominal concentration of the internal standard.

In summary, the recommendations in our March 16 and 23, 2011 memoranda remain unchanged:

- The accuracy of the reported bioanalytical data for serum asparaginase activities and plasma asparagine concentrations cannot be assured.
- The lack of records demonstrating proper handling and storage of samples between blood collection and analyses precludes reconstruction of these data, even if future method validation experiments are conducted properly.

OSI is drafting a regulatory letter addressed to the inspected firm [redacted] (b) (4) to address the uncorrected deficiencies documented during the inspection.

Charles R. Bonapace, Pharm.D.

Michael F. Skelly, Ph.D.

Final Classification:
OAI - [redacted] (b) (4)

cc:
OSI/Ball/Moreno/Salewski/Cooper
OSI/Haidar/Yau/Dejernett/Skelly/Bonapace/CF
DBOP/Keegan/Dinndorf/Demko/Laughner
DCP5/Yang/Zhao/Booth
Draft: MFS 7/12/11
Draft: CRB 7/13/11
Edit: MKY 7/13/11
OSI file: 6168
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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/

CHARLES R BONAPACE
07/14/2011

MICHAEL F SKELLY
07/15/2011

MARTIN K YAU
07/15/2011

Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research

Office of Biotechnology Products
Federal Research Center
Silver Spring, MD
Tel. 301-796-4242

Memorandum

PROJECT MANAGER'S REVIEW

Application Number:	STN 125359/0
Name of Drug:	Erwinaze™ (proper name)
Sponsor:	EUSA Pharma (USA), Inc.
Material Reviewed:	Erwinaze™ (proper name) Carton and Container Labels
Submission Date:	September 8, 2010 and February 28, 2011

EXECUTIVE SUMMARY

The carton and container labels for Erwinaze™ (proper name) were reviewed and found to comply with the following regulations: 21 CFR 610.60 through 21 CFR 610.67; 21 CFR 201.2 through 21 CFR 201.25; 21 CFR 201.50 through 21 CFR 201.57, 21 CFR 200.100 and United States Pharmacopeia, 2/1/11-5/1/11, USP 33/NF 28. Labeling deficiencies were identified. Please see comments in the conclusions section.

Background

STN 125359/0 for proper name is an original Biologic License Application (BLA) indicated for as a component of a multi-agent chemotherapeutic regimen for the treatment of patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to *E. coli*. The product is available as a sterile lyophilized powder in 10,000 international units per vial.

Labels Reviewed:

Erwinaze™ (proper name) Container Labels
    Vial labels
Erwinaze™ (proper name) Carton Labels
    Five Vial Carton

Start of Sponsor Material

Vial Labels

(b) (4)

(b) (4)

End of Sponsor Material

I. Container

A. 21 CFR 610.60 Container Label

1. Full label. The following items shall appear on the label affixed to each container of a product capable of bearing a full label:
   a. The proper name (established name) of the product, proper name– is displayed along with the Trade name (proprietary name), Erwinaze™. This conforms to the regulation.

   b. The name, addresses, and license number of the manufacturer – The complete address should be listed, along with the U.S. license number. "EUSA Pharma (USA), Inc., Langhorne, PA 19047, License No. ???? is listed. This does not conform to the regulation. Revise to read US License No.

c. The lot number or other lot identification – The lot number is located on the container label. This conforms to the regulation.

d. The expiration date – The expiration date is displayed on the container label. This conforms to the regulation.

e. The recommended individual dose, for multiple dose containers – This product is supplied in a single use vial. This regulation does not apply.

f. The statement "Rx only" for prescription biologicals – The statement "Rx Only" is located on the label. This conforms to the regulation.

g. If a Medication Guide is required under part 208 of the chapter, the statement required under §208.24(d) of this chapter instructing the authorized dispenser to provide a Medication Guide to each patient to whom the drug is dispensed and stating how the Medication Guide is provided, except where the container label is too small, the required statement may be placed on the package label – A medication guide is not required. This regulation does not apply.

2. Package label information. If the container is not enclosed in a package, all the items required for a package label shall appear on the container label. – The container is enclosed in a package (carton). This regulation does not apply.

3. Partial label. If the container is capable of bearing only a partial label, the container shall show as a minimum the name (expressed either as the proper or common name), the lot number or other lot identification and the name of the manufacturer; in addition, for multiple dose containers, the recommended individual dose. Containers bearing partial labels shall be placed in a package which bears all the items required for a package label. – the product bears a full label. This regulation does not apply.

4. No container label. If the container is incapable of bearing any label, the items required for a container label may be omitted, provided the container is placed in a package which bears all the items required for a package label. – This container bears a label. This regulation does not apply.

5. **Visual inspection. When the label has been affixed to the container, a sufficient area of the container shall remain uncovered for its full length or circumference to permit inspection of the contents.** – This does not conform to the regulation. Information requested.

B. **21 CFR 201.2 Drugs and devices; National Drug Code numbers – The National Drug Code (NDC) number is located at the top of the label. Per 21 CFR 207.35, the last five digits of the NDC number represent the Product-Package Code configuration in either a 3-2 or 4-1 configuration. The NDC does not appear on the container label and is requested but not required. This conforms to the regulation.**

C. **21 CFR 201.5 Drugs; adequate directions for use – A reference to the prescribing information is not stated on the container label, however it does appear on the carton label. This conforms to the regulation.**

D. **21 CFR 201.6 Drugs; misleading statements – The only names that appear on the label are the trade name (proprietary name), Erwinaze™ and the proper name (established name), proper name. This conforms to the regulation.**

E. **21 CFR 201.10 Drugs; statement of ingredients –This conforms to the applicable parts of the regulation.**

F. **21 CFR 201.15 Drugs; prominence of required label statements – This conforms to the applicable parts of the regulation.**

G. **21 CFR 201.17 Drugs; location of expiration date – The expiration date appears under the lot number. This conforms to 21 CFR 610.60 and 21 CFR 201.17.** Recommend requesting format of dating and changing expiry to exp.

H. **21 CFR 201.25 Bar code label requirements –A bar code is not displayed on the label.** This does not conform to the regulation.

I. **21 CFR 201.50 Statement of identity – The proper name (established name), proper name is stated on the label with the trade name (proprietary name), Erwinaze. This conforms to the regulation.**

J. **21 CFR 201.51 Declaration of net quantity of contents – The net quantity is declared, "10,000 International Units per vial". This conforms to the regulation.**

K. **21 CFR 201.55 Statement of dosage – No statement appears on the container label with a statement of dosage or a reference to a statement of**

dosage. A dosage statement is provided on the carton. This conforms to the regulation.

L. 21 CFR 201.100 Prescription drugs for human use – The label bears statements of "Rx Only" and other pertinent information. This conforms to the regulation.

(b) (4)

(b) (4)

End of Sponsor Material

II. Carton

A. 21 CFR 610.61 Carton/Package Label –

a. **The proper name (established name) of the product (proper name) – is displayed along with the (proprietary name), Erwinaze™. This conforms to the regulation.**

b. The name, addresses, and license number of the manufacturer – The complete address should be listed, along with the U.S. license number. "EUSA Pharma (USA), Inc., Langhorne, PA 19047, License No. ????" is listed. This does not conform to the regulation. Revise to read US License No.

c. **The lot number or other lot identification – The lot number is located on the carton label. This conforms to the regulation.**

d. **The expiration date – The expiration date is below the lot number. The format is not provided. This conforms to the regulation.** Revise the term "Expiry Date:" to "Exp date:" and provide format for dating.

e. **The preservative used and its concentration, if no preservative is used and the absence of a preservative is a safety factor, the words "no preservative" –The statement "No Preservative" is not displayed on the carton.** This does not conform to the regulation.

f. **The number of containers, if more than one –The product is supplied with five single-use vials per carton. The number of containers is provided on the side panel and on the primary panel. This conforms to the regulation.** Recommend revising quantity statement and moving to primary panel.

g. **The amount of product in the container expressed as (1) the number of doses, (2) the volume, (3) units of potency, (4) weight, (5) equivalent volume (for dried product to be reconstituted), or (6) such combination of the foregoing as needed for an accurate description of the contents, whichever is applicable – The amount of product is expressed as 10,000 International Units per vial. This conforms to the regulation.**

h. **The recommended storage temperature – The statement "Store at 2° to 8°C (36° to 46°F) is displayed on the primary panel of the carton. This conforms to the regulation.**

i. The words "Do not Freeze" or the equivalent, as well as other instructions, when indicated by the character of the product –This does not conform to the regulation. Add the statements, "Store in carton until use to protect from light".

j. **The recommended individual dose if the enclosed container(s) is a multiple-dose container –The product is supplied in single -use vials. This regulation does not apply.**

k. **The route of administration recommended, or reference to such directions in and enclosed circular – The recommended dosage statement states the route of administration is intramuscular. This conforms to the regulation.** Recommend adding route of administration with primary presentation of Trade name and Strength.

l. **Known sensitizing substances, or reference to enclosed circular containing appropriate information –none listed. This conforms to the regulation.**

m. **The type and calculated amount of antibiotics added during manufacture – none listed. This conforms to the regulation.**

n. **The inactive ingredients when a safety factor, or reference to enclosed circular containing appropriate information. The inactive ingredients are listed on the primary panel of the carton. This conforms to the regulation.** Per USPC, inactive ingredients should be listed in alphabetical order.

o. **The adjuvant, if present –none listed. This conforms to the regulation.**

p. **The source of the product when a factor in safe administration –The source of the product is not listed on the carton. This conforms to the regulation.**

q. **The identity of each microorganism used in manufacture, and, where applicable, the production medium and the method of inactivation, or reference to an enclosed circular containing appropriate information. – *Erwinia chrysanthemi* (nee *carotovora)* is listed in the package**

insert. This conforms to the regulation. Recommend adding to the carton.

r. Minimum potency of product expressed in terms of official standard of potency or, if potency is a factor and no U.S. standard of potency has been prescribed, the words "No U.S. standard of potency" – "No U.S. Standard of Potency" is displayed on the carton. This does not conform to the regulation.

s. The statement "Rx only" for prescription biologicals – The statement "Rx Only" is located on the carton. This conforms to the regulation.

t. If a Medication Guide is required under part 208 of this chapter, the statement required under §208.24(d) of this chapter instructing the authorized dispenser to provide a Medication Guide to each patient to whom the drug is dispensed and stating how the Medication Guide is provided, except where the container label is too small, the required statement may be placed on the package label –A medication guide is not required. This conforms to the regulation.

B. 21 CFR 610.62 Proper name; package label; legible type : This product is a not a "specified" biological product and is subject to this regulation. Proper name shall be placed above any trade name and shall be at least as prominent as the point size and typeface used in designating the trade name. This does not conform to the regulation.

C. 21 CFR 610.63 Divided manufacturing responsibility to be shown –This regulation does not apply.

D. 21 CFR 610.64 Name and address of distributor
The name and address of the distributor of a product may appear on the label provided that the name, address, and license number of the manufacturer also appears on the label and the name of the distributor is qualified by one of the following phrases: "Manufactured for _____". "Distributed by _____", "Manufactured by _____ for _____", "Manufactured for _____ by _____", "Distributor: _____", or 'Marketed by _____". The qualifying phrases may be abbreviated. This regulation does not apply.

E. 21 CFR 610.65 Products for export – This product will be distributed outside the US. This conforms to the regulation.

F. 21 CFR 610.67 Bar code label requirements

Biological products must comply with the bar code requirements at §201.25 of this chapter. – A bar code appears on the carton label. This conforms to the regulation.

G. 21 CFR 201.2 Drugs and devices; National Drug Code numbers – The National Drug Code (NDC) number is located near the bottom of the primary panel. Per 21 CFR 207.35, the last five digits of the NDC number represent the Product-Package Code configuration in either a 3-2 or 4-1. The NDC configuration appears as, "NDC 57902-XXX-XX". This conforms to the regulation.

H. 21 CFR 201.5 Drugs; adequate directions for use – The label states "Use sterile procedures for product reconstitution and administration. See Package Insert for details and conditions for use after reconstitution." This conforms to the regulation. Recommend revising to usual dosage statement and removing references to reconstitution.

I. 21 CFR 201.6 Drugs; misleading statements – The only names that appear on the label are the trade name (proprietary name), Erwinaze and the proper name (established name), proper name. This conforms to the regulation.

J. 21 CFR 201.10 Drugs; statement of ingredients – Regulated by 21 CFR 610.62. This regulation does not apply.

K. 21 CFR 201.15 Drugs; prominence of required label statements – This conform to the regulation.

L. 21 CFR 201.17 Drugs; location of expiration date – The expiration date appears under the lot number on the bottom of the label. This conforms to 21 CFR 610.61 and 21 CFR 201.17.

M. 21 CFR 201.25 Bar code label requirements – A bar code appears on the carton label. This conforms to the regulation.

N. 21 CFR 201.50 Statement of identity – The proper name (established name), proper name is stated on the label with the trade name (proprietary name), Erwinaze. This conforms to the regulation.

O. 21 CFR 201.51 Declaration of net quantity of contents – The net quantity is declared, "10,000 International Units (IU) per vial, 5 vials". This conforms to the regulation.

P. 21 CFR 201.55 Statement of dosage –The statement "Use sterile procedures for product reconstitution and administration. See Package Insert for details and conditions for use after reconstitution." appears on

the label. This conforms to the regulation. Recommend revising the statement

Q. 21 CFR 201.100 Prescription drugs for human use – The label bears statements "Rx Only" and other pertinent information. This conforms to the regulation.

Conclusions

The following deficiencies and recommendations were noted in the review of the Erwinaze™ container and carton labels.

1. Container
   a. Please indicate how the label is affixed to the vial and where the visual area of inspection is located per 21 CFR 610.60.
   b. Please see below for recommended format of trade name, proper name, strength, and dosage form presentation. *See recommended format
   c. Please add a bar code to the label per 21 CFR 201.25.

2. Carton label
   a. (b) (4)
   b.
   c.
   d. Per USPC Official 2/1/11-5/1/11, USP 33/NF 28, <1091> Labeling of Inactive ingredients, please list the names of the inactive ingredients in alphabetical order in the following format: inactive ingredient (amount)
   e. The statement, "5 Vials per Pack" is currently located on the top flap of the carton, please move the statement to the principle display panel. * See recommended principle display format below
   f. Revise the statement, "License No:" to "U.S. License No" per 21 CFR 610.60 and 21 CFR 610.61.
   g. Remove the all references to reconstitution instructions from the principle display panel. The usual dosage statement will refer the user to complete instructions for reconstitution, dilution, and dosage.
   h. Consider adding the microorganism used in the manufacturer of the product per 21 CFR 610.61 to the carton in addition to the listing in the prescribing information.
   i. (b) (4)

3. Carton and Container

a.

b.

c.

d.

e.

(b) (4)

***Recommended formats**

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

6/16/11

Kimberly Rains, Pharm.D
Regulatory Project Manager
CDER/OPS/OBP

Comment/Concurrence:

06/17/2011

Jacek Cieslak, Ph.D.
Product Reviewer
Division of Therapeutic Proteins
CDER/OPS/OBP

6-17-11

Barry Cherney, Ph.D.
Deputy Director
Division of Therapeutic Proteins
CDER/OPS/OBP

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration
Center for Drug Evaluation and Research
OPS/OBP/ Division of Therapeutic Proteins

CONSULT REQUESTED ON:	Immunogenicity (Method validation)
REVIEWER:	Faruk Sheikh, Ph.D., Laboratory of Immunology, Bldg 29A, 3B-15, Rockville Pike, Rockville-20892 Faruk Sheikh, 6/3/11
APPROVED BY:	Susan Kirshner, Ph.D, Associate Chief, Laboratory of Immunology Susan R. Kirshner 6/1/11
STN:	STN125359 (Erwinaze)
PRODUCT:	L-Asparaginase for injection (*Erwinia chrysanthemi*)
ROUTE OF ADMIN:	Intramuscular Injection (IM); alternate dosing by s.c. & i.v. routes
DOSE REGIMEN:	25,000 IU/m2 IM MWF 6 doses for each dose of pegasparaginase
SPONSOR:	Eusa Pharma (USA) Inc. One Summit Square 1717 Langhorne Newtown Road Langhorne, PA-19047
INDICATION:	(b) (4) acute lymphoblastic leukemia (ALL), as a substitute for *E. coli*-derived enzymes.

REVIEW TEAM:

Yang, Jun:	Clinical Pharmachology Reviewer
Laughner, Erik S:	Regulatory Project Manager
Cieslak, Jacek:	Product Quality Reviewer
Dinndorf, Patricia A:	Clinical Reviewer

COMMENT TO THE SPONSOR

You have not provided information demonstrating that you have validated assays to detect anti-drug: binding antibodies; neutralizing antibodies; IgE antibodies. This information along with the test results from clinical samples should be submitted to the BLA. We recommend that you submit to the IND complete validation reports with relevant developmental data along with SOPs for routine assay performance to the Agency for review prior to testing critical clinical samples.

RECOMMENDATION:

The Sponsor submitted information in an attempt to support the validation of an anti-erwinase antibody (anti-drug antibody, ADA) screening assay followed by a confirmatory assay using immunodepletion. The Sponsor also submitted data in an attempt to support to validate a neutralizing antibody assay. All assays were deficient in several critical areas and there were major flaws in the development of screening assay. Therefore it was determined that the screening assay needed to be redesigned. The neutralizing assay may not need to be redesigned to address our concerns.

The initial submission of these assays was to the IND, and the reviews were submitted to the IND. None of the concerns identified in the IND review were addressed in the original BLA submission. Following our review of the IND, we sent an advice/information request letter to the Sponsor (May 6, 2010 see Appendix I *for specific comments) in which additional data to support the use of the total anti-Erwinase antibody assay and the development of a neutralizing antibody assay were requested. In addition, we suggested the sponsor to develop an IgE assay to assess the hypersensitivity rate because hypersensitivity reactions have been observed with E.coli-derived asparaginases.*

In response to our information requests the Sponsor provided an amendment to the Erwinase IND (IND 290) on 27-th July, 2010 and requested a Type-C meeting. However, after reviewing the meeting request package we held a teleconference with the sponsor on 17th December, 2010, in order to discuss the deficiencies in the validation of their assays and to provide them guidance to develop appropriate assays to detect anti-erwinase antibodies.

During this meeting we suggested: 1) the screening assay be redesigned as the Sponsor chose an invalid approach to setting the cut-point; 2) the Sponsor to develop an erwinase-specific positive control antibody by hyperimmunizing appropriate animals since the Sponsor used an inappropriate positive control antibody raised against E. coli asparaginase, which shows limited cross-reactivity to Erwinia asparaginase (see appendix II and appendix III for specific comments and suggestions). We also discussed the deficiencies in the neutralizing antibody assay development and the necessity of having an IgE assay. The Sponsor acknowledged our suggestions and submitted a timeline (see below) to the agency with brief outline for redeveloping these assays (screening assay, neutralizing antibody assay and IgE assay) starting from hyperimmunizing animals with erwinase, purification of erwinase-specific antibodies, completion of assay validation and to begin with clinical sample analysis in early 2012. Given the scope of the project, the time required may be justified. No progress report for the current projects was submitted to the Agency until now for review. Therefore, we do not have any specific comments regarding the new assays at this time.

However, we recommend the Sponsor to submit full validation reports with relevant developmental data along with SOPs for routine assay performance to the Agency for review prior to testing critical clinical samples. These reports may be submitted to the IND.

PROPOSED TIMELINE FOR ASSAY DEVELOPMENT AND VALIDATION, JANUARY 19, 2011:

	Immunization of Animals	ELISA for Total Erw Abs	Neutralizing Abs	IGE Assay
Timeline submission to FDA	25Jan11	25Jan11	25Jan11	25Jan11
FDA Feedback	04Feb11	04Feb11	04Feb11	04Feb11
(b) (4)	18Feb11	18Feb11	18Feb11	18Feb11
Start of 8 Week Immunization	28Feb11			
Completion of Immunization and Start of Assay Validation	09May11	09May11	09May11	09May11
Final Report of Immunization	13June11			
Final Assay Report to FDA		03Oct11	03Oct11	03Oct11
6 Week FDA review time		07Nov11	07Nov11	07Nov11
Samples Run and Results Reported to FDA		**15Jan12**	**15Jan12**	**15Jan12**

PRODUCT BACKGROUND:

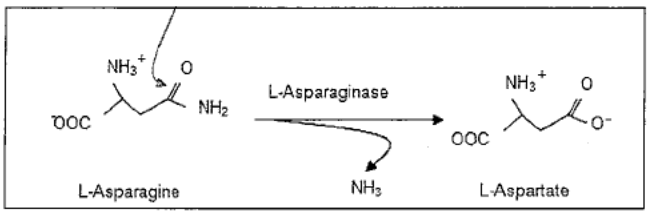
Erwinase, an L-asparaginase derived from non-human pathogen *Erwinia chrysanthemi*, has been proposed as a substitute for *E coli*-derived asparaginases (*E. coli* asparaginase or Elspar and PEG asparaginase or Oncaspar). L-asparaginase is an important component of multi-agent chemotherapy for childhood acute lymphoblastic leukemia (ALL). ALL is the most frequent cancer in children representing nearly 25% of all cancer in the age group younger than 15 years.

Erwinia chrysanthemi is a gram-negative bacillus and belongs to the family of plant pathogens. Erwinase is licensed in Europe, Canada and Asia, but has never been commercially available in the United States. *E. coli*-derived L-asparaginases have been approved and used in the United States. Dana-Farber Cancer Institute ALL Consortium has demonstrated that about 30% of patients are hypersensitive to asparaginase and these responses are frequently associated with the development of neutralizing antibodies. PEG–asparaginase was developed with the objective of reducing the immunogenic

potential. When given intramuscularly, PEG-asparaginase has a longer half-life and is associated with a lower rate of antibody formation than the native enzyme.

Studies have found that Erwinia L-asparaginase has comparable efficacy and no cross reactivity with E. coli L-asparaginase. Thus, it may be an acceptable substitute for patients who are allergic to the E. coli protein. In addition, Erwinia asparaginase has a much shorter biological half-life of asparaginase activity (0.65 days) than either native E. coli (1.44 days) or PEG asparaginase (5.73 days.).

Asparaginase works by converting the extracellular pool of L-asparagine into aspartic acid and ammonia which is required by cells for protein synthesis. Normal cells can synthesize their own L-asparagine. However, the tumor cells are defective in producing L-asparagine and are dependent on the extracellular pool. Tumor cells require huge amounts of asparagine to keep up with their rapid growth, and otherwise die when the dietary supply is cut off by asparaginase.



The Erwinase enzyme is a homo-tetramer and each monomer has 35kDa MW.
The lyophilized Erwinase powder is freely soluble in water and the product is more inherently stable at alkaline pH than *E. coli*-derived asparaginase. This enzyme has more basic amino acid composition and it has less aspartic acid and arginine than the *E. coli*-derived protein. This protein also lacks cysteine and tryptophan.

The sponsor stated that each vial contains 10,000 U of Erwinia L-asparaginase, 0.50 mg NaCl EP and 5 mg of dextrose (glucose) monohydrate EP. The lyophilized drug product is reconstituted with 2 ml Sodium Chloride Injection USP and is suitable for intramuscular injection.

The investigators have chosen a dose of *Erwinia*, 25,000 IU/m2 IM MWF for 6 doses as replacement for 1 dose of PEG-asparaginase. They hypothesize that this dose and schedule of Erwinase will provide a 48 hour trough Erwinase activity of ≥ 0.1 IU/mL in patients with PEG-asparaginase allergy.

Comment to File: *This information is provided for background. The product quality review is deferred to the DTP Laboratory of Chemistry.*

OBJECTIVE: The objective was to validate the assays to quantitate anti-Erwinase antibody and to assess neutralizing antibodies developed in human serum by Erwinase.

TESTING FACILITY:

(b) (4)

(b) (4)

IMMUNOGENICITY REVIEW:

Study Title 1: Validation of the Assay to Quantitate Anti-Erwinase Antibody in Human Serum.

MATERIALS:

Positive Control: The positive control was rabbit anti-*E.coli* Asparaginase (Rockland, cat. #100-4171).

Comments: *The sponsor used rabbit anti-E.coli Asparaginase as a positive control to screen anti-erwinase antibody. This is not be appropriate because of limited cross-reactivity between these asparaginases.*

Negative Control: Twenty normal human sera pooled and diluted at 0.002%.
Standard Diluent: 0.002% pooled normal human sera with 0.1%BSA, 0.01% Tween-20 in PBS.
Quality Control (QC) Samples: The anti-asparaginase was diluted to 140, 70 and 35ug/mL in Standard Diluents as single-use High, Mid, and Low QC Samples.
Standard Curve Samples: The standard samples were in seven concentrations ranging 1.667 ug/ml to 4000 ug/ml in human serum.

METHOD:

The sponsor used an ELISA assay to measure anti-erwinase antibodies in human serum. In this assay, the ELISA plate wells were coated with 1 μg/mL Erwinase in 0.05M carbonate/bicarbonate buffer for 60±10 minutes at 36-38°C or 18 ± 6 hours at 2-8°C. The plates were washed with PBS-T (PBS, 0.01% Tween-20) and were blocked for 60±10 minutes with 1% BSA in PBS at 36-38°C. After washing, the wells were incubated for 60±10 minutes at 36-38°C on a shaker with either of the following solutions;

(1) Anti-asparaginase (positive control) titrated in Standard Diluent for a standard curve,
(2) High, Mid, and Low QC Sample sets of anti-asparaginase in Standard Diluent and
(3) Negative Control sample, derived from twenty pooled normal human sera (diluted in 0.1% BSA, 0.01% Tween-20 in PBS at 0.002% to normal serum concentration).

After washing unbound antibodies from the wells, protein A/G-HRP conjugate was added (1:250,000 in PBS with 0.1% BSA, 0.01% Tween-20) and the plates were incubated on a shaker for 60 ± 10 minutes at 36-38°C. The plates were again washed and TMB

(3,3′,5,5′-tetramentylbenzidine) Substrate was added for 15 ± 2 minutes at room temperature. TMB is a chromogenic substrate for horseradish peroxidase. Once oxidized by the enzyme, this substrate yields a blue product. TMB Stop Solution (sulfuric acid) stopped the reaction. The absorbance for a blue color was measured at 630 nm and yellow color developed after the addition of the stop solution was measured at 450nm. The difference of absorbance at 450 nm and 630 nm is calculated to correct for optical imperfections in the microplate, which is proportional to the quantity of the antibody bound to Erwinase. The sponsor indicated that a 4-parameter curve analysis was used to analyze each standard curve using GraphPad Prism.

Note: The dual wavelength analysis can help to overcome any non-wavelength specific imperfections in the plate. In theory, the use of dual wavelength analysis should provide better precision. In practice, because the optical quality of the microtiter plates is excellent, there is usually no significant statistical improvement in the data by subtracting out the reference absorbance from the test absorbance.

VALIDATION PARAMETERS REPORTED:

1) **Precision:**
   a) Intra-plate precision (variability within a plate).
   b) Inter-assay precision (repeatability or variability within one plate).
   c) Intermediate precision (variability from run to run).
2) **Accuracy:**
3) **R^2 value:**

Comment to Sponsor: *The sponsor did not submit data for the sensitivity and specificity of the assay. Please submit sensitivity, specificity and the limit of detection of your assay.*

PRECISION:

Intra-plate precision: The sponsor stated that the validation was assessed by comparing the measured anti-asparaginase concentrations from eight individual wells of each QC sample (High, Mid, Low). The standard deviation between these eight concentrations was used to calculate the variability (CV%). The following table reported the result (reproduced from the submission).

Table: Summary of Intra-plate Precision %CV Values

	Experiment #1:%CV of the QC Sample Tested			Experiment #2:%CV of the QC Sample Tested			Experiment #3:%CV of the QC Sample Tested			All Values <25%
QC Sample	Plate #1	Plate #2	Plate #3	Plate #1	Plate #2	Plate #3	Plate #1	Plate #2	Plate #3	
High QC	10	11	10	5	8	8	4	4	8	Yes
Mid QC	7	9	7	5	3	3	11	3	4	Yes
Low QC	6	8	5	3	3	3	9	3	2	Yes

Inter-assay precision: According to the sponsor this assay validation was performed by comparing the mean of anti-asparaginase concentrations of QC samples tested on 3 plates on the same day and the CV% was demonstrated from the SD values of the mean of the QC samples.

Table: Repeatability

	Experiment #1: Measured Anti-asparaginase, ug/mL						
QC Sample	Plate #1	Plate #2	Plate #3	Mean for Experiment #1	S.D.	%CV	%CV <25%
High QC	115.9	115.1	112.8	114.6	1.61	1	Yes
Mid QC	65.5	62.5	64.1	64.0	1.50	2	Yes
Low QC	43.4	39.5	42.9	41.9	2.12	5	Yes

	Experiment #2: Measured Anti-asparaginase, ug/mL						
QC Sample	Plate #1	Plate #2	Plate #3	Mean for Experiment #2	S.D.	%CV	%CV <25%
High QC	107.9	118.3	121.5	115.9	7.11	6	Yes
Mid QC	57.0	61.0	58.2	58.7	2.05	3	Yes
Low QC	36.9	38.5	36.6	37.3	1.02	3	Yes

	Experiment #3: Measured Anti-asparaginase, ug/mL						
QC Sample	Plate #1	Plate #2	Plate #3	Mean for Experiment #3	S.D.	%CV	%CV <25%
High QC	126.7	121.0	121.5	123.1	3.16	3	Yes
Mid QC	71.3	66.9	69.0	69.1	2.20	3	Yes
Low QC	43.3	42.0	43.6	43.0	0.85	2	Yes

Intermediate precision: The sponsor stated that the mean concentration for the same QC samples on different days (3 days) was used to assess intermediate precision. The CV% between experiments were calculated and shown in the table below (from the submission).

Table: Intermediate Precision

QC Sample	Mean for Experiment #1	Mean for Experiment #2	Mean for Experiment #3	Overall Mean for the Validation	S.D.	%CV	%CV <25%
High QC	114.6	115.9	123.1	117.9	4.6	4	Yes
Mid QC	64.0	58.7	69.1	63.9	5.2	8	Yes
Low QC	41.9	37.3	43.0	40.7	3.0	7	Yes

Comment to File: *The measured values of the QC samples were quantified against the standard curve and deduced the precision within a plate (Intraplate), between plates on a single day (repeatability) or between days (intermediate). The variability of these assays as measured by CV%, showed within acceptable limit (1-8%). Therefore, the precision of these studies were met. However, how erwinase coated plate reacted with rabbit anti-E. . coli asparaginase needs to be seen.*

ACCURACY: The accuracy of a QC Standard was validated by quantifying the High, Mid, and Low QC Standard concentrations against the standard curve and calculating the percent accuracy of the measured concentration to the expected concentration of the High, Mid, and Low QC Standard (140, 70 and 35 mg/mL). The result is shown below.

Table: Summary of Percent Accuracy

	Experiment #1: Mean Measured Anti-asparaginase, ug/mL			Experiment #2: Mean Measured Anti-asparaginase, ug/mL			Experiment #3: Mean Measured Anti-asparaginase, ug/mL			%Accuracy
QC Sample	Plate #1	Plate #2	Plate #3	Plate #1	Plate #2	Plate #3	Plate #1	Plate #2	Plate #3	100% ± 25%
High QC	116	115	113	108	118	122	127	121	122	Yes
Mid QC	66	63	64	57	61	58	71	67	69	Yes
Low QC	43	40	43	37	39	37	43	42	44	Yes

Comment to File: *The sponsor provided raw data of each experiment containing 8 read outs for each QC samples per plate with the submission. I reviewed all of them. The back calculated values were compared with the stated values and calculated percent accuracy of the samples, which were within acceptable limit (±25%). Therefore, the assessment is acceptable.*

R^2 Values:

The **R^2 value** is used to establish the strength of the trend. In this study the R-squared of the 4 PL analyses for each plate tested during the validation are presented in the table below which are greater than 0.99, which is good.

Table: Summary of R2 values.

	Experiment #1			Experiment #2			Experiment #3			All Values >0.98
	Plate #1	Plate #2	Plate #3	Plate #1	Plate #2	Plate #3	Plate #1	Plate #2	Plate #3	
R_2 Value	0.9944	0.9894	0.9935	0.9954	0.9943	0.9937	0.9970	0.9971	0.9992	Yes

Comment to Sponsor: *The sponsor developed an ELISA assay. The controls and QC samples used in this assay were demonstrated. However, the sponsor did not establish a limit of detection to select anti-erwinase positive samples. Generally, a cut-point of the assay is determined using normal and or patient's serum sample to identify antibody positive samples. Please develop a qualified assay and define cut-point of the assay to screen drug-induced antibody in patient's serum and submit to the agency.*

<u>Study Title 2:</u> Neutralizing Antibody Assay for Erwinase in Human serum.

Critical Reagents:

Normal human serum; Source: Bioreclamation or equivalent.
Asp-AMC (7-amino-4-methylcourmarin); Source: Sigma.
L-asparagine; Source: Sigma.

Background: The sponsor demonstrated an enzymatic inhibition assay to detect neutralizing antibodies to Erwinase in this submission. The sponsor stated that commercially available rabbit polyclonal antibody raised against the *E. coli*-asparaginase could not be used as a positive control because it was able to bind to Erwinase, but could not neutralize the enzyme activity. Their studies showed that the antibody is capable of inhibiting Erwinase activity by approximately 50% in PBS as well as in 0.5% serum (see Fig.1a). However, the Erwinase enzyme activity was inhibited by 60% in the presence of 1% serum, while addition of the anti-*E.coli* asparaginase did not cause further inhibition (see Fig1b below). This limited the utility of this assay for samples containing higher concentrations of serum.
Comment: *This antibody has limited cross-reactivity which could be the reason that the sponsor did not get an inhibition.*

Fig 1a

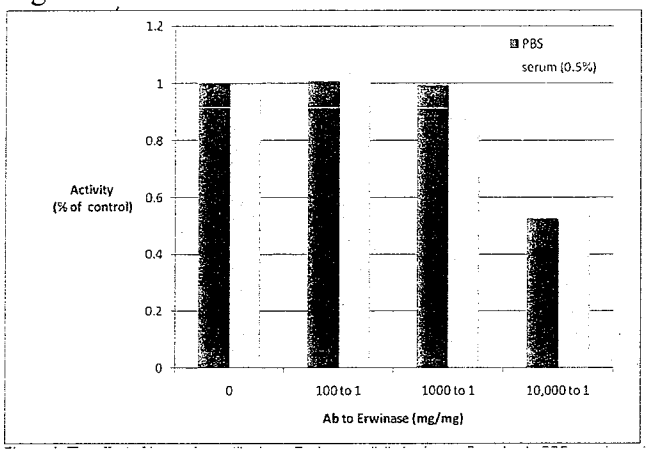
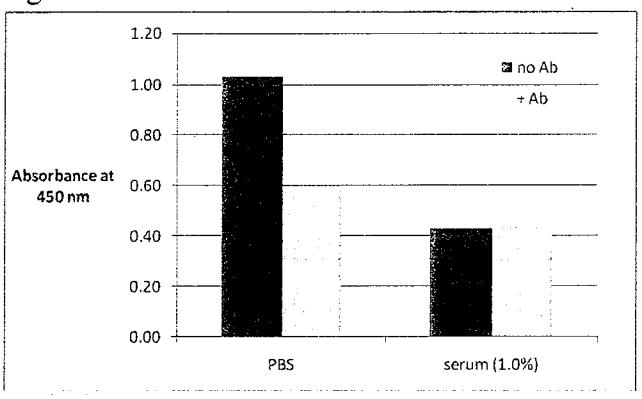


Fig 1b



The sponsor demonstrated that the serum also interferes with Nessler's Reagent (I reviewed the data provided with the submission, but it is not included in this review). The Nessler's reagent is commonly used to quantify ammonia that produces color when incubated with ammonium. Ammonia is produced by asparaginase that converts L-asparagine to aspartic acid and ammonia. Therefore, the sponsor developed a neutralizing antibody assay which utilizes a fluorescent substrate (Asp-AMC) for the enzyme Erwinase (see the assay below).

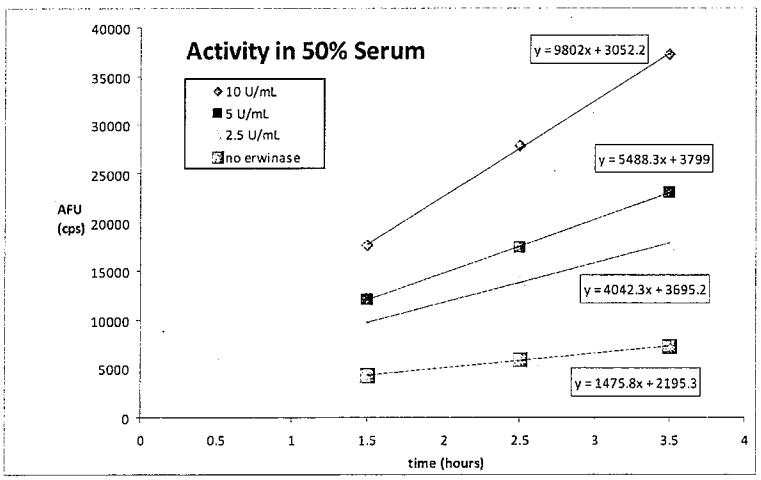
Assay Design: The sponsor developed an Enzyme Activity Assay to detect neutralizing antibodies to Erwinase in human patient serum using a fluorescent substrate of Erwinase (Asp-AMC) in a 96-well format. In this assay, Erwinase activity generates a product 7-amino-4-methylcourmarin (AMC) whose fluorescence is measured and is 200-fold greater than the substrate (Asp-AMC). This is a modified assay method originally published by Ylikangas and Mononen in Analytical Biochemistry in 2000 (**280,** 42–45). This assay used 50% serum to measure inhibition of Erwinase activity by Asp-AMC. For the analysis of patient serum samples, the assay employs a known Erwinase spiked serum sample compared to the unspiked serum sample, where the increase in fluorescence due to the spike would be inhibited by the presence of any neutralizing antibodies. L-asparagine (Asp-AMC) was used as an inhibitor to demonstrate enzyme inhibition in the assay and detected as decreased fluorescence. To demonstrate the cut-point assay the

sponsor used the following controls to normalize the patient serum samples: (1) a pool of normal human sera (U_P), (2) the same serum pool spiked with known amount of Erwinase (S_P) and (3) the same spiked serum pool with L-asparagine, the enzyme inhibitor (I).

In order to make the assay sensitive the sponsor determined (1) the concentration of Erwinase to be used and (2) the reaction time. Since, the titer in serum of neutralizing antibodies is unknown; a minimal concentration of Erwinase was required for increased sensitivity. The fluorescence increases over time as a result of enzymatic activity. The sponsor determined an optimum reaction time to get sufficient fluorescence signal that could be measured.

1) Final **Erwinase spike concentration** to be used 5 Units/mL.
2) The **reaction time** to be used at 37°C was 2 hrs to produce a detectable signal above the background.

Erwinase concentration and reaction time determination:
The sponsor stated that a varying amount of Erwinase (1.25-10 U/mL) was added to a serum pool in this assay and the change in fluorescence over time was measured. The samples were incubated at 37^0C in 50% serum (see effect of serum below). The sponsor stated that the 50% serum itself has some intrinsic activity. This background activity was subtracted from the Erwinase-containing samples in each reaction.



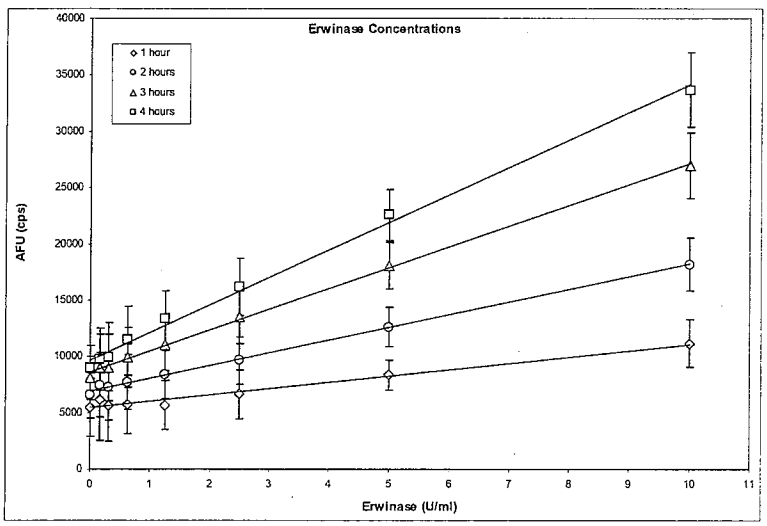
Comment to File: *The signal for all concentrations increased linearly over time including the serum control which lacks any added Erwinase. The increase for the samples containing serum alone likely comes from the activity of the endogenous enzyme glycosyl-asparaginase. The resolution in the signal differences between varying amounts of Erwinase increases with increase in time of incubation. So, to detect lower amount of the antibody by inhibition of enzyme activity, the sample has to incubate for longer time. The sponsor chose 2 hours reaction time, which should be okay for this assay and the approach is acceptable.*

The same data plotted (below) to show that Erwinase is proportional to the signal produced. The sponsor tested the signal produced at each time point by various concentrations of Erwinase and expressed by the number of SD above background. The sponsor argued that the SD equal or greater than 3 could be accurately measured (highlighted, see table below). At 2 hr time point 5U/ml Erwinase generated 3 SD above background. This approach is acceptable because the minimum of 2 hour and 5U/ml could generate detectable differences as indicated by the plots below. However, the sponsor has to show that this condition can detect neutralizing antibody consistently.

Comment to Sponsor: *According to your data regarding Erwinase spike determination, the resolution of different amount of Erwinase was more at 3 and 4 hour time point than 2 hour time point. However, you chose to use 5U/ml of Erwinase as the spike concentration and 2 hours as the reaction time. Please provide data to show that your condition would detect neutralizing antibody consistently and submit data showing the minimum amount of the neutralizing antibody that could be detected by this condition.*

SD above background:

	time (hours)			
Erw. (U/mL)	1	2	3	4
10	2.2	5.7	10.0	12.6
5	1.1	3.0	5.3	6.9
2.5	0.4	1.5	2.9	3.7
1.25	0.1	0.9	1.6	2.2
0.625	0.1	0.6	1.0	1.3
0.313	0.1	0.3	0.5	0.5
0.156	0.3	0.5	0.5	0.4

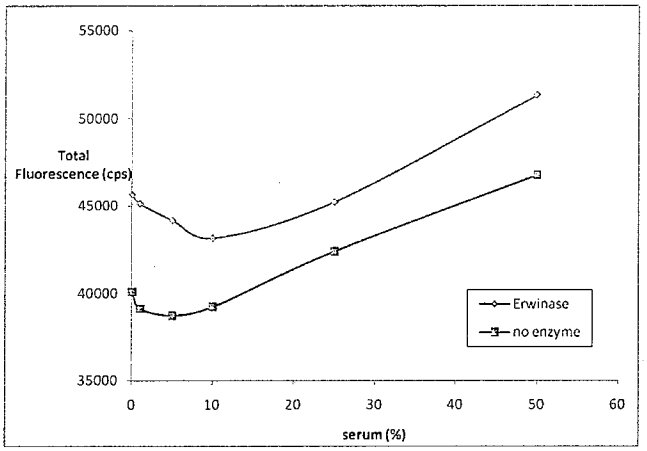


Effect of Serum:

According to the literature this assay was performed in the presence of 50% serum. The sponsor used various percentage of serum (up to 50%) to confirm that 50% serum was optimum to use in their assay. The fluorescent signal as a function of serum concentration is shown below. The sponsor stated that the samples containing either Erwinase (20U/mL) or PBS only were incubated with varying amounts of serum for 30 minutes at

37°C before addition of the substrate (Asp-AMC) and the measurements were taken after 1 hour at 37°C.

Comment to Sponsor: *Regarding the assay on the effect of serum, the fluorescent signal with 10% serum and 50% are similar. Please provide data showing that your assay can effectively detect low levels of neutralizing antibody in presence of 50% serum than 10% serum.*

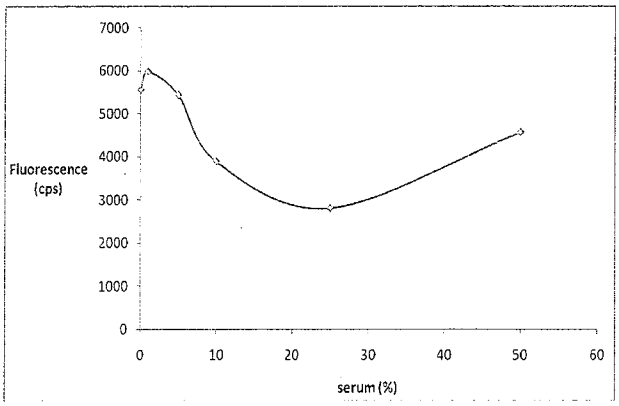


Comment to File: *Up to 10% serum there showed little effect on the fluorescence signal. Above 10% the contribution of serum appears to be proportional to its concentration. The control samples which contain only PBS and no added enzyme have high background signal as well as with Erwinase sample. The data indicated that the fluorescence signal increases with increase in serum concentration in control as well as in test samples. No explanation was provided. Nevertheless, when this background was subtracted from Erwinase activity, the Erwinase activity was detectable (see Fig. below) in 20-50% serum which increases linearly with serum concentration.*

The data indicated that the samples containing 1.0% serum or no serum produced the highest signal. This was not unexpected since compounds in serum that could potentially interfere with fluorescence are either diluted or not present.

However, as the serum concentration increases to 25% the signal declines. After 25% it again increases and the signal returns to a higher reading at 50% serum. Although the cause of this was not explained, 50% serum was used in the development of this type assay earlier by Ylikangas and Mononen (Analytical Biochemistry, (2000) ***280,*** *42–45). So, the use of 50% should be okay. However, the following graph indicated that about 10% serum can also generate same signal as it with 50% serum. More supporting data is necessary if the sponsor intend to use 50% serum in their assay, eg. Sensitivity of the assay.*

The following figure shows the Erwinase activity after the background signal has been subtracted.



Assessment of variability among individual serum:

The sponsor stated that they used 50 individual serum samples to test the variability associated with different sera spiked with 5U/ml Erwinase measuring their fluorescence after 2 hours at 37°C. The data were normalized by the following equation:

% Activity = 100X(S-U)/U
(S and U are spike and unspike for each individual serum Sample).

% Pool = 100X(S-U)/(Sp-Up)
(Sp and Up are spike and unspike of the pool)

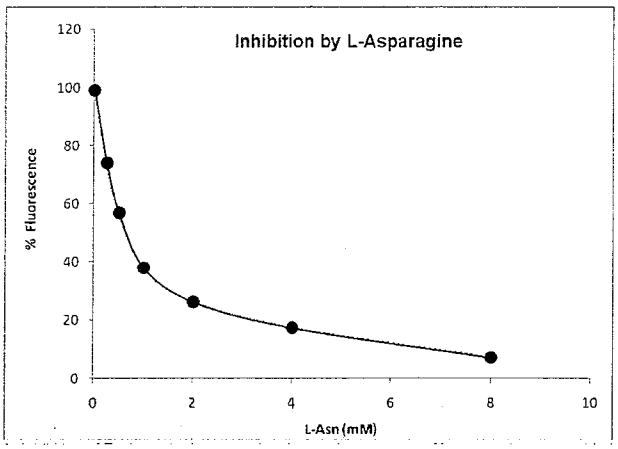
Sample	U	S	% Activity	% of Pool	
1		(b) (4)	41	131	
2			29	96	
3			33	108	
4			32	104	
5			27	86	
6			28	96	
7			36	128	
8			38	128	
9			39	161	
10			30	104	
11			41	143	Plate 1
12			43	144	
13			37	149	
14			33	103	
15			41	135	
16			43	127	
17			30	118	
18			36	123	
19			37	124	
20			34	116	
21			30	95	
22		(b) (4)	33	95	
23			30	94	
24			34	92	
25			22	63	
26			27	72	
27			33	85	
28			33	86	
29			28	69	
30			27	65	
31			34	87	
32			30	76	Plate 2
33			24	78	
34			28	68	
35			27	79	
36			29	68	
37			23	55	
38			28	77	
39			31	72	
40			29	79	
41			30	74	
42			31	76	
43			22	48	
44			20	45	
45			39	108	
46			23	51	Plate 3
47			29	66	
48			21	47	
49			37	90	
50			36	102	
mean	26634	39000	32	94	
Stdev	2995	4116	6	29	
%CV	11	11	18	31	

Comment to File: *The Erwinase spike at a concentration of 5U/ml produced a mean value of activity of 32% and the variability is 18% (CV%). The variability (CV%) in this assay with individual serum samples is within acceptable limit. However, the % activity value is low. In an alternative approach the sponsor normalized the data from 50 individual serum samples against data obtained from a pool of serum samples. The mean value is 94% demonstrating a good recovery with 31% variability. The higher variability could be due to few samples (25, 37, 43, 44, 46 and 48) that generated lower individual activity% as well as the value of pool %. Removing these samples from the calculation might result a lower variability. This approach could be acceptable due to good recovery of the data.*

Inhibition by L-Asparagine (Specificity):

A titration was presented to assess the inhibition of Erwinase activity by L-Asparagine. An increasing amount of L-Asparagine was added to a sample containing 0.5 mM

substrate Asp-AMC and 5 U/mL Erwinase. According to the sponsor the fluorescence was recorded after 2 hours at 37°C. The experiment was performed in duplicates in 50% serum. The sponsor stated that 0.25-1.0 mM L-asparagine can provide sufficient inhibition permitting signal adequate for detection.



Comment to File: The study presented in the following figure indicated that an inhibition of Erwinase activity by L-Asparagine is achievable with the current reaction condition. The amount of L-asparagine (0.25-1mM) chosen from this inhibition curve belongs to the linear part of the curve and so the inhibition is achievable within this concentration of L-asparagine.

The sponsor reported another assessment with 21 individual serum samples that showed 0.5mM L-asparagine could be used as inhibitor concentration. Three different concentrations (0.25, 0.50 and 1mM) of L-asparagine were used in this study and the result is presented in the following table.

	% of Activity Remaining			
	L-Asn (mM)			
Sample #	0.25	0.5	1	
pool	68.4	47.4	30.8	Plate 1
1	66.0	50.9	31.3	
2	70.0	52.0	37.0	
3	64.1	43.3	28.5	
4	66.3	47.6	31.6	
5	66.3	46.9	28.8	
6	66.9	47.8	30.2	
7	67.8	51.4	32.3	
pool	74.0	54.4	38.5	Plate 2
8	78.0	67.1	48.0	
9	76.3	61.7	42.3	
10	73.2	72.1	42.8	
11	76.0	58.6	49.9	
15	88.8	69.1	47.0	
16	82.9	61.4	42.2	
17	70.0	54.6	28.2	
pool	69.0	51.2	31.1	Plate 3
18	65.6	53.3	30.3	
19	70.0	60.0	42.6	
20	60.5	50.7	30.5	
21	69.1	53.8	37.3	
22	64.6	53.9	35.6	
24	73.7	59.2	38.1	
25	67.6	55.1	32.5	
SD	6.4	7.3	6.7	
Mean	69.0	53.8	34.1	
%CV	9.2	13.5	19.6	

Comment to file: *The data indicated that at concentrations of 0.25 mM, 0.50 mM and 1.0 mM, L-asparagine inhibited Erwinase activity by 31%, 46% and 66% respectively. The precision of the assay as measured by CV% ranges from 9% to 20%. So, using these data 0.50 mM-1.0 mM L-asparagine can be used as the inhibitor concentration for the assay.*

Cut Point Selection:

The sponsor stated that the cut-point was determined by normalizing the individual activities of these 21 individual serum samples (above) to control serum sample pool (generated by pooling 50 individual normal human serum samples). The result was presented as % of pool and calculated by the equation below.

% of Pool=100x(S-U)/(Sp-Up)

Where **S** and **U** are spiked and unspiked respectively for the individual serum sample and **Sp** and **Up** are spiked and unspiked respectively for the serum pool control.

Sample #	% Activity	% of Pool	
pool	70		plate 1
1	83	113.2	
2	86	108.0	
3	73	95.8	
4	71	94.2	
5	72	95.3	
6	78	103.2	
7	79	101.9	
pool	67		plate 2
8	70	94.1	
9	65	105.9	
10	65	94.9	
11	68	93.6	
15	60	81.5	
16	59	73.1	
17	54	97.1	
pool	57		plate 3
18	56	98.9	
19	65	101.0	
20	58	105.7	
21	49	85.1	
22	64	105.6	
24	53	87.6	
25	53	103.7	
SD	**10**	**9.5**	
Mean	**66**	**97.1**	
%CV	**15**	**9.8**	

Comment to File: *The mean value for % of Pool for these 21 samples was 97.1% with a standard deviation of 9.5. The precision looks good for the assay which is expressed as CV % (9.8%). The highlighted sample had a pool value of 73.1% which represents approximately 2.5XSD below the mean. The cut point is determined as a ratio of the actual reading as expected. Any sample showing a pool value below to this cut point (97.1%) should be considered as a sample likely to contain neutralizing antibody. However, given to the considerations to sample #16 which is normal serum sample, the sponsor selected a value of 68% (3XSD below the mean) as the cut point for % of pool. Samples that produce values below this cut point should be considered inhibitory towards Erwinase activity and likely to contain neutralizing antibodies. This approach to define the cut point by mean ± 3XSD is normally practiced in the industry. Moreover, the sample 16 showed 59% activity which is more than the pool activity (54%) of that plate. Therefore, this assessment is acceptable.*

Comment to Sponsor: *This approach to establish the cut-point is acceptable. However, it will be good if the sponsor compared and verified the cut-point obtained from the normal serum sample to that of the serum sample of the target patient population. Please verify this cut-point in sera from the serum of the target patient population.*

Serum Pool Control:

The sponsor stated that a serum pool will be included in each 96-well plate as an internal control. So, an acceptance criterion for the serum pool was established by the sponsor on the basis of the % Activity and % Inhibition of the serum pool from 3 different plates (See Table below). The mean value for the % Activity was 67% with a SD of 7% and the % Inhibition was 51% with a SD of 4%. The percent inhibition was calculated by the following equation:

% Inhibition= 100x(S-I)/(S-U)

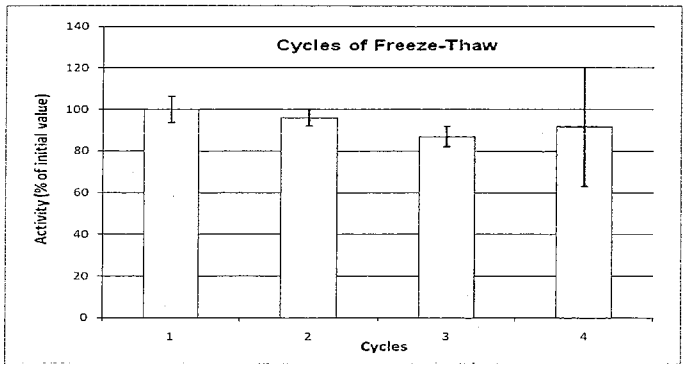
In this equation, **S** and **U** are spiked and unspiked and **I** is spiked with 0.50 mM L-asparagine.

pool	% Activity	% Inhibition
plate 1	70	47
plate 2	67	54
plate 3	57	51
SD	7	4
Mean	67	51
%CV	10.2	6.9

Comment to File: *The sponsor estimated the acceptance criterion for Erwinase spiked controls (S) containing inhibitor (I) on the basis of % inhibition as mean % inhibition minus 2XSD which is, in this case 43%. Similarly, the acceptance criterion for Erwinase spiked (S) controls is described by the calculated value of % increase in fluorescence signal due to enzymatic activity over unspiked (U) Controls ((100x(S-U))/U) and is defined as % Activity. This value should fall between 2SD above and below the mean values which is 53 and 81%. This value is much higher than the mean % activity found in the assay with 50 individual serum sample (mean was 32%, see data above). In addition, this calculation is based on current industry standards for this type of assay. Therefore, this approach is acceptable.*

STABILITY ASSESSMENT:

Stability of Erwinase Stock:
The sponsor stated that they performed 4 cycles of freeze-thaw experiments on 10,000 U/mL Erwinase stock solutions which had been aliquoted and frozen at -70°C. The results are shown below.



# of F/T	Fluorescence (cps)	% of Initial	%CV
1	13121	100	6
2	12609	96	4
3	11416	87	5
4	12039	92	29

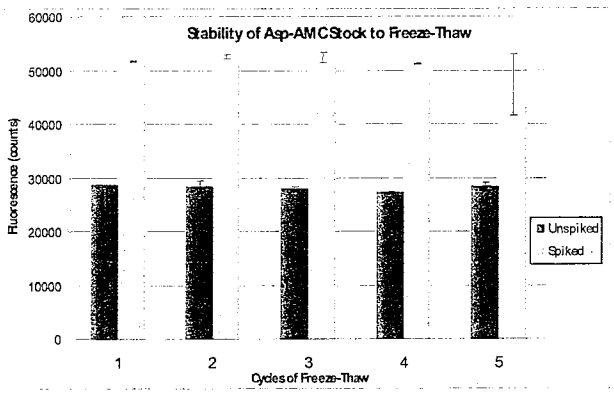
Comment to File: *The data shows that the activity declines slightly with increase in the number of freeze-thaw cycles. The variability was within acceptable limit until 3rd freeze-thaw cycle. So, the samples can be used up to three freeze-thaw cycles without much loss in activity. The Erwinase is a homo-tetramer enzyme, so this enzyme may loose integrity with increase in number of free-thaw cycle.*

Comment to Sponsor: *The stability data of the Erwinase stock solutions indicated that the sample maintained its integrity up to four freeze-thaw cycles without significant loss in activity. However, the precision of the fourth freeze-thaw cycle indicated by CV% was high. Please consider this data to limit freeze/thaw cycle to a minimum during clinical sample analysis.*

Stability of Substrate (Asp-AMC):

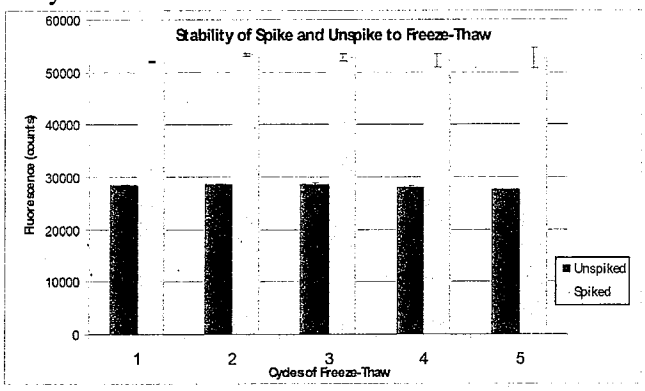
The sponsor stated that they performed a series of freeze-thaw experiments on the substrate Asp-AMC. Aliquots of concentrated Asp-AMC were stored at -20°C. In this assay, the sponsor stated that the substrate was diluted to working concentrations and added to both spiked and unspiked serum samples and after 2 hours at 37°C fluorescence was measured and compared. The result is shown in Fig. below.

Comment to File: *The data indicated no appreciable decrease in stability of the substrate after 5 cycles of freeze-thaw assessment. Although the sponsor indicated that they do not anticipate these many cycles of freeze thawing of the substrate, their data indicate that the substrate may be used after up to five freeze-thaw cycles.*



Stability of Spike and Unspiked samples:

The sponsor reported that they tested the stability of the spiked and unspiked serum pool to cycles of freeze-thaw. The data is shown in the figure below.



Comment to File: *The data indicated no significant loss in activity up to 5 cycles of freeze-thawing in either the spiked or unspiked sera. So, spiked and unspiked samples can be used up to five cycles under current experimental conditions of freeze-thaw cycles without significant loss in activity.*

Plate Acceptance Criteria: The sponsor presented data of the plate acceptance criteria in three occasions. The sponsor stated that each plate contained three controls: unspiked (U), spiked with 5U/ml Erwinase (S) and spiked sample with 0.5 mM L-asparagine inhibitor (I). All of these samples were made in 50% human serum.

OCCASION 1					
	% Activity	Between 53-81%?	% Inhibition	>/= 43%?	Pass Yes/No
Plate 1	59	yes	44	yes	yes
Plate 2	65	yes	48	yes	yes
Plate 3	72	yes	48	yes	yes

OCCASION 2					
	% Activity	Between 53-81%?	% Inhibition	>/= 43%?	Pass Yes/No
Plate 1	58	yes	53	yes	yes
Plate 2	60	yes	60	yes	yes
Plate 3	62	yes	62	yes	yes

OCCASION 3					
	% Activity	Between 53-81%?	% Inhibition	>/= 43%?	Pass Yes/No
Plate 1	67	yes	49	yes	yes
Plate 2	64	yes	45	yes	yes
Plate 3	65	yes	49	yes	yes

Position Effect: The sponsor demonstrated that the data were not affected by placing controls or test samples in different places within the plate. The result is shown below.

Appendix 3: Position Effects - Three Occasions

Study/Report Number: 828-5P5R-IM 9.29.08

OCCASION 1

Plate 1

U	S	I		
Unspike	Spike	Spike+Inh	% Activity	% Inhibition
30533	49597	41023	62	45
31122	48579	40983	56	44
30465	48558	40702	59	43
29734	50143	40436	69	48
30082	50075	40587	66	47
29754	49634	40768	67	45
29106	48958	40434	68	43
29118	49486	40731	70	43
28836	48977	39801	70	46
		Mean	65	45
		SD	5	2
		%CV	8	4
		Less or equal to 20%?	YES	YES

Plate 2

U	S	I		
Unspike	Spike	Spike+Inh	% Activity	% Inhibition
29955	50559	41538	69	44
30359	49003	39808	61	49
30015	49356	39547	64	51
29474	51016	41383	73	45
29124	49095	40302	69	44
29070	48151	39443	66	46
28723	48795	40003	70	44
28667	49564	40268	73	44
27852	46350	38201	66	44
		Mean	68	46
		SD	4	3
		%CV	6	6
		Less or equal to 20%?	YES	YES

Plate 3

U	S	I		
Unspike	Spike	Spike+Inh	% Activity	% Inhibition
29835	51304	42651	72	40
29721	51888	40506	75	51
29719	50571	39634	70	52
28907	47953	39196	66	46
28980	49401	39031	70	51
28403	48105	38920	69	47
28650	49883	39684	74	48
28056	50863	41318	81	42
28370	49069	39135	73	48
		Mean	72	47
		SD	4	4
		%CV	6	9
		Less or equal to 20%?	YES	YES

OCCASION 2

Plate 1

U	S	I		
Unspike	Spike	Spike+Inh	% Activity	% Inhibition
29577	44558	38043	51	43
28393	46494	36060	64	58
28760	45681	36199	59	56
27500	42782	37443	56	35
27877	46139	36293	66	54
28024	45850	37513	64	47
26784	45367	37827	69	41
27858	44581	36299	60	50
27307	45088	37244	65	44
		Mean	61	47
		SD	6	8
		%CV	9	16
		Less or equal to 20%?	yes	yes

Plate 2

U	S	I		
Unspike	Spike	Spike+Inh	% Activity	% Inhibition
29712	47671	38573	60	51
29904	48243	38112	61	55
30038	47513	40642	58	39
29407	46951	39156	60	44
28737	46361	37490	61	50
28883	47477	38520	64	48
27814	44762	38956	61	34
28681	45323	37006	58	50
28184	46360	38301	64	44
		Mean	61	46
		SD	2	6
		%CV	4	14
		Less or equal to 20%?	yes	yes

Plate 3

U	S	I		
Unspike	Spike	Spike+Inh	% Activity	% Inhibition
29954	49236	39022	64	53
29864	48833	39106	64	51
30196	47919	39361	59	48
27915	47425	37861	70	49
28088	48295	39271	72	45
28456	48297	39254	70	46
28478	47462	38956	67	45
28346	48041	38727	69	47
29460	47517	37482	61	56
		Mean	66	49
		SD	4	4
		%CV	7	8
		Less or equal to 20%?	yes	yes

OCCASION 3

Plate 1

U	S	I		
Unspike	Spike	Spike+Inh	% Activity	% Inhibition
30655	51498	42045	68	45
29844	49988	40637	67	46
30229	50184	39293	66	55
29221	49512	39354	69	50
28974	48533	39316	68	47
29062	47553	38074	64	51
30289	48251	40001	59	46
29705	48047	39562	62	46
29352	48510	38805	65	51
		Mean	65	49
		SD	3	3
		%CV	5	6
		Less or equal to 20%?	YES	YES

Plate 2

U	S	I		
Unspike	Spike	Spike+Inh	% Activity	% Inhibition
30136	50218	42680	67	38
30591	48243	40516	58	44
29772	49981	39215	68	53
30235	50540	42198	67	41
29707	46851	40511	58	37
29662	46947	38250	58	50
28333	48328	40384	71	40
28339	48637	38645	72	49
28157	48536	39841	72	43
		Mean	66	44
		SD	6	6
		%CV	9	13
		Less or equal to 20%?	YES	YES

Plate 3

U	S	I		
Unspike	Spike	Spike+Inh	% Activity	% Inhibition
32736	54177	43788	65	48
31870	52284	42059	64	50
31351	52243	42299	67	48
30616	50376	41593	65	44
30428	50767	43394	67	36
32215	52842	42405	64	51
31655	51153	42446	62	45
30738	49487	40992	61	45
31583	50218	41214	59	48
		Mean	64	46
		SD	3	4
		%CV	4	9
		Less or equal to 20%?	YES	YES

Comment to File: *The data presented from the assessment on the effect of position of the Controls and Test Samples in the test plate indicated that there was no positional effect on the samples. The mean value and % CV was within acceptable limit. So, this assay is acceptable for the validation purposes.*

Intra-plate Precision or Repeatability:

The sponsor stated that the precision of the assay was obtained across three plates within one occasion in triplicates and the data were evaluated by calculating % Activity and % Inhibition of the Controls only.

Appendix 4: Reapeatability-Three Occasions

Study/Report Number: 828-5P5R-IM 9.29.08

OCCASION #1, PLATES #1,2,3					
U	S	I			
Unspike	Spike	Spike+Inh	% Activity	% Inhibition	
30533	49597	41023	62	45	plate 1
31122	48579	40983	56	44	
30465	48558	40702	59	43	
29955	50559	41538	69	44	plate 2
30359	49003	39808	61	49	
30015	49356	39547	64	51	
29835	51304	42651	72	40	plate 3
29721	51888	40506	75	51	
29719	50571	39634	70	52	
			65	47	Mean
			6	4	SD
			10	9	%CV
		Did it pass?	YES	YES	</= 25%?

OCCASION #2, PLATES #1,2,3					
U	S	I			
Unspike	Spike	Spike+Inh	% Activity	% Inhibition	
29577	44558	38043	51	43	plate 1
28393	46494	36060	64	58	
28760	45681	36199	59	56	
29712	47671	38573	60	51	plate 2
29904	48243	38112	61	55	
30038	47513	40642	58	39	
29954	49236	39022	64	53	plate 3
29864	48833	39106	64	51	
30196	47919	39361	59	48	
			60	51	Mean
			4.2	6.0	SD
			7	12	%CV
		Did it pass?	YES	YES	</= 25%?

OCCASION #3, PLATES #1,2,3					
U	S	I			
Unspike	Spike	Spike+Inh	% Activity	% Inhibition	
30655	51498	42045	68	45	plate 1
29844	49988	40637	67	46	
30229	50184	39293	66	55	
30136	50218	42680	67	38	plate 2
30591	48243	40516	58	44	
29772	49981	39215	68	53	
32736	54177	43788	65	48	plate 3
31870	52284	42059	64	50	
31351	52243	42299	67	48	
			66	47	Mean
			3	5	SD
			5	11	%CV
		Did it pass?	YES	YES	</= 25%?

Comment to File: *The % activity and the inhibition by the presence of inhibitor in all three occasions of the assessment were within the acceptance limit. The variability (CV%) of all assays were also between 4-12%, within acceptable limit. Therefore, the assay precision met the acceptance limit.*

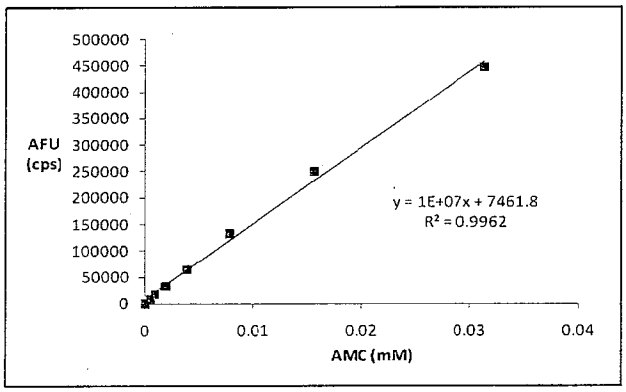
Inter-plate precision or Intermediate Precision: The sponsor determined intermediate precision by comparing values for % Activity and the % Inhibition for only the Controls across three occasions for a set of conditions (one unspiked well, one spiked well and one spiked with inhibitor well in the same row). The data were presented below.

Table: Intermediate Precision-Occasion-to-Occasion.

U	S	I				
Unspike	Spike	Spike+Inh	% Activity	% Inhibition		Occasion
30533	49597	41023	62	45	plate 1	1
31122	48579	40983	56	44		
30465	48558	40702	59	43		
29955	50559	41538	69	44	plate 2	
30359	49003	39808	61	49		
30015	49356	39547	64	51		
29835	51304	42651	72	40	plate 3	
29721	51888	40506	75	51		
29719	50571	39634	70	52		
29577	44558	38043	51	43	plate 1	2
28393	46494	36060	64	58		
28760	45681	36199	59	56		
29712	47671	38573	60	51	plate 2	
29904	48243	38112	61	55		
30038	47513	40642	58	39		
29954	49236	39022	64	53	plate 3	
29864	48833	39106	64	51		
30196	47919	39361	59	48		
30655	51498	42045	68	45	plate 1	3
29844	49988	40637	67	46		
30229	50184	39293	66	55		
30136	50218	42680	67	38	plate 2	
30591	48243	40516	58	44		
29772	49981	39215	68	53		
32736	54177	43788	65	48	plate 3	
31870	52284	42059	64	50		
31351	52243	42299	67	48		
			64	48	Mean	
			5	5	SD	
			8	11	%CV	
		Did it pass?	YES	YES	<= 25%?	

Comment to File: *The sponsor demonstrated intermediate precision by comparing values for % Activity and the % Inhibition for the Controls across three occasions. The individual values for % activity and % inhibition look good in comparison to corresponding mean values. The CV% for % activity as well as for % inhibition was 8% and 11% respectively, which is within the acceptance limit. Therefore, the intermediate precision is acceptable.*

Standard Curve of the product AMC: The fluorescence of known concentrations of AMC was plotted in the following graph.



Comment to File: *The sponsor deduced a standard curve for AMC which can be useful for the determination of AMC in the sample. The sponsor stated that varying amounts of the product (AMC) were titrated into wells containing buffer (50 mM Tris-HCl, 1% ethylene glycol, pH 7.5) to determine linear region. The data showed that concentrations of AMC up to 30 μM fit well to a linear equation (Fig. above) permitting quantification of product.*

APPENDIX I: Comments sent to the Sponsor May 6, 2010

Comments to the Sponsor:

1. *Your screening assay for the assessment of total anti-Erwinase antibodies induced in patient's serum is deficient. Please submit additional data supporting the use of this assay to detect the production of antibodies to Erwinase (IgM and all subisotypes of IgG). To validate the screening assay you need to address the following issues;*

    a. *Please determine the absolute sensitivity of your assay in mass units (eg. ng/mL) and provide the data that supports it. The sensitivity of the assay should be determined in the matrix that will be used for the screening of patients.*

    b. *Provide the data and calculations used to establish the cut-point for the screening assay utilized to decide whether a sample is positive and verify the cut-point with treatment-naïve ALL patient sera, if available. The cut-point should be set so as to minimize false negative results. Define acceptance criteria for negative and low positive quality controls to ensure that the sensitivity of the assay remains constant. A high positive quality control is recommended to ensure that the range of the assay is consistent.*

    c. *Please establish the specificity of your assay.*

    d. *Guidance for the development and validation of assays may be found in ICH Q2 and in Mire Sluis, et al, "Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products." J Immunol Methods. 2004 June: 289(1-2):1-16; Shankar G, et al, "Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharmaceutical and Biomedical Analysis. 2008: 48:1267 – 1281; Gupta S, et al. "Recommendations for the design, optimization, and qualification of cell-based assays used for the detection of neutralizing antibody responses elicited to biological therapeutics. J Immunol Methods. 2007: 321:1 – 18; and docket number FDA-2009-D-0539 Draft Guidance for Industry on Assay Development for Immunogenicity Testing of Therapeutic Proteins: Availability.*

    e. *Please identify the critical reagents for your assay and submit their corresponding qualification and stability programs to ensure the reproducible performance of the assay.*

    f. *Please design your studies so that the serum samples to test for product immunogenicity are obtained at times when antibodies would be expected to peak and serum levels of Erwinase study drug to wane.*

g. *We recommend you design a confirmatory assay to exclude false positive samples in your screening assay.*

2. *Please design, validate and submit for evaluation a separate assay to screen for the presence of IgE antibodies to the product in patient sera.*

3. *Please address the following items regarding your neutralizing antibody assay;*

   a. *Develop a suitable positive control for your neutralization assay. This control can then be used to optimize and validate your assay. It is important that you supply data demonstrating the sensitivity of the assay and its ability to detect the presence of neutralizing antibodies in sera of treated patients. In your analysis, please parse the data between naïve patients and those that were previously treated with E. coli asparaginase (native and PEG-modified).*

   b. *Establish the sensitivity (LOD) of the assay and provide the corresponding data to support it.*

   c. *Please verify the cut point determined in your neutralization assay with serum from the target population of ALL patients including those that developed an immune response to E.coli asparaginase, if available.*

   d. *Develop appropriate quality controls in the neutralizing antibody assay and establish acceptance criteria based on these controls.*

   e. *Please validate the serum concentrations chosen for use in your assay to demonstrate that they minimize matrix interference.*

   f. *Regarding Erwinase spike determination, you chose to use 5U/ml of Erwinase as the spike concentration and 2 hours as the reaction time. Please provide data showing that your condition would detect neutralizing antibody consistently.*

   g. *In the neutralizing antibody assay report submitted to the file (reference the SDN), the stability data for the Erwinase stock solutions was reduced after the fourth freeze-thaw cycle resulting in lower precision, as indicated by a high %CV. Please consider this data when specifying a limit for freeze-thaw cycles for the stock solutions used in your assay.*

APPENDIX II:

Comments to Sponsor:

Screening Assay:

1. *Your antibody screening assay is unacceptable. Please see our specific comments below as well as the references for guidance to develop the assay.*

   a. *Your positive control Ab is inappropriate as it was raised against E. coli asparaginase, which shows limited cross-reactivity to Erwinia asparaginase. You should develop an erwinase-specific antibody to use as a positive control. Please develop erwinase-specific positive control antibody by hyperimmunizing an appropriate animal, e.g. rabbit or goat, and affinity purifying the hyperimmune serum.*
   b. *Establish an assay cut-point to screen anti-erwinase antibody positive samples using normal human serum (for example, 50-100 Individual serum samples) and verify the assay cut-point in treatment naïve ALL patients' serum. The FDA does not recommend using interpolation to a standard curve to establish cut-points as there must be dilutional parallelism between the reference standard and test article for the method to be valid. Furthermore, in your case, the limited cross-reactivity of the positive control Ab makes your assay unacceptably insensitive.*
   c. *The specificity of your assay should be established by pre-incubating an appropriate positive control antibody with excess erwinase prior to use in the screeing assay. Please see the references below on the development and validation of screening assays.*
   d. *Validate your assay using an appropriate positive control Ab and cut-point strategy. [References: (1) CDER 2009. Draft Guidance for Industry: Assay development for immunogenicity testing of therapeutic proteins. (2) Mire-Sluis et al., J Immunol Methods. 2004. 289:1 – 16. (3) Shankar et al., J Pharmaceu Biomed Anal. 2008. 48:1267 – 1281. (4) Gupta et al. J Immunol Methods. 2007. 321:1 - 18.]*

Confirmatory Assay:

2. *Provide the validation data for the confirmatory assay. The confirmatory assay cut-point (% immunodepletion) should be demonstrated based on assay precision using your appropriate anti-erwinase antibody in normal human serum.*

Neutralizing antibody assay:

Please address the following concern about the neutralizing antibody assay.

3. *In case of erwinase spike determination, you chose 5U/ml erwinase and 2 hour reaction time as optimum although longer reaction time generated better resolution and dynamic range. Please justify.*
4. *Regarding effect of serum study,*
   a. *You spiked serum with 20U/ml and incubated for 1 hour instead of the optimum reaction condition of 5U/ml erwinase and 2 hour reaction time. Justify your change in reaction protocol.*

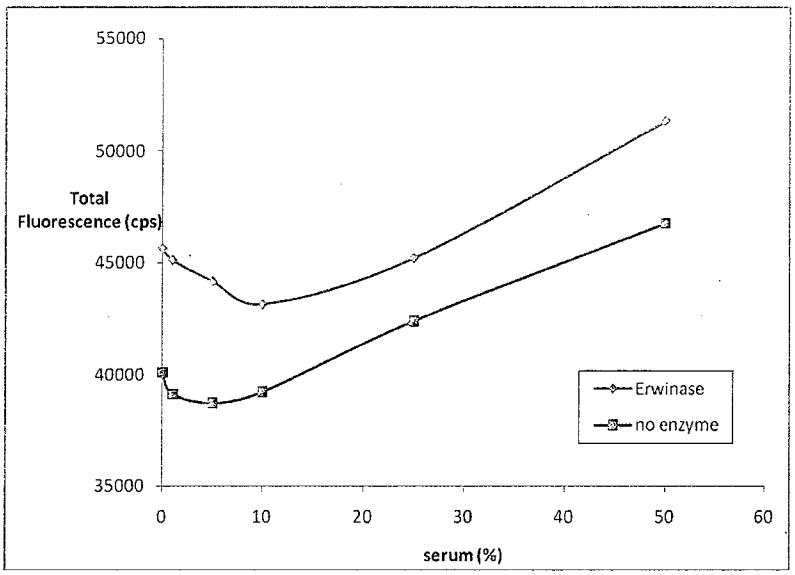


Figure 3: The fluorescent signal as a function of serum concentration is shown. Samples containing either Ewrinase (20 U/mL) or PBS only were incubated with varying amounts of serum for 30 minutes at 37°C before addition of the substrate (Asp-AMC). Measurements were taken after 1 hour at 37°C. Each point represents the average of 3 data points.

A.

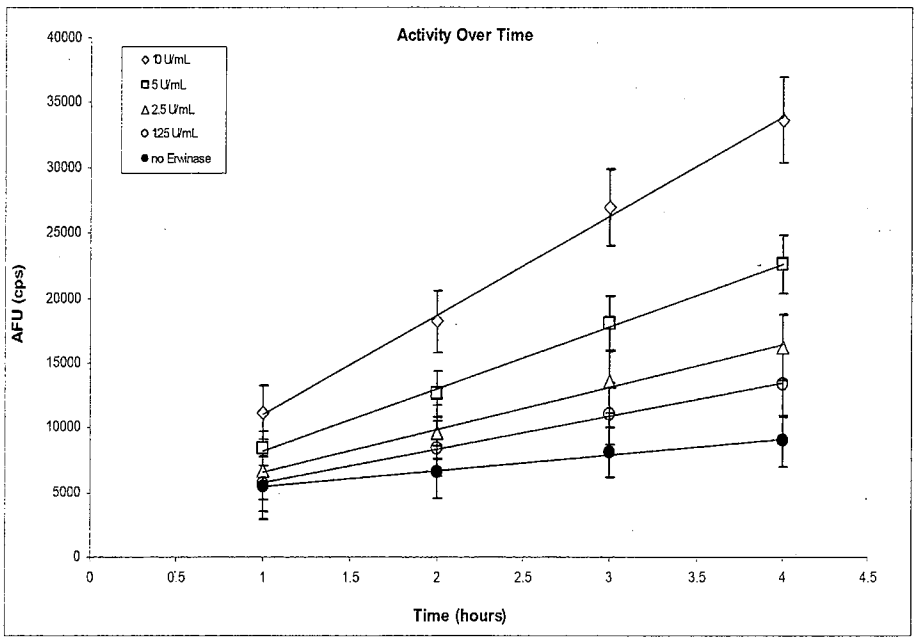


Figure 1: Increase of fluorescence over time of varying amounts of Erwinase at 37°C in **50% serum** (A) and signal produced as a function of Erwinase concentration (B).

b. *Serum sample without erwinase has 8-10 times (5000 vs 46000 cps) higher fluorescence count at one hour time point in compare to the same sample used during erwinase spike determination. Explain this difference.*
c. *At 1% serum concentration the dynamic range as defined by the difference in fluorescence count was better (5984cps) than at 50% serum study (4575cps). However, you chose to use 50% serum over better resolution with 1%. Please justify.*

5. *Please develop a positive control neutralizing antibody using the methods suggested in comment 1a. Once you have developed such an antibody you should use that antibody to confirm assay performance.*

IgE Assay:

6. *As you have had type 1 hypersensitivity responses to erwinase please develop and validate a sensitive IgE- anti-erwinase antibody detection assay. Please make sure that you use a positive anti-erwinase IgE control in the assay and specify the sensitivity of the assay.*

   You should be aware that clinically relevant levels of antigen specific IgE are in the ng/ml to sub-ng/ml range. Therefore you must develop an assay with this level of sensitivity. In the presence of a robust IgG response it is generally necessary to remove IgG from the test article to accurately measure IgE.

   It is also difficult to develop an appropriate positive control antibody (i.e. anti-Erwinase IgE). However companies have used antigen specific IgG conjugated to a human IgE specific for a non-relevant protein as a surrogate positive control for the development and routine implementation of IgE assays.

7. *Once validated you may submit the assay to the Agency for review prior to testing serum from hypersensitivity patients.*

APPENDIX III:

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Memorandum

Date: 12/17/10

From: Erik Laughner, RPM DBOP/OODP/CDER/FDA

Subject: IND 290; Telecon with EUSA Pharma to discuss immuno assays for Erwinaze based on review EUSA Pharma's July 27 and August 3, 2010 submissions (see DARRTS S/N 925 and 927).

FDA Attendees:

Susan Kirshner, Ph.D.	CMC Team Leader (Immunogenicity), DTP/OBP
Faruk Sheik, Ph.D.	CMC Reviewer (Immunogenicity), DTP/OBP
Suzanne Demko, PA-C.	Clinical Team Leader, DBOP/OODP
Patricia Dinndorf, M.D.,	Clinical Reviewer, DBOP/OODP
Jun Yang, Ph.D.	Clinical Pharmacology Reviewer, DCP5/OCP
Hong Zhao, Ph.D.	Clinical Pharmacology Team Leader, DCP5/OCP
Patricia Keegan, M.D.	Division Director, DBOP/OODP
Erik Laughner, M.S.	Senior Regulatory Health Project Manager, DBOP/OODP
Jennifer Shen, Ph.D.	IOTF Fellow, DCP5/OCP

EUSA Pharma Attendees:

Paul Plourde MD SVP EUSA
Tim Corn, MD CMO EUSA
Harriette Nadler Sr Dir Regulatory
(b) (4)
(b) (4)
(b) (4)

Background: FDA requested this tcon to discuss ongoing problems EUSA Pharma apparently has with the development and validation of screening and neutralizing assays for Erwinaze. On 12/14/10, FDA provided EUSA Pharma written comments (blue) to facilitate the tcon discussion. Discussion is incorporated under each comment below.

Screening Assay:

1. Your antibody screening assay is unacceptable. Please see our specific comments below as well as the references for guidance to develop the assay.

    a. Your positive control antibody (Ab) is inappropriate as it was raised against *E. coli* asparaginase, which shows limited cross-reactivity to *Erwinia asparaginase*. You should develop an erwinase-specific antibody to use as a positive control.

Please develop erwinase-specific positive control antibody by hyperimmunizing an appropriate animal, e.g. rabbit or goat, and affinity purifying the hyperimmune serum.

DISCUSSION: EUSA Pharma acknowledged and agreed to affinity purify a new antibody and establish a cut-point. This would take ~2-3 months to develop the antibody and ~1 month to develop the assay.

b. Establish an assay cut-point to screen anti-erwinase antibody positive samples using normal human serum (for example, 50-100 Individual serum samples) and verify the assay cut-point in treatment naïve ALL patients' serum. The FDA does not recommend using interpolation to a standard curve to establish cut-points as there must be dilutional parallelism between the reference standard and test article for the method to be valid. Furthermore, in your case, the limited cross-reactivity of the positive control Ab makes your assay unacceptably insensitive.

DISCUSSION: EUSA Pharma acknowledged the comment and proposed that only Erwinze naïve patients be tested. FDA agreed this was acceptable with the caveat that if there was any future indication expansion for Erwinaze this may need to be re-evaluated. The FDA emphasized that a qualitative cut-point established based on OD obviates the need to for dilutional parallelism between a reference standard and test article. Therefore that aspect of assay development and validation can be dropped.

c. The specificity of your assay should be established by pre-incubating an appropriate positive control antibody with excess erwinase prior to use in the screeing assay. Please see the references below on the development and validation of screening assays.

DISCUSSION: EUSA Pharma acknowledged.

d. Validate your assay using an appropriate positive control Ab and cut-point strategy. [References: (1) CDER 2009. Draft Guidance for Industry: Assay development for immunogenicity testing of therapeutic proteins. (2) Mire-Sluis et al., J Immunol Methods. 2004. 289:1 – 16. (3) Shankar et al., J Pharmaceu Biomed Anal. 2008. 48:1267 – 1281. (4) Gupta et al. J Immunol Methods. 2007. 321:1 - 18.]

DISCUSSION: EUSA Pharma acknowledged.

<u>Confirmatory Assay:</u>

2. Provide the validation data for the confirmatory assay. The confirmatory assay cut-point (% immunodepletion) should be demonstrated based on assay precision using your appropriate anti-erwinase antibody in normal human serum.

DISCUSSION: EUSA Pharma acknowledged. FDA clarified that the cut-point method was preferred over interpolation to a standard curve. In addition, titering was advantageous over a positive/negative value as this information could lead to better potential correlation with efficacy/safety issues.

Neutralizing antibody assay:

Please address the following concern about the neutralizing antibody assay.

3. In the case of erwinase spike determination, you chose 5U/ml erwinase and 2 hour reaction time as optimum although the longer reaction time generated better resolution and dynamic range. Please justify.

   DISCUSSION: EUSA Pharma acknowledged.

4. Regarding effect of serum study,

   a. You spiked serum with 20U/ml and incubated for 1 hour instead of the optimum reaction condition of 5U/ml erwinase and 2 hour reaction time. Justify your change in reaction protocol.

      DISCUSSION: EUSA Pharma acknowledged.

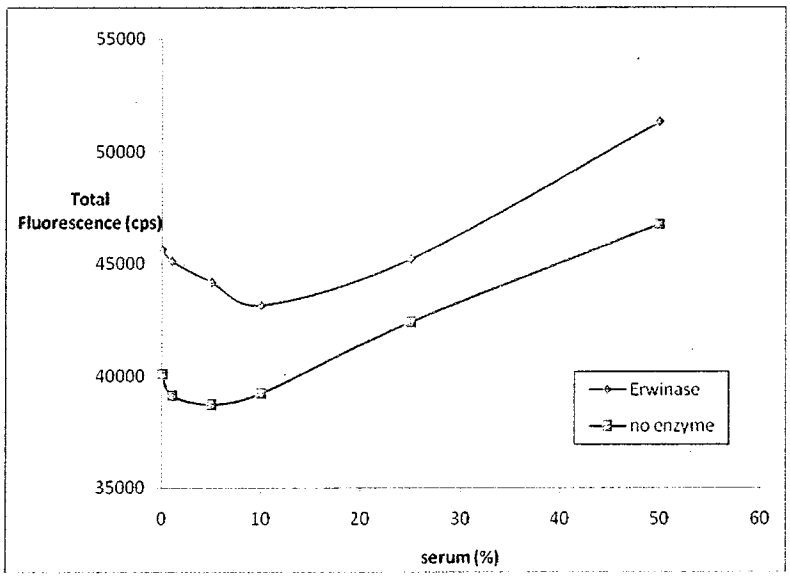


Figure 3: The fluorescent signal as a function of serum concentration is shown. Samples containing either Ewrinase (20 U/mL) or PBS only were incubated with varying amounts of serum for 30 minutes at 37°C before addition of the substrate (Asp-AMC). Measurements were taken after 1 hour at 37°C. Each point represents the average of 3 data points.

A.

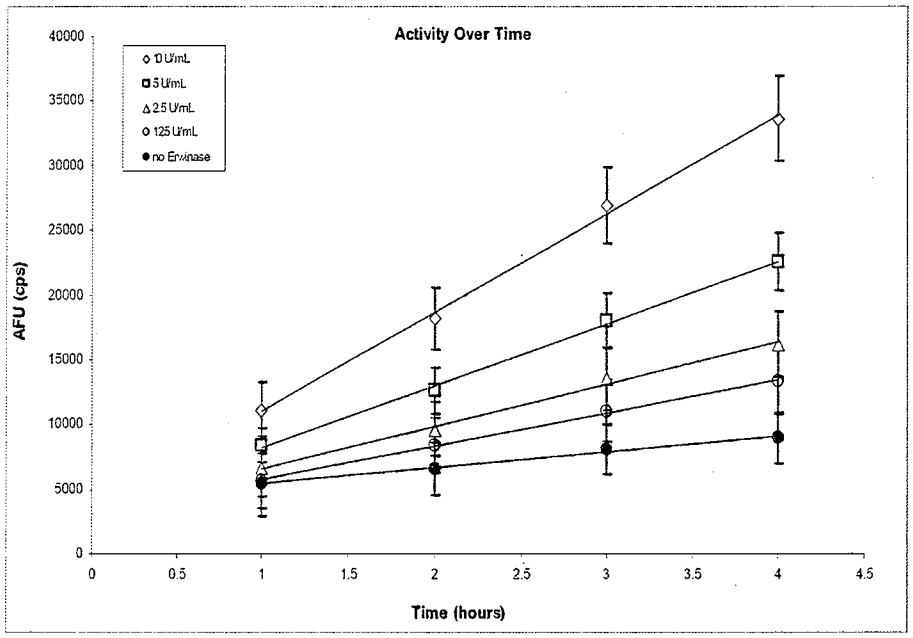


Figure 1: Increase of fluorescence over time of varying amounts of Erwinase at 37°C in **<u>50% serum</u>** (A) and signal produced as a function of Erwinase concentration (B).

b. Serum sample without erwinase has 8-10 times (5000 vs 46000 cps) higher fluorescence count at one hour time point in compare to the same sample used during erwinase spike determination. Explain this difference.

DISCUSSION: EUSA Pharma acknowledged.

c. At 1% serum concentration the dynamic range as defined by the difference in fluorescence count was better (5984cps) than at 50% serum study (4575cps). However, you chose to use 50% serum over better resolution with 1%. Please justify.

DISCUSSION: EUSA Pharma acknowledged.

5. Please develop a positive control neutralizing antibody using the methods suggested in comment 1a. Once you have developed such an antibody you should use that antibody to confirm assay performance.

DISCUSSION: EUSA Pharma acknowledged.

<u>IgE Assay:</u>

6. As you have had type 1 hypersensitivity responses to erwinase please develop and validate a sensitive IgE- anti-erwinase antibody detection assay. Please make sure that you use a positive anti-erwinase IgE control in the assay and specify the sensitivity of the assay.
You should be aware that clinically relevant levels of antigen specific IgE are in the ng/ml to sub-ng/ml range. Therefore you must develop an assay with this level of sensitivity.
In the presence of a robust IgG response it is generally necessary to remove IgG from the

test article to accurately measure IgE. It is also difficult to develop an appropriate positive control antibody (i.e. anti-Erwinase IgE). However companies have used antigen specific IgG conjugated to a human IgE specific for a non-relevant proten as a surrogate positive control for the development and routine implementation of IgE assays.

DISCUSSION: EUSA Pharma acknowledged and agreed to consider the conjugation method. FDA noted that a guidance document published thru the Clinical Laboratories Standard Institute might be available. It may be undergoing final revisions for publication. FDA agreed that the IgE assay should be used on those patients that show hyper-sensitivity.

7. Once validated you may submit the assay to FDA for review prior to testing serum from hypersensitivity patients.

DISCUSSION: EUSA Pharma acknowledged and agreed that samples would be archived until FDA agreed with validation of the assays.

ADDITIONAL DISCUSSON/ACTION ITEMS:

EUSA Pharma would provide their tcon summary to FDA. FDA requested that EUSA Pharma provide a plan by the week of January 20th which describes the timeframe to develop and validate the assays described above. FDA requested that when EUSA Pharma provides the validation information to FDA for review, the supporting data and the actual SOPs for the assays should be included. FDA expressed the importance of getting this finished to allow for appropriate information to be into an eventual label for the BLA.

1 INTRODUCTION

This review evaluates the container labels and carton labeling received on February 28, 2011 for Erwinaze (b) (4) in a response to a request from the Division of Biologic Oncology Products.

1.1 PRODUCT INFORMATION

Erwinaze contains the enzyme L-asparagine amidohydrolase (L-asparaginase) derived in crystalline from the non-human (plant) pathogen *Erwinia chrysanthemi*. Erwinaze is indicated as a component of a multi-agent chemotherapeutic regimen for the treatment of patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to *E. coli* derived asparaginase. The recommended dose is 25,000 International Units per m^2 intramuscularly three times a week (Monday/Wednesday/Friday) for two weeks to replace each dose of pegaspargase, or each course of asparaginase treatment. Erwinaze must be reconstituted with 1 mL to 2 mL of 0.9% sodium chloride for injection. The maximum volume of Erwinaze at any one injection site is 2 mL. If the volume of reconstituted Erwinaze to be injected is greater than 2 mL, use multiple injection sites. Erwinaze will be supplied in vials containing 10,000 International Units and packaged in cartons containing five vials.

(b) (4)

2 METHODS AND MATERIALS

DMEPA conducted a search of the FDA Adverse Event Reporting System (AERS) database. We also reviewed the proposed label and labeling.

2.1 AERS SELECTION OF MEDICATION ERROR CASES

Since Elspar (Asparaginase) and Oncaspar (Pegaspargase) are currently marketed products that are similar to Erwinaze, DMEPA searched the FDA Adverse Event Reporting System (AERS) for medication error reports involving these products that might indicate risk related to Erwinaze.

2.1.1 Elspar

An AERS search conducted on February 17, 2011 used the MedDRA High Level Group Terms "Medication Errors" and "Product Quality Issues", and trade name "Elspar".

The reports were manually reviewed to determine if a medication error occurred. Duplicate reports were combined into cases. Cases that described a medication error were categorized by type of error. We reviewed the cases within each category to identify factors that contributed to the medication errors. If the root cause(s) were associated with the labels, labeling, or packaging of the product, the cases were considered pertinent to this review. Those cases that did not describe a medication error or did not describe an error applicable to this review (e.g. adverse drug event not resulting from a medication error, product quality complaints, etc.), were excluded from further analysis.

2.1.2 Oncaspar

An AERS search conducted on February 17, 2011 used the MedDRA High Level Group Terms "Medication Errors" and "Product Quality Issues" and trade name "Oncaspar".

The reports were manually reviewed to determine if a medication error occurred. Duplicate reports were combined into cases. Cases that described a medication error were categorized by type of error. We reviewed the cases within each category to identify factors that contributed to the medication errors. If the root cause(s) were associated with the labels, labeling, or packaging of the product, the cases were considered pertinent to this review. Those cases that did not describe a medication error or did not describe an error applicable to this review (e.g. adverse drug event not resulting from a medication error, product quality complaints, etc.), were excluded from further analysis.

2.2 LABEL AND LABELING RISK ASSESSMENT

DMEPA uses Failure Mode and Effects Analysis (FMEA) to evaluate container labels and carton labeling. This review summarizes our evaluation of the container label and carton labeling submitted by the Applicant on February 28, 2011 (see Appendices D through F).

3 RESULTS AND DISCUSSION

The following sections describe the findings and assessment of the AERS data and the label and labeling review.

3.1 FINDINGS FROM THE FDA ADVERSE EVENTS REPORTING SYSTEM (AERS)

3.1.1 Elspar

The AERS search retrieved 11 cases (see Appendix A for the ISR numbers). Following exclusion of cases as described in Section 2.1.1, six cases remained for our evaluation:

Wrong route of administration (n=4)

- The recommended routes of administration for Elspar are intramuscular or intravenous. In three of the four cases, Elspar was administered subcutaneously. No outcome was reported in these cases. In the remaining case, the subcutaneous route was prescribed but Elspar was administered intravenously. The patient developed a rash, chest tightening, and shortness of breath. None of the reports stated a specific causality. Although the labels and labeling were not cited as sources for these errors, we note the route of administration is not stated on the proposed container label and carton labeling of Erwinaze. Therefore, the route of administration will be added.

Wrong dose (n=2)

- Two cases reported administration of an overdose of Elspar. In one case, the reporter stated a child received a 10-fold overdose of Asparaginase because the pharmacist entered information into the computer incorrectly. The outcome was unknown. In the second case, the reporter stated the patient was accidentally administered 68,000 units rather than the intended 18,000 units. The patient developed a fever and was subsequently discharged home. The causality was not stated. However, ensuring the dosage and administrations sections of the insert labeling are clear may help to minimize the potential for such errors to occur.

3.1.2 Oncaspar

The AERS search retrieved 4 cases (see Appendix A for the ISR numbers). Three cases were excluded for the reasons stated in Section 2.1.2 (see Appendix C). The remaining case describes the potential for a wrong dose due to the way the strength is presented on the container label.

Potential wrong dose (n=1)

- The lack of a total drug content statement was reported as a potential source of error.

We note the entire drug content of the vial (strength) is stated on the container label and carton labeling of Erwinaze, but the resultant concentration per mL following reconstitution is not stated (see our recommendations in Section 4).

3.2 LABEL AND LABELING RISK ASSESSMENT

The following deficiencies were noted in the container label and/or carton labeling:

- The tradename and proper name lack prominence.
- A route of administration statement is not present.
- The dangerous abbreviation "IU" is in the statement of strength on the principal display panel of the carton labeling.
- The dosage and reconstitution information is not complete. Additionally, it clutters the labeling.
- The layout of the dosage form and net quantity statement is not optimal.
- The "Rx Only" statement is too prominent.

We reserve review of and recommendations for the insert labeling for the labeling meetings scheduled with the Division of Biologic Oncology Products. Our recommendations will be made in the labeling meetings and/or to the working insert labeling that is available on the shared (N) drive.

4 CONCLUSION AND RECOMMENDATIONS

Our evaluation noted areas where information on the container label and carton labeling can be improved to minimize the potential for medication errors. Section 4.1 *Comments to the Applicant* contains our recommendations for the container label and carton labeling. We request the recommendations in Section 4.1 be communicated to the Applicant prior to approval.

Please copy the Division of Medication Error Prevention and Analysis on any communication to the Applicant with regard to this review. If you have further questions or need clarifications, please contact OSE Regulatory Project Manager, Sue Kang, at 301-796-4216.

4.1 COMMENTS TO THE APPLICANT

A. General Comment for the Container Label and Carton Labeling

   1. The tradename, proper name, and strength lack prominence. Increase the size and prominence of the tradename, proper name and strength.
   2. The "Rx Only" statement is too prominent. Decrease the size of the "Rx Only" statement.

B. Container Label

   1. Revise the statement of strength to read "10,000 International Units per vial".
   2. If space permits, describe how many units per mL are provided once the product is reconstituted. For example: "Following reconstitution each mL provides XXX International Units per mL" or "Following reconsitution with XXX each mL contains XXX International Units"

3. The route of administration is not present. Add the route of administration "For Intramuscular Use" to the label and position it below the statement of strength, per 21 CFR 201.100(b)(3).
4. The container label does not have a barcode. Add a barcode to the container label.

C. Carton Labeling (Principal Display Panel)

1. See Comments A.2, B.2 and B.3, above.
2. The abbreviation "IU" is in the statement of strength on the principal display panel of the carton labeling. The abbreviation "IU" is on the ISMP List of Error-Prone Abbreviations, Symbols, and Dose Designations" because it has been confused as "IV" or the number "10". As part of a national campaign to reduce medication errors related to error prone medical abbreviations and dose designations, the FDA agreed not to approve labels and labeling that include the use of error-prone abbreviations or dose designations. Therefore, replace "IU" with "International Units".
3. The dosage and reconstitution information are not complete. Since limited space does not allow for placement of the complete dosage and reconstitution information, we recommend this information be deleted from the carton labeling and replaced with a standard usual dosage statement instead (e.g., "Usual Dosage: See package insert" or similar verbiage). Additionally, this statement should be moved to the side panel to minimize crowding on the principal display panel.
4. The net quantity statement is too close to the strength, which may lead to confusion. Relocate the net quantity statement to a less prominent area on the principal display panel such as the upper right corner.
5. There is no statement on the carton that the vials are for "single use". Add the statement "Single Use Vial—Discard Unused Portion" to the principal display panel.
6. Delete the fold line from the carton flap and use separate labels for the top panel and principal display panel.

D. Carton Labeling (Side Label)

See Comment B.3, above.

REFERENCES

Adverse Events Reporting System (AERS)

AERS is a database application in CDER FDA that contains adverse event reports for approved drugs and therapeutic biologics. These reports are submitted to the FDA mostly from the manufacturers that have approved products in the U.S. The main utility of a spontaneous reporting system that captures reports from health care professionals and consumers, such as AERS, is to identify potential post marketing safety issues. There are inherent limitations to the voluntary or spontaneous reporting system, such as underreporting and duplicate reporting; for any given report, there is no certainty that the reported suspect product(s) caused the reported adverse event(s); and raw counts from AERS cannot be used to calculate incidence rates or estimates of drug risk for a particular product or used for comparing risk between products.

APPENDICES

Appendix A: AERS Database ISR Report Numbers

Elspar	
Report	**ISR Number**
1	4536051
2	4536009
3	1469547 (MFG report #)
4	4579874
5	3403307
6	41173062
7	3731927
8	4313418
9	5605459
10	will fill in; no ISR # on rpt
11	will fill in; no ISR # on rpt

Oncaspar	
Report	**ISR Number**
1	3219214
2	3515076
3	4356009
4	7150234

Appendix B: Excluded AERS Search Results for Elspar

The AERS search conducted on February 17, 2011 for medicaton errors involving Elspar yielded 11 cases. Five of these cases were excluded from further evaluation for the reasons below:

- Adverse drug reactions not related to a medication error (n=4)
- Medication error due to name confusion (n=1)

Appendix C: Excluded AERS Search Results for Oncaspar

The AERS search conducted on February 17, 2011 for medicaton errors involving Oncaspar yielded 4 cases. Three of these cases were excluded from further evaluation for the reasons below:

- Wrong drug error due to name confusion (n=1)
- Wrong drug errors not related to the labels or labeling of Oncaspar (n=1)
- Potential wrong technique error not related to the labels and labeling of Oncaspar (n=1)

Appendix D: Container Label

(b) (4)

Appendix E: Carton Labeling

(b) (4)

Principal Display Panel

Appendix E: Carton Labeling (cont'd)

(b) (4)

Side Panel

Appendix F: Mock-ups of the carton labeling

(b) (4)

(b) (4)

Appendix F: Mock-ups of the carton labeling (cont'd)

(b) (4)

(b) (4)

Appendix F: Mock-ups of the carton labeling (cont'd)

(b) (4)

(b) (4)

Division of Drug Marketing,
Advertising, and Communications

Internal Consult

****Pre-decisional Agency Information****

To: Erik Laughner, Regulatory Project Manager
Division of Biologic Oncology Products
Office of Oncology Drug Products

From: Carole C. Broadnax, R.Ph., Pharm.D., Regulatory Review Officer
Division of Drug Marketing, Advertising, and Communications, CDER

C. Broadnax 5/2/11

Date: May 2, 2011

Re: **ERWINAZE** (b) (4) **[proper name tentative]) for injection**
STN BL 125359
DDMAC Comments on proposed labeling

In response to the Division of Biologic Oncology Products' (DBOP) March 8, 2011, consult request, DDMAC has reviewed proposed labeling (PI, carton and container) for Erwinaze (b) (4) [proper name tentative]).

DDMAC's comments for the PI are based on the draft marked-up labeling that DBOP sent via email dated April 5, 2011, to DDMAC. DDMAC's PI comments are provided directly in the attached document. Please note that for the PI we hid DBOP's comments, deletions, and formatting changes so that our comments are easier to read.

The carton and container labeling used in this review can be found in the original application at: \\cber-fs3\m\eCTD Submissions\STN125359\125359.enx. DDMAC's comments for the carton are provided below.

- (b) (4)

(b) (4)

- [redacted]

DDMAC does not have comments for the container labeling at this time.

Thank you for your consult. If you have any questions regarding the PI or the carton/container labeling, please contact Carole Broadnax at 301-796-0575 or Carole.Broadnax@fda.hhs.gov.

11 PAGES OF DRAFT LABELING HAVE BEEN WITHHELD IN FULL AS b4 (CCI/TS) IMMEDIATELY FOLLOWING THIS PAGE

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Pediatric and Maternal Health Staff
Office of New Drugs
Center for Drug Evaluation and Research
Food and Drug Administration
Silver Spring, MD 20993
Tel 301-796-0700
FAX 301-796-9744

Maternal Health Team Labeling Review

Date: April 11, 2011 **Date Consulted:** January 24, 2011

From: Leyla Sahin, M.D.
Medical Officer, Maternal Health Team (MHT)
Pediatric and Maternal Health Staff

For Dr. Leyla Sahin

Through: Karen Feibus, M.D.
Team Leader, Maternal Health Team (MHT)
Pediatric and Maternal Health Staff

Lisa Mathis, M.D.
Associate Director, Office of New Drugs
Pediatric and Maternal Health Staff

To: Division of Biologic Oncology Products

Drug: Erwinaze (Erwinia L-asparigase); BLA 125,359

Subject: Pregnancy and Nursing Mothers labeling

Materials Reviewed: Pregnancy and Nursing Mothers subsections of Erwinaze labeling

Consult Question: Please review sections of the proposed label as they relate to pregnancy and lactation.

INTRODUCTION

On November 1st, 2010, EUSA Pharma, Inc. submitted a biologic licensing application (BLA) to the Division of Biologic Oncology Products (DBOP) for Erwinaze (Erwinia L-asparaginase). Erwinaze is an enzyme that hydrolyzes circulating asparagine, resulting in the starvation and death of the malignant cells. Normal cells, which can synthesize asparagine de novo, are not affected. The sponsor's proposed indication for Erwinaze is for the treatment of patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to (b) (4) E.coli derived asparaginase.

DBOP consulted the Maternal Health Team (MHT) to review the pregnancy and nursing mothers section of the Erwinaze package insert, and provide comment. This review provides revisions to the sponsor's proposed Pregnancy and Nursing Mothers subsections of Erwinaze labeling.

BACKGROUND

The Pregnancy and Nursing Mothers section of labeling should describe available animal and human data in a manner that allows clinicians, who are prescribing medication for pregnant patients and female patients of reproductive potential, to balance the benefits of treating the patient with the potential risks to the mother, fetus and/or infant. PMHS- maternal health labeling recommendations comply with current regulations but incorporate "the spirit" of the Proposed Pregnancy and Lactation Labeling Rule (published on May 29, 2008). Usually the first paragraph in the pregnancy subsection of labeling summarizes available data from published literature, outcomes of studies conducted in pregnant women (when available), and outcomes of studies conducted in animals, as well as the required regulatory language for the designated pregnancy category. The paragraphs that follow provide more detailed descriptions of the available human and animal data, and when appropriate, clinical information that may affect patient management.

SUBMITTED MATERIAL

(b) (4)

(b) (4)

DISCUSSION AND CONCLUSIONS

Based on the Division's assessment that the sponsor's referenced nonclinical reproductive toxicology studies are not acceptable, MHT concurs with the Division's request for these studies as post-marketing requirements. Therefore based on the lack of nonclinical reproductive toxicology data, pregnancy category C is appropriate for labeling.

RECOMMENDATIONS

Provided below are the MHT's recommended revisions to the sponsor's proposed labeling.

(b) (4)

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: March 16, 2011

TO: Patricia Keegan, M.D.
Director
Division of Biologic Oncology Products (DBOP)

FROM: Charles R. Bonapace, Pharm.D.
Pharmacologist, GLP & Bioequivalence Investigations Branch, Division of Scientific Investigations (HFD-45)
and
Michael F. Skelly, Ph.D.
Pharmacologist, GLP & Bioequivalence Investigations Branch, Division of Scientific Investigations (HFD-45)

THROUGH: Martin K. Yau, Ph.D.
Acting Team Leader, Bioequivalence Team, GLP & Bioequivalence Investigations Branch, Division of Scientific Investigations (HFD-45)
and
Sam H. Haidar, R.Ph., Ph.D. Sam H. Haidar
Branch Chief, GLP & Bioequivalence Investigations Branch, Division of Scientific Investigations (HFD-45)

SUBJECT: Preliminary Review of EIR Covering BLA 125359, *Erwinia* L-Asparaginase, sponsored by EUSA Pharma (USA), Inc.

At the request of the Division of Biologic Oncology Products, the Division of Scientific Investigations (DSI) conducted an audit of bioanalytical portions of the following study:

> ***COG Study AALL07P2***: *"Pharmacology and Toxicity of Erwinia* Asparaginase (Erwinaze; Crisantaspase: IND 290) Following Allergy to Pegaspargase in Treatment of Children with Acute Lymphoblastic Leukemia (ALL)"

The clinical portions of the study were conducted at multiple sites. A request for inspection of clinical portions was directed to DSI-GCPII; see the separate report. The analytical portions of this study were conducted at the (b) (4) (b) (4) The sponsor's "Clinical Study Report (Initial)"

dated 8/23/10 was based in part on "an updated summary of the assay results for the serum asparaginase and plasma asparagine samples" delivered to EUSA by [redacted] (b)(4) on 7/6/10. It did not include the "final data summary" delivered to the sponsor on 1/11/11, with additional data generated in late 2010. During the inspection, [redacted] (b)(4) opined to us that our questions should more appropriately be addressed to the sponsor rather than to him. Apparently this was conveyed to DBOP by the sponsor that the FDA inspectors were asking inappropriate questions. Please also note that the analyst who conducted asparaginase activity assays at [redacted] (b)(4) committed suicide[1] a few days before the inspection. We did not have access to her original notebooks and records, and we could not inspect her laboratory workspace. Instead, we relied on photocopies of her records at [redacted] (b)(4) [redacted] (b)(4) was informed that standard FDA practice was followed in the inspection in all regards.

Provided below is our evaluation of inspectional findings at [redacted] (b)(4). The inspection has not closed, and Form FDA 483 has not been issued, so the firm has not had an opportunity to respond to these observations. DSI will review and forward any responses when they are received.

Bioanalytical Portions - Study AALL07P2

1. **Failure to reject analytical runs when the quality control (QC) samples failed the acceptance criterion at one or two of the three QC concentrations. Examples: Asparagine runs on 3/9/10, 3/11/10, 3/15/10, 3/25/10, 3/30/10, 4/2/10, 4/8/10, 4/15/10, and 12/8/10 and asparaginase run on 4/8/10.**

Each asparagine assay run included one sample each of blank plasma containing endogenous asparagine (approximately 5 µg/mL), and freshly-prepared singlet aliquots of the same blank plasma fortified with additional 2, 8, and 40 µg/mL asparagine. Note in item 4a that contrary to the analytical SOP, the blank plasma actually was expired transfusion plasma, ordinarily diluted approximately 30% from native plasma with anticoagulant containing citrate, phosphate, and dextrose. We cannot evaluate whether dilution and these pH/redox buffers affected accuracy and precision of the assay. Nevertheless, without at least one

[redacted] (b)(6)

QC passing at each of the three concentrations, the accuracy of asparagine data within the runs is not assured. Each asparaginase run assayed duplicate QCs at three concentrations but each QC pair was averaged to determine a mean response, and treated as a singlet value. Since the low QC mean result on 4/8/10 was outside the acceptance criteria, the run should have been rejected.

2. **Failure to reject chromatograms when no asparagine internal standard was detected, or when peaks could not be accurately integrated.**

It cannot be verified whether residual asparaginase in the serum samples, particularly those without timely and complete acidification at the clinical sites (item 3), might have degraded the ^{15}N-labeled asparagine used as internal standard in this assay. Nevertheless, the chromatograms without integratable asparagine and internal standard peaks cannot be assessed for asparagine in the samples.

3. **Failure to exclude serum and plasma samples thawed or unacidified when received from clinical sites.**

Inactivation of *E. coli* asparaginase required acidification to pH 2.0 or lower to preserve asparagine in plasma, even for short periods of time. Stability of asparaginase in thawed serum (for asparaginase assays), and of asparagine in thawed or unacidified plasma (for asparagine assays) during shipment conditions was not evaluated. Therefore, these samples cannot be assessed for asparaginase or asparagine.

4. **Failure to demonstrate stability of samples under the conditions of the study. Examples:**
   a) **The blank plasma used in method development, validation, and QC samples for the asparagine assay was citrate-phosphate-dextrose transfusion plasma, not heparin plasma as in study samples.**

Transfusion plasma is diluted approximately 30% from native plasma and contains pH and redox buffers. It has not been demonstrated whether these conditions affect accuracy, precision, or stability of asparagine in the assay system.

   b) **There was no evaluation of freeze/thaw or long-term frozen stability of samples for the asparagine assay. Most plasma samples were**

stored frozen for 3 to 11 months before assay for asparagine.

The stability of asparagine during these conditions and times has not been demonstrated.

c) **Records of freezer temperatures for storage of samples for asparaginase and asparagine were not retrievable in an auditable form. The alarm system for temperatures outside -70°C to -90°C did not record the extreme excursions of temperature and durations of the excursions when the alarm triggered, including the event on 3/4/10 when the majority of study asparagine samples were in this freezer.**

The institutional alarm system for freezer temperatures did not enable retrieval of actual or extreme (min/max) temperatures. It recorded only a few minutes of the several hours when the storage freezer was observed to be between -70°C and -60°C. Therefore, it cannot be assured that the samples were stored under conditions of demonstrated stability.

d) **Some samples were received thawed (7 shipments), or without acid preservative for asparagine (61 samples), or with documented delays between sample collection and plasma acidification (multiple examples longer than 10 minutes).**

These samples were compromised by handling and storage conditions outside the protocol and method validations. The accuracy of measurements using these compromised samples cannot be assured.

e) **The effectiveness of hydrochloric acid in preserving asparagine in plasma was tested only for *E. coli* asparaginase, not for *Erwinia* asparaginase.**

It has not been established that hydrochloric acid addition is effective in preserving asparagine in plasma in the presence of *Erwinia* asparaginase.

f) **The times when samples were removed from frozen storage for analysis were not recorded.**

It cannot be assessed whether serum and plasma samples were stable during the undocumented times when samples were removed from the freezer to thaw before assay, and then returned to the freezer. There is no documentation of conditions or times during processing, other than the method SOPs calling for room temperature thawing.

5. **Between-run accuracy and precision for the asparagine assay were not evaluated, in that fresh QC samples were prepared for each of three days of pre-study "between-day" evaluation, and during the entire study.**

The three days of "between-day" experiments for evaluation of accuracy and precision were in fact repeated within-day experiments only. They did not incorporate conditions of multi-day storage under refrigerated or frozen conditions.

6. **There was no evaluation of variability in recovery of asparagine in more than one plasma sample in a run.**

A single donor's lot of transfusion plasma was used in individual validation experiments. The so-called "matrix effects" on inter-lot variability of recovery and detection of asparagine have not been evaluated.

7. **There was no evaluation of stability of asparagine in stock solutions or extracts.**

The stability of asparagine in stock solutions and extracts has not been evaluated. However, we note that chromatographic areas for asparagine and ^{15}N-asparagine did not vary appreciably between runs.

8. **Failure to adequately document preparation and storage of asparaginase stock solutions.**

The laboratory notebook did not record details of preparation of new stock solutions of *E. coli* asparaginase, to assure accurate preparation of calibration standards. Based on purchase records of *E. coli* asparaginase and stability data of *E. coli* asparaginase stock solutions stored in a refrigerator, insufficient vials of *E. coli* asparaginase (100 units/vial) were purchased to cover the period of time when serum samples were analyzed for asparaginase activity.

9. **Only a single stock solution of asparagine was used for both calibrators and QC samples, rather than**

independently-prepared stock solutions, in both pre-study validation and within-study conduct.

and

10. **There was no verification (by balance printer or witness) of the weights of asparagine used for calibrator and QC stock solutions.**

The accuracy of weights and dilutions in calibrators and QC samples is not assured by comparison of independent solutions.

11. **Failure to adjust nominal asparaginase concentrations in calibrator and quality control solutions for the acutal content of commercial vials.**

The laboratory prepared stock solutions of *E. coli* asparaginase for calibrators and QC samples by reconstituting commercial vials without adjusting for their actual content.

12. **Failed to perform assessments of incurred sample reproducibility (ISR) for any of the incurred samples for asparagine or asparaginase assays.**

There was no evaluation of within- or between-run "incurred sample reproducibility," in actual study plasma samples collected with heparin anticoagulant for asparagine assay, in the presence of *Erwinia* asparaginase, or in actual study serum samples for asparaginase assays.

13. **Reported serum asparaginase activities less than the lower limit of quantitation. Specifically, the LLOQ of the asparaginase assay is 0.025 IU/mL. However, asparaginase concentrations ranging from 0.009 to 0.024 IU/mL were reported.**

According to the SOP and conventional FDA guidance, values below the lowest calibrator should have been reported as BLQ (below limits of quantitation). The occasional mentions of "below limits of detection" are not germane to this type of assay.

Due to the multiple deficiencies observed during this inspection, the serum asparaginase activities and plasma asparagine concentrations reported in the BLA, or in the last (1/11/11) draft tabulation by [redacted] (b)(4) cannot be assured. Although [redacted] (b)(4) expected to issue a final analytical report within a few weeks, he did not reveal an intention to exclude the questionable data, or to re-evaluate them in light of the incomplete validations.

Conclusions:

Following the above inspection, the Division of Scientific Investigations recommends the following:

- The accuracy of the reported and not-yet-reported bioanalytical data for serum asparaginase activities and plasma asparagine concentrations cannot be assured.
- The lack of records demonstrating proper handling and storage of samples between blood collection and analyses precludes reconstruction of these data, even if future method validation experiments are conducted properly.

After you have reviewed this transmittal memo, please append it to the original BLA submissions.

Charles R. Bonapace, Pharm.D.

Michael F. Skelly, Ph.D.

Final Classifications:
pending [redacted] (b) (4)

cc:
DSI/GLPBB/Haidar/Yau/Lee/Dejernett/CF
DBOP/Dinndorf/Demko/Laughner
DBOP/Jun Yang/Hong Zhao
OPS/OBP/DTP/Beaucage
ORA/HFR-NE250/Noe/Murphy
OC/DSI/Ball/Viswanathan
OC/Cooper
Draft: MFS 3/16/11
Draft: CRB 3/16/11
Edit: MKY 3/17/11
DSI: 6168
O:\BIOEQUIV\EIRCOVER\125359
FACTS: 1252967

Email:
CDER DSI PM TRACK

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

CLINICAL INSPECTION SUMMARY

DATE: March 7, 2011

TO: Patricia Dinndorf
Erik Laughner

FROM Robert Young
Good Clinical Practice Branch II
Division of Scientific Investigations

THROUGH: Tejashri Purohit-Sheth, M.D.
Branch Chief
Good Clinical Practice Branch II
Division of Scientific Investigations

SUBJECT: Evaluation of Clinical Inspections

BLA: 125359

APPLICANT: EUSA

DRUG: Erwinaze (Erwinia L-asparaginase)

NME: No

THERAPEUTIC CLASSIFICATION: Standard

INDICATION: Acute Lymphoblastic Leukemia (ALL) patients (b) (4)

CONSULTATION REQUEST DATE: January 28, 2011

Inspection Summary Goal Date: March 30, 2011
DIVISION ACTION GOAL DATE: May 3, 2011

I. BACKGROUND:

EUSA Pharma submitted this BLA for approval of ERWINAZE for use in the treatment of acute lymphoblastic leukemia in patients with hypersensitivity to E. coli derived asparaginase. Because of allergic reactions to different species derived asparaginases, the availability of alternate species derived asparaginases can be therapeutically useful. Available at the present time is a native and a modified *E coli* product. The subject of the present BLA is an *Erwinia* product.

For approval of the present application a PK study was submitted: Study AALL07P2. The reviewing division requested a single site visit inspection of one of the participating clinical sites to review study execution including proper dosing, collection of samples and reporting of adverse reactions.

II. RESULTS:

CI	Protocol # and # of Subjects	Inspection Date	Final Classification
Patrick A. Brown, M.D. Johns Hopkins University Pediatric Oncology 1650 Orleans St. CRB 2M49 Baltimore, Maryland 21231	AALL07P2 3 subjects	22 Feb – 1 Mar 2011	Pending

Key to Classifications

NAI = No deviation from regulations.
VAI = Deviation(s) from regulations.
OAI = Significant deviations from regulations. Data unreliable.
Pending = Preliminary classification based on information in 483 or preliminary communication with the field; EIR has not been received from the field and complete review of EIR is pending.

1. Patrick A. Brown

Note: This CIS was based on written communications between the FDA field investigator and DSI. The EIR had not yet been received. An inspection summary addendum will be generated if conclusions change upon receipt and review of the EIR.

a. What was inspected: Case histories were reviewed for all three subjects participating in the trial.

b. General observations/commentary: The records were found to be in order. Some samples and EKGs were missed and some adverse reaction reports were submitted late. Corrective actions had been implemented. Otherwise, no substantial regulatory violations were identified and no Form FDA 483 was

issued.

c. Assessment of data integrity: Although isolated regulatory violations were noted as above, based on preliminary communication with the field, it is unlikely that these findings would significantly impact data reliability. The data submitted is acceptable in support of the pending application.

IV. OVERALL ASSESSMENT OF FINDINGS AND RECOMMENDATIONS

The clinical site of Dr. Patrick Brown was inspected in support of this BLA. No significant regulatory violations were noted and the data generated appear acceptable in support of the pending application.

Note: The EIR for the inspection of Dr. Patrick Brown has not yet been received. The inspectional findings noted above are based on written communications between the FDA field investigator and DSI. The An inspection summary addendum will be generated if conclusions change upon receipt and review of the EIR.

Robert Young

/Robert Young/
Robert Young
Good Clinical Practice Branch II
Division of Scientific Investigations

CONCURRENCE:

/Tejashri Purohit-Sheth, M.D./
Tejashri Purohit-Sheth, M.D.
Branch Chief
Good Clinical Practice Branch II
Division of Scientific Investigations

REGULATORY PROJECT MANAGER LABELING REVIEW (PHYSICIAN LABELING RULE)

Review Date: January 14, 2011

Division of Biologic Oncology Products

Application Number: BLA STN 125359

Name of Drug: ERWINAZE

Applicant: EUSA Pharma (USA), Inc.

Material Reviewed:

Initial Proposed Labeling Submitted with Clinical Portion of Rolling Application: September 8, 2010

Submission Date of Structure Product Labeling (SPL): September 8, 2010

Type of Labeling Reviewed: WORD/SPL

Background and Summary

This review provides a list of revisions for the proposed labeling that should be conveyed to the applicant. These comments are based on Title 21 of the Code of Federal Regulations (201.56 and 201.57), the preamble to the Final Rule, Guidance(s), and FDA recommendations to provide for labeling quality and consistency across review divisions. When a reference is not cited, consider these comments as recommendations only.

Review

This is a preliminary format review of the proposed labeling submitted in this application. The attached label contains the review comments to go to the Applicant in the 74-day letter.

Recommendations

I completed a preliminary review of the proposed PLR labeling submitted in this application largely based on 21 CFR Parts 201.56 and 201.57, the preamble to the Final Rule, and FDA Guidance documents. The applicant complied with the major requirements for a PLR label in terms of required sections, headings/sub-headings, font size, etc. A search for the most common formatting deficiencies routinely encountered identified a few issues that will have to be addressed

by the Applicant. A list of deficiencies are embedded as comments in the Applicant's initial proposed package insert which will be attached to the 74-day letter. I note that the proposed label contains a Proper Name deemed unacceptable by FDA. FDA and the Applicant will have to work on an agreed to Proper Name prior to any approval action. Substantial team review and revision of this label would begin after the mid-cycle meeting

Erik Laughner, M.S. RAC (US)
Senior Regulatory Health Project Manager

Filename: CSO Labeling Review Template (updated 1-16-07).doc
CSO LABELING REVIEW OF PLR FORMAT

13 PAGES OF DRAFT LABELING HAVE BEEN WITHHELD IN FULL AS b4 (CCI/TS) IMMEDIATELY FOLLOWING THIS PAGE