CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125359Orig1s000

PHARMACOLOGY REVIEW(S)
MEMORANDUM

Erwinaze (L-asparaginase)

Date: October 28, 2011
To: File for BLA 125359/000
From: John K. Leighton, PhD, DABT
       Director, Division of Hematology Oncology Toxicology
       Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review by Dr. Kufrin and
supervisory memorandum and labeling provided by Dr. Pilaro. I concur with their
conclusions that Erwinaze may be approved for the proposed indication. I also concur
with the proposed labeling and the requested post marketing requirements for
reproduction toxicity evaluation.
ADDENDUM TO REVIEW MEMORANDUM

TO: The file
CC: Patricia Keegan, M.D., Director, Division of Oncology Products 2, Office of Hematology and Oncology Drug Products (OHOP), Center for Drug Evaluation and Research (CDER)
John K. Leighton, Ph.D., D.A.B.T., Director, Division of Hematology and Oncology Toxicology, OHOP, CDER
FROM: Anne M. Pilaro, Ph.D., Supervisory Toxicologist, Division of Hematology and Oncology Toxicology, OHOP, CDER

BLA #: 125359/000
SPONSOR: EUSA Pharma USA Inc. (EUSA)
PRODUCT: Erwinaze™ (non-proprietary name not yet assigned; L-asparaginase enzyme)
SUBMISSION TYPE: original BLA application
DATE: October 27, 2011

SYNOPSIS
This memorandum provides documentation of the post-marketing requirements for EUSA to conduct additional nonclinical studies to support the potential use of Erwinaze™ during pregnancy (Section 8.1 of the labeling), and its use in males and females of reproductive potential.

There were no nonclinical reproductive or developmental toxicity studies included in the original BLA submission for Erwinaze™. This BLA was submitted under the Public Health Services Act 351(a) regulatory pathway, as a new molecular entity biologic therapy. The sponsor had initially provided language in the labeling that

Following passage of the recent Patient Protection and Affordable Health Care Act in 2010 and establishment of the Public Health Services Act 351(k) regulatory pathway for biosimilar biologic therapies, reliance on published literature or findings from developmental and reproductive toxicity studies conducted by another (i.e. innovator) sponsor is not permitted to support approval of a BLA submitted under the 351(a) regulatory pathway. Therefore, to support the 351(a) application for Erwinaze™, EUSA will be required to conduct the complete battery of fertility, embryo-fetal and pre-postnatal nonclinical developmental toxicity studies with Erwinaze™, and provide the results from these studies as a post-approval supplement to the BLA.

The following draft language for the following post-marketing requirements (PMR) for these nonclinical studies was communicated to the sponsor on October 14, 2011:

The resulting nonclinical reproductive and developmental toxicity findings will be incorporated into the labeling following completion of the studies as post-marketing requirements, and review of the resulting data by the FDA. Additionally, FDA advised EUSA in a separate communication that should a high rate embryolethality or teratogenic effects (i.e. greater than 50% malformation rate or embryofetal loss) occur in the range-finding embryofetal developmental toxicity (EFD) studies of Erwinia-derived asparaginase, these findings would be incorporated in the labeling and the definitive EFD studies could be waived.

The following response to FDA's draft PMR language was received back from EUSA on October 21, 2011, and is quoted directly from their e-mail communication:
MEMORANDUM

TO: The file
CC: Patricia Keegan, M.D., Director, Division of Oncology Products 2, Office of Hematology and Oncology Products (OHOP), Center for Drug Evaluation and Research (CDER)
FROM: Anne M. Pilaro, Ph.D., Supervisory Toxicologist, Division of Hematology and Oncology Toxicology, OHOP, CDER
BLA #: 125359/000
SPONSOR: EUSA Pharma USA Inc. (EUSA)
PRODUCT: Erwinaze™ (non-proprietary name not yet assigned1; L-asparaginase enzyme)
SUBMISSION TYPE: original BLA application
DATE: October 27, 2011

SYNOPSIS:
EUSA Pharma USA Inc. (EUSA) has submitted an original biologics licensing application (BLA) for their recombinant, L-asparaginase enzyme derived from Erwinia chrysanthemi (Erwinaze™). 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."2

L-asparaginase is an enzyme that catalyzes the deamination of L-asparagine to L-aspartic acid, with the release of ammonia. Acute lymphoblastic leukemia cells require high plasma levels of asparagine for survival, but are unable to synthesize asparagine themselves. When an exogenous asparaginase enzyme, e.g. Elspar® is supplied, ALL cells are deprived of circulating asparagine and are unable to survive. Elspar® has been approved for use as part of a multi-drug chemotherapy regimen for treatment of ALL since 1994, and has an established clinical safety record. However, some ALL patients develop an allergic or hypersensitivity reaction to Elspar® or its pegylated derivative Oncaspar® (pegaspargase), and in these patients, an asparaginase derived from other sources is needed. Erwinaze™ L-asparaginase is derived from Erwinia chrysanthemi (facultative anaerobic, gram-negative bacteria of the family Enterobacteriaceae), and is similar in catalytic activity to the other approved asparaginases derived from Escherichia coli (i.e. Elspar®, Oncaspar®).

Nonclinical studies investigating the pharmacology, pharmacokinetics and toxicity of Erwinia-derived asparaginase3 in rats, mice, rabbits, beagle dogs and Rhesus monkeys in support of the safety of Erwinaze™ for the proposed indication were reviewed by the primary reviewer, D. Dubravka Kufrin, Ph.D., and are briefly summarized in the “Executive Summary” and “Integrated Summary and Safety Evaluation” sections of her

1 As of the date of this memorandum, the non-proprietary name for this product has not yet been accepted by FDA. Where appropriate, this product will be referred to throughout this review by the FDA accepted tradename Erwinaze™, or the descriptive name “Erwinia-derived asparaginase.”
2 From the indication statement in the current, draft labeling language for Erwinaze™
3 Many of the original toxicology studies on Erwinia-derived asparaginase reviewed by Dr. Kufrin were conducted with enzyme derived from the bacterium commonly known as Erwinia carotovora. Erwinia carotovora was subsequently reclassified in the 1980s as Erwinia chrysanthemi, and in 2007 the strain was reclassified again as Dickeya zeae.
review. Initial nonclinical studies of Erwinia-derived asparaginase were conducted prior to establishment of the Good Laboratory Practice regulations (GLP; 21 CFR part 58), and were therefore not conducted to the standards by which FDA expects nonclinical safety studies to be performed. Thus, Dr. Kufin's review of the sponsor's nonclinical information submitted to the BLA consists of her evaluation of previously conducted nonclinical pharmacology and acute and repeat-dose toxicology studies with Erwinia-derived asparaginases (generally from studies conducted in the 1960's and 1970's), combined with a review of the open literature of Erwinia sp. asparaginase pharmacology studies.

The major toxicity observed in mice, rats and rabbits treated with Erwinia-derived asparaginase was mortality of undetermined cause, although in some cases cerebral, subdural or meningeal hemorrhage was reported in early decedent animals. Inappetence, decreased food consumption (qualitative), occasional incidences of hyperglycemia, and loss of body weight/failure to gain body weight were also reported in both single and repeat-dose toxicity studies with Erwinia-derived asparaginases. Repeat-dose toxicology studies in rabbits, dogs and Rhesus monkeys could not establish dose-response relationships for asparaginase toxicity, since most of these studies employed only a single dose level of the enzyme. Some liver toxicities including hepatocellular vacuolization, elevated transaminase levels, fatty liver and impaired bromosulphthalein clearance were reported in rabbits and Rhesus monkeys treated with asparaginase derived from either E. coli or Erwinia carotovora, and were less severe in the Erwinia-derived asparaginase treated groups. Occasional findings in the kidneys (e.g. pale kidneys, dilated renal tubules), lungs (e.g. congestion), and increased oxyphil cells in the thyroid or parathyroid glands were also reported in some studies in rabbits, but were not consistently related to either the dose or source of the asparaginase enzyme.

Comment: Comparison of the doses tested in the animal studies to the doses of Erwinaze™ tested clinically is not possible. The nonclinical doses were calculated based on specific activity of the Erwinia-derived asparaginase enzyme (i.e. International Units [IU]/kg) and the recommended clinical dosing is based on IU/m², but the specific activity of Erwinaze™ is calculated using a different potency assay than was used to establish the activity of the Erwinia-derived enzyme used in the toxicity studies.

There were no nonclinical genotoxicity or carcinogenicity studies performed with Erwinia-derived asparaginase, as per the guidance provided in ICH S6 "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" and ICH S9 "Nonclinical Safety Evaluation for Anticancer Pharmaceuticals." Nonclinical studies to evaluate the potential teratogenicity, effects on fertility and reproductive function, and effects on late pregnancy, delivery and juvenile development of L-asparaginase derived from Erwinia chrysanthemi were not submitted in this BLA. EUSA has been informed that conducting the full range of reproductive and developmental toxicity studies, as outlined in the ICH S5(R2) Guidance: "Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility" and submitting the resulting data to the BLA will be a post-approval requirement.
**Comment:** Pediatric ALL has a “cure” rate of approximately 80% with long-term survival rates of about 45-60%, and many of these patients subsequently have children of their own. Because of the potential for long-term survival, this patient population does not fit the scope that is addressed by the ICH S9 guidance document (i.e. late-stage, advanced cancers with short-term survival). Therefore, the ICH M3(R2) “Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation” for Pharmaceuticals applies to this product in this indication, and the full battery of developmental and reproductive toxicity testing as outlined in the ICH S5(R2) guidance is required.

In summary, the nonclinical studies provided by EUSA in support of this BLA showed that Erwinia-derived asparaginases were generally well-tolerated in animals. Inappetence and weight loss were the most frequent toxicities, with fatty liver, changes in liver function, hyperglycemia and mortality at relatively high doses also reported. Although the nonclinical studies were not conducted in full compliance with the GLP regulations and the doses tested were established using different calculations of specific activity than are used at the present time, these studies did support the initial safety of Erwinia-derived asparaginases for human testing. There are over 40 years of clinical experience with EUSA’s product (i.e. the initial IND for this product, BB IND 290 was submitted in 1968), and Erwinase (tradename) is approved in the European Union for the same indication as proposed in this BLA. The major clinical toxicities reported with Erwinaze™ and other, E. coli-derived asparaginases are well documented and include hypersensitivity reactions, coagulopathies, pancreatitis, glucose intolerance and liver abnormalities (which for the most part were not predicted by the nonclinical studies). Therefore, together with the known clinical safety record, the nonclinical studies submitted are adequate to conclude that Erwinaze™ is reasonably safe for use in ALL patients who are hypersensitive to asparaginases isolated from E. coli, or pegylated asparaginase products. EUSA will not be required to repeat the general toxicology studies; however, there will be post-marketing requirements for additional nonclinical embryo-fetal developmental and reproductive toxicity studies. EUSA will be required to conduct the complete battery of fertility, embryo-fetal and pre-post-natal nonclinical developmental toxicity studies with Erwinaze™, and provide the results from these studies as a post-approval supplement to the BLA.

**Recommendation:** In summary, I concur with Dr. Kufnir’s conclusions regarding the nonclinical findings for Erwinaze™ and her current recommendations that the licensing application be approved for marketing for its proposed indication. I also concur with the proposed post-marketing requirement for EUSA to conduct additional nonclinical reproductive and developmental toxicity studies, to obtain data to convey the risks of Erwinaze™ use during pregnancy to patients and prescribing physicians. A copy of Dr. Kufnir’s review, with supervisory sign-off, has been conveyed to the regulatory project manager for inclusion in the final action package.

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Application number: BLA 125359
Supporting document/s: 0000
Applicant’s letter date: September 8, 2010
CDER stamp date: September 8, 2010
Product: Erwinaze™ (Erwinia chrysanthemi L-asparaginase)
Indication: Acute lymphoblastic leukemia (ALL)
Applicant: EUSA PHARMA (USA) INC
One Summit Square
1717 Langhorne Newtown Road
Suite 201
Langhorne, PA 19047

Review Division: Division of Biologic Oncology Products
Reviewer: Dubravka Kufrin, PhD
Supervisor/Team Leader: Anne M. Pilaro, PhD
Division Director: Patricia Keegan, MD
Project Manager: Erik Laughner, MS

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of STN BLA #125359 are owned by EUSA PHARMA (USA) Inc. or are data for which EUSA PHARMA has obtained a written right of reference. Any information or data necessary for approval of STN BLA #125359 that EUSA PHARMA does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that EUSA PHARMA (USA) Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of STN BLA #125359.
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1 Executive Summary

The nonclinical studies conducted on Erwinia sp. asparaginases that were submitted with this biologics licensing application (BLA) pre-dated the Good Laboratory Practice (GLP) regulations 21 CFR Part 58, and are therefore not GLP-compliant. Since Erwinase™¹ is approved in Europe on the basis of its safety and effectiveness shown in European clinical studies together with the same nonclinical studies submitted here, this reviewer reviewed the submitted nonclinical studies even though they were not GLP-compliant. Together with the known clinical safety record, the pharmacology/toxicology nonclinical studies submitted are adequate to conclude that Erwinaze™² is reasonably safe for use in acute lymphoblastic leukemia patients who are hypersensitive to asparaginases isolated from E. coli, or to pegylated asparaginase products.

1.1 Recommendations

1.1.1 Approvability—Yes, from the nonclinical perspective. However, there were multiple deficiencies identified by the Chemistry, Manufacturing and Controls, Facilities, and Clinical Pharmacology disciplines that will result in a Complete Review letter being issued for this submission.

1.1.2 Additional Nonclinical Recommendations

There will be post-marketing requirements for additional nonclinical embryofetal developmental and reproductive toxicity studies. Under the new legislation (Patient Protection and Affordable Care Act, 2010), reliance on published literature for developmental and reproductive toxicity studies to support a BLA in the 351(a) regulatory pathway is not possible. Therefore, FDA requires that the complete battery of fertility, embryo-fetal and pre-post-natal nonclinical developmental toxicity studies be conducted with Erwinaze™, and the results from these studies be provided as a post-approval supplement to the BLA. The sponsor has provided plans and protocols to be evaluated by the FDA for all of the necessary studies as an amendment to the IND (IND #290, supporting document #990, date of submission March 22nd, 2011).

1.1.3 Labeling

At present, there are no nonclinical findings to be included in the draft labeling. Updated labeling will be provided at the time of re-submission of the BLA in response to

¹ Erwinase—approved product name in Europe
² Erwinaze—proposed US trade name for the product that is the subject of this BLA
the deficiencies identified by the other disciplines. The nonclinical reproductive and developmental toxicities will be incorporated into the labeling following completion of the studies as post-marketing requirements, and review of the resulting data by the FDA.

1.2 Brief Discussion of Nonclinical Findings

L-asparaginase (L-asp) is an enzyme that catalyzes the deamination of L-asparagine to L-aspartic acid, with the release of ammonia. The L-asparaginase enzyme reviewed here is derived from *Erwinia chrysanthemi*, (a facultative anaerobic, gram-negative bacteria [family *Enterobacteriaceae*]), and is similar to approved asparaginases derived from a close phylogenetic relative, *Escherichia coli* (Elspar, Oncaspar). Elspar has been used for treatment of acute lymphoblastic leukemia (ALL) since 1994, and has an established clinical safety record. However, some ALL patients develop an allergic or hypersensitivity reaction to Elspar, or its pegylated derivative Oncaspar (pegaspargase), and in these patients, asparaginase derived from other sources are needed.

The pharmacology/toxicology studies conducted in support of the Erwinaza™ drug development program pre-dated the GLP regulations. Since Erwinaza™ is approved in Europe on the basis of the safety and effectiveness shown in European clinical studies together with the same nonclinical studies submitted here, this reviewer reviewed the submitted nonclinical studies even though they were not GLP-compliant. Some of the nonclinical studies used L-asparaginase derived from different bacterial sources, i.e., *Erwinia chrysanthemi, Erwinia carotovora*, or sometimes the test article was identified in this review as *Erwinia sp.*, to denote that it was an L-asparaginase from unidentified *Erwinia* source, or to refer to the *Erwinia*-derived L-asparaginases as a group of related products.

The acute toxicity of L-asparaginase derived from *Erwinia sp.* was assessed in six animal studies in rats and rabbits, with the maximum nonlethal dose identified between 100,000 and 200,000 IU/kg³. Maximum lethal doses were between 200,000 and 500,000 IU/kg. Lower tolerance rates were seen in rabbit studies, where the maximum nonlethal dose was 1,000 IU/kg while the minimum lethal dose was approximately 2,000 IU/kg. Subdural hemorrhages were observed in some early decedent rabbits, although the true cause of death was not elucidated. In the acute studies in both rodents and rabbits, antemortem muscle weakness and ataxia were observed in those animals with early deaths.

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³ The IU/kg is the dose reported by the sponsor and used in the sponsor-submitted, non-GLP pharmacology/toxicology studies. The sponsor has not specified how the units of activity (IU) were determined. Based on the new CMC data (submitted November 1, 2010), the specific activity of Erwinaza™ should be in the range of [b]4 [b] However, the previous batches used for the pharmacology/toxicology studies did not use the same detection method to assay for enzyme activity (Please refer to Appendix section, Table 3).
Repeat-dose toxicity studies in rabbits, dogs and monkeys could not establish a dose response relationship, since three of the studies tested only a single dose level. One rabbit that received repeat dosing with 1,000 IU/kg/day for 5 days died 12 days post-dosing of uncertain causes, while the four remaining rabbits in this study that were identically treated tolerated the experience.

Submitted single and repeat-dose toxicology studies with L-asparaginase from *Erwinia* sp. were done using intraperitoneal or intravenous injection routes, which differ from the intramuscular route that is proposed for the clinical development. However, substantial human safety data is available using Erwinase™ injected IM, and therefore additional animal data would not be useful or informative.

Nonclinical studies to evaluate the potential teratogenicity, effects on fertility and reproductive function, and effects on late pregnancy, delivery and juvenile development of L-asparaginase derived from *Erwinia chrysanthemi* were not submitted in this BLA. The sponsor has been informed that conducting the full range of reproductive and developmental toxicity studies and submitting the data to the BLA will be a post-approval requirement. Carcinogenicity and mutagenicity studies for this protein therapeutic have not been requested, as per the ICH S9 and ICH S6 guidelines.

### 2 Drug Information

#### 2.1 Drug: Erwinaze™ is a type II L-asparaginase, isolated from the bacteria *Erwinia* (also known as *Erwinia chrysanthemi*). It is similar to *Escherichia coli* and *Erwinia carotovora*-derived asparaginases in that all of these enzymes utilize L-asparagine as a substrate for catalysis of L-asparagine in its conversion to L-aspartate and ammonia. The biochemical reaction of L-asparaginase is depicted below:

\[
\text{L-asparagine} \xrightarrow{\text{L-asparaginase}} \text{L-aspartic acid + NH}_3
\]

<table>
<thead>
<tr>
<th>2.1.1</th>
<th>CAS Registry Number</th>
<th>9015-68-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.2</td>
<td>Generic Name</td>
<td>not yet specified (USAN name is currently under discussion)</td>
</tr>
<tr>
<td></td>
<td>Other non-proprietary names:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>British Approved Name (BAN): Crisantaspsase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tentative US Adopted Name (USAN): Asparaginase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other Names: L-asparaginase from <em>Erwinia chrysanthemi</em> (<em>Er. carotovora</em>), <em>Erwinia</em> asparaginase</td>
<td></td>
</tr>
<tr>
<td>2.1.3</td>
<td>Code Name</td>
<td>NSC 106977 (The American National Service Center reference number for <em>Erwinia</em> L-asparaginase)</td>
</tr>
</tbody>
</table>
### 2.1.4 Chemical Name
L-asparaginase amidohydrolase EC

### 2.1.5 Molecular Formula
140 kDa (4 X 35 kDa of tetrameric, identical polypeptide chain subunits)

### 2.1.6 Structure
Erwinaze™ L-asparaginase is a homotetramer with four active sites. Each tetramer consists of [Redacted]. The secondary structure of the monomer is presented in Figure 1 (provided by the sponsor’s non-clinical overview, p 6).

### 2.1.7 Pharmacologic class
L-asparaginase

#### 2.2 Relevant IND/s, NDA/s, and DMF/s

There are a number of active INDs within OODP that are currently investigating L-asparaginase. These include the original IND #290 from EUSA Inc. for the product submitted in this BLA, as well as INDs [Redacted] 100594 and [Redacted] for L-asparaginases derived from different bacterial sources.
2.3 Clinical Formulation

2.3.1 Drug Formulation

Erwinaze™ is supplied as a sterile freeze-dried preparation of 10,000 units L-asparaginase derived from Er. chrysanthemi, in sodium chloride. When reconstituted with injectable physiological saline the resulting solution for injection contains 10,000 units/mL of asparaginase, 0.5 mg/mL sodium chloride and 5 mg/mL glucose monohydrate.

**Reviewer comment:** From the CMC section of the BLA: “Erwinaze™ (Erwinia L-asparaginase for Injection) is produced from Erwinia chrysanthemi subsequent to produce the Drug Substance (DS). The DS is stored The proposed shelf-life is when stored under these conditions. When required for manufacture of the Drug Product (DP), the DS is

2.3.2 Comments on Novel Excipients

There are no novel excipients in the Drug Product formulation. The excipients used are sodium chloride and glucose monohydrate.

2.3.3 Comments on Impurities/Degradants of Concern

Application of the new methodologies suggested by the agency during the May 30th, 2008 pre-BLA meeting and implemented by the manufacturer of Erwinaze™, revealed product heterogeneity. Upon further examination, the sponsor concluded that the available data concerning impurities/degradants have not identified any safety issues associated with impurities or degradation products of Erwinaze™.

2.4 Proposed Clinical Population and Dosing Regimen

Erwinaze™ is for the treatment of patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to E. coli derived asparaginase. The proposed dosing regimen for Erwinia asparaginase is. This regimen would substitute for 4 doses of E. coli asparaginase (each at 10,000 IU/m²) in re-induction treatment, in order to maintain a mean trough L-asparaginase activity level of ≥0.1 IU/mL in a majority of patients with median
trough asparagine levels <1.5 μM.

2.5 Regulatory Background

Reviewer comment: In the United States of America (US), there are 2 asparaginase preparations approved by the FDA and available for clinical use: 1) native L-asparaginase derived from *E. coli* (Elispar®); and 2) pegasparase (Oncaspar®), a modified (pegylated) form of the *E. coli* enzyme. A third preparation, *Erwinia* asparaginase (Crisantaspase, Erwinase™), derived from *Erwinia chrysanthemi*, is licensed in some countries in Europe and in Canada but is not commercially available in the US. Prior to 2002, it was available to patients with hypersensitivity to the *E. coli* derived preparations on a named (single) patient basis. Because of manufacturing difficulties, the Erwinase™ product was removed from the worldwide market in 2003. In 2004, the issues were resolved and Erwinase™ was again made available outside the US. In 2006, the distribution of this product was taken over by EUSA Pharma Inc., who are pursuing the registration of Erwinaze™ in the US under the Erwinaze™ IND (IND #290). A compassionate use protocol was begun by the Children’s Oncology Group (COG; the Erwinaze™ Master Treatment Protocol) to enable US patients with hypersensitivity to the other forms of L-asparaginase to receive the medication prior to its approval by FDA.

2 Studies Submitted

A tabulated overview of the submitted studies is depicted in the following pages (taken from the submission):

<table>
<thead>
<tr>
<th>Type of Study</th>
<th>Species and Strain</th>
<th>Route</th>
<th>Dose of Dosing</th>
<th>Dose*</th>
<th>GLP</th>
<th>Testing Facility</th>
<th>Study Number</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-Dose Toxicity</td>
<td>Mice: (b) 14</td>
<td>Intravenous</td>
<td>Single Dose</td>
<td>1000,000, 150,000 and 1,000,000 IU/kg BW (mice and rats), 500, 1000 and 2500 IU/kg BW (rabbits)</td>
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<td>(b) (4)</td>
<td>631-667-668</td>
<td>4.3.1</td>
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<tr>
<td>Single-Dose Toxicity</td>
<td>Rat, Rabbit, Hamster (consumption specific)</td>
<td>Intravenous</td>
<td>Single Dose</td>
<td>1,000 IU/kg BW IV (mice and rats), 1,000 IU/kg BW IP (rabbits)</td>
<td>No</td>
<td>(b) (4)</td>
<td>631-667-71-4</td>
<td>4.3.1</td>
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<td>Single-Dose Toxicity</td>
<td>Cynomolgus Monkey</td>
<td>Intraperitoneal</td>
<td>Single Dose</td>
<td>100,000, 150,000 and 1,000,000 IU/kg BW</td>
<td>No</td>
<td>(b) (4)</td>
<td>631-667-668</td>
<td>4.3.1</td>
</tr>
<tr>
<td>Single-Dose Toxicity</td>
<td>Cynomolgus Monkey</td>
<td>Intraperitoneal</td>
<td>Single Dose</td>
<td>100,000, 150,000 and 1,000,000 IU/kg BW</td>
<td>No</td>
<td>(b) (4)</td>
<td>631-667-668</td>
<td>4.3.1</td>
</tr>
</tbody>
</table>

* GLP = Good Laboratory Practice

Notes:
- QS = quick screen
- COG = Children’s Oncology Group

For general, repeated-dose toxicity, the 90-day NOAEL is 500 IU/kg BW (mice) and 50 IU/kg BW (rabbits).
<table>
<thead>
<tr>
<th>Type of Study</th>
<th>Species and Strain</th>
<th>Route</th>
<th>Duration of Dosing</th>
<th>Dose(s)</th>
<th>GLP</th>
<th>Testing Facility (b) (c)</th>
<th>Study Number</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-Dose Toxicity</td>
<td>CD-1 mice</td>
<td>Intravenous</td>
<td>Single Dose</td>
<td>1000, 2000, 5000 and 10,000 IU/kg BW</td>
<td>No</td>
<td>4016-71-173</td>
<td>4.2.3.1</td>
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<tr>
<td>Single-Dose Toxicity</td>
<td>CD-1 mice</td>
<td>Intravenous</td>
<td>Single Dose</td>
<td>1000, 2000, 5000 and 10,000 IU/kg BW</td>
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<tr>
<td>Single-Dose Toxicity</td>
<td>New Zealand White Rabbit</td>
<td>Intravenous</td>
<td>Single Dose</td>
<td>5000, 10,000 and 20,000 IU/kg BW</td>
<td>No</td>
<td>15-71-D-111</td>
<td>4.2.3.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of Study</th>
<th>Species and Strain</th>
<th>Route</th>
<th>Duration of Dosing</th>
<th>Dose(s)</th>
<th>GLP</th>
<th>Testing Facility (b) (c)</th>
<th>Study Number</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-Dose Toxicity</td>
<td>New Zealand White Rabbit</td>
<td>Intravenous</td>
<td>Single Dose</td>
<td>2000, 5000, and 10,000 IU/kg BW</td>
<td>No</td>
<td>4016-71-173</td>
<td>4.2.3.1</td>
<td></td>
</tr>
<tr>
<td>Repeat-Dose Toxicity</td>
<td>Rabbit</td>
<td>Intravenous</td>
<td>One day and Five days</td>
<td>1000, 2000, and 5000 IU/kg dose/day and 1000 IU/kg day (five days)</td>
<td>No</td>
<td>09/14-595-2-02</td>
<td>4.2.3.2</td>
<td></td>
</tr>
<tr>
<td>Repeat-Dose Toxicity</td>
<td>Single Dogs</td>
<td>Intravenous</td>
<td>Four Weeks</td>
<td>5000 IU/kg day</td>
<td>No</td>
<td>SESC 1069</td>
<td>4.2.3.2</td>
<td></td>
</tr>
<tr>
<td>Repeat-Dose Toxicity</td>
<td>Phobus monkey</td>
<td>Intravenous</td>
<td>Five days</td>
<td>1000 IU/kg day</td>
<td>No</td>
<td>09/14-595-3-02</td>
<td>4.2.3.2</td>
<td></td>
</tr>
</tbody>
</table>
3.1 Studies Reviewed

All single dose and repeat-dose toxicity studies submitted with this BLA have been reviewed.

3.2 Studies Not Reviewed

Studies provided in the BLA as “other toxicity studies” (Studies # (3) DPH-70-07, “Pyrogenicity” and #HRC 97) have not been reviewed.

3.3 Previous Reviews Referenced

None.

4 Pharmacology

4.1 Primary Pharmacology

Acute lymphoblastic leukemia (ALL) cells require a high amount of asparagine for survival, are but are unable to synthesize this amino acid on their own. The anti-tumor effects of asparaginase were discovered more than a half century ago, when it was observed that guinea pig serum had an inhibitory effect on certain neoplasias. This

4 Kidd JG (1953a). Regression of transplanted lymphomas induced in vivo by means of normal guinea pig serum. I. Course of transplanted cancers of various kinds in mice and
activity was eventually defined as resulting from the L-asparaginase mediated catalysis and removal of plasma asparagine. When exogenous asparaginase enzyme is supplied as a drug, leukemic cells are deprived of circulating asparagine and are unable to survive. Further studies showed that asparaginases derived from other sources have been equally effective in neoplastic growth inhibition.

Many of the original studies on Erwinia species (Er. sp.) asparaginase toxicity have been conducted with asparaginase derived from the bacterium commonly known as Er. carotovora. Er. carotovora was isolated in 1901, and was reclassified as Pectobacterium carotovora in the 1980s. The strain was subsequently reclassified as Erwinia chrysanthemi, and in 2007 the strain was reclassified again as Dickeya zeae. The asparaginases derived from multiple different strains of Er. carotovora have about 77% -99% sequence homology with the enzyme from Er. chrysanthemi. The Er. carotovora derived enzymes exhibit similar biochemical specificity for L-asparagine, and are considered adequate surrogates for L-asparaginase from Er. chrysanthemi. Studies using asparaginase from Er. carotovora were considered relevant to assessing the nonclinical effects of Erwinaze™.

The effect of asparaginase and asparagine deprivation on the growth of mouse leukemia L5178Y cells and cells selected for asparagine-independent growth (L5178Yr) was studied in vitro. Asparaginase (10 IU/ml) inhibited cell growth and protein synthesis in wild type cells, but not in L5178Yr cells. Asparagine deprivation inhibited protein synthesis in wild type L5178Y cells, resulting in an inhibition of growth. Cancer cells able to grow in the absence of asparagines (i.e. L5178Yr) were able to grow in the presence of L-asparaginase, suggesting that the therapeutic action of L-asparaginase was due to depletion of asparagine.

The anti-tumor activity of E. coli and Er. carotovora asparaginases against lymphoid leukaemias EARAD-1 and L5178Y/CA55 was compared in BDF1 mice. The mice were inoculated with tumor cells on day 1 and treated with 0, 125 or 250 IU/kg asparaginase IP for 5 days starting on Day 1 or 11 (EARAD-1 cells) or Day 9 (L5178Y/CCA 55 cells). Both asparaginases significantly prolonged survival compared to untreated controls, but the E. coli asparaginase was more effective (see Table 2).

---

Table 2 Effect of *E. coli* and *Er. carotovora* asparaginase on tumor growth in mice

<table>
<thead>
<tr>
<th>Treatment (IU/kg)</th>
<th><strong>EARAD-1</strong></th>
<th></th>
<th><strong>L5178Y/CA 55</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated Days 1-5</td>
<td>Treated Days 11-15</td>
<td>Treated Days 9-13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MST (days ±SE)</td>
<td>90 Day survival</td>
<td>MST (days ±SE)</td>
<td>90 Day survival</td>
</tr>
<tr>
<td>Saline</td>
<td>16 ± 0.8</td>
<td>0/10</td>
<td>16 ± 0.8</td>
<td>0/5</td>
</tr>
<tr>
<td><em>E. coli</em> 125</td>
<td>&gt;90</td>
<td>9/10</td>
<td>29 ± 2.1</td>
<td>0/5</td>
</tr>
<tr>
<td><em>E. coli</em> 250</td>
<td>&gt;90</td>
<td>10/10</td>
<td>41 ± 2.9</td>
<td>2/5</td>
</tr>
<tr>
<td><em>Erwinia</em> 125</td>
<td>32 ± 3.2</td>
<td>2/10</td>
<td>23 ± 1.0</td>
<td>0/5</td>
</tr>
<tr>
<td><em>Erwinia</em> 250</td>
<td>48 ± 2.0</td>
<td>5/10</td>
<td>29 ± 2.4</td>
<td>0/5</td>
</tr>
<tr>
<td><em>Erwinia</em> 500</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

MST = Median survival time; ND = Not done.

The reason for this discrepancy was not completely elucidated. Both enzymes reduced plasma asparagine concentrations to undetectable levels by 24 hours post-dose (250 IU/kg IP). The *E. coli*-derived asparaginase had a slightly longer plasma half life (28 hours) than the enzyme from *Er. carotovora* (24 hours). The authors speculated that *E. coli* asparaginase may have had a greater distribution to the extravascular region, although there is no data to support this hypothesis.

### 4.2 Secondary Pharmacology

The $K_m$ for L-asparagine of *Er. chrysanthemi*-derived enzyme is 115-fold lower than the $K_m$ for glutamine\(^8\). The *Er. chrysanthemi*-derived enzyme showed a lower $K_m$ for L-asparagine as compared to other asparaginases from *Er. carotovora*\(^9\) whereas a higher $K_m$ value was found for glutamine as compared to asparaginase from *Er. carotovora*\(^10\). For example, the $K_m$ for glutamine is 3.4 mM for asparaginase from *Er. carotovora*. Low affinity for glutamine may be important, since the toxicity of the enzyme is partially attributable to the L-glutaminase activity\(^11\), and it has been suggested that asparaginases with high affinity for asparagine and low affinity for glutamine exhibit less


toxicity during the course of anti-cancer therapy. By contrast, it has also been suggested that glutaminase activity contributes to the clinical efficacy of asparaginase.

4.3 Safety Pharmacology

No non-clinical safety pharmacology studies have been submitted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The half-life of intraperitoneally (IP) administered *Er. carotovora*-isolated L-asparaginase in male mice after dosing with 10 IU (400 IU/kg, assuming a 25 gr weight) was calculated as 5 hours. When 40 IU (1,600 IU/kg, assuming a 25 gr weight) was intraperitoneally injected in C3H mice the half-life of the enzyme was 7.5 hours. The half-life in rabbits was 10.5 hours, 1.5 hours in rats, and 1.6 hours in Rhesus monkeys. The bioavailability of Erwinaze™ after intravenous administration is 100%; while there are no data available for different routes of administration.

6 General Toxicology

6.1 Single-Dose Toxicity

Study #031-665-6881. A study on the acute toxicity and pyrogenicity of asparaginase.

The acute toxicity and mortality rate of three batches of asparaginase were tested in mice, rats and rabbits over a three week period, and sub-batch B-asparaginase passed the British Pharmacopoeia (B.P.) test for pyrogens (See Appendix 1 at the end of this review for rabbit results).

Ten C.D. strain mice/sex at each dose level were injected IP with a single dose of 200,000, 500,000 or 1,000,000 (1 M) U/kg L-asparaginase, and were observed daily for three weeks. One male mouse died on study day 4, and one female on study day 2, (1 M U/kg/day, total mortality 20%), one female on day 3 and another on day 4 (500 000 U/kg/day, total mortality rate 20%).

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Five albino, Swiss rats (sex [female]) per dose level were injected intravenously (IV) with a single dose of 200,000, 500,000, 1 M U/kg of L-asparaginase. Four male and 4 female rats each in the 1 M U/kg dose group, and two female rats injected with 500,000 U/kg died on study day 2. Additionally, the final female rat injected with 1 M U/kg L-asparaginase died on study day 3.

| Dose in u/kg | Sex | Route of Injection | Number In Group | DAY | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 4,000       | ♂   | IV                | 3               |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 4,000       | ♀   | IV                | 3               |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 8,000       | ♂   | IV                | 3               |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 8,000       | ♀   | IV                | 3               |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 12,000      | ♂   | IV                | 3               |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 12,000      | ♀   | IV                | 3               |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

**KEY:**  
Day 0 = day of injection (5, 10, 17)  
IV = Intravenous

**NOTE:** On day 21 all surviving animals were killed and the investigation terminated.

The standard B.P. test for pyrogens was performed on three, previously unused female rabbits at 2,000 U/kg body weight by IV administration. Results are presented in Appendix 1.
Study #11/71/D409. Acute toxicity of Erwinia asparaginase (Batch 29 Jan 71).
Ten males and ten female mice per group were injected IP with 200,000 (low); 500,000 (mid) or 1,000,000 (high dose) IU/kg L-asparaginase isolated from Erwinia sp. Deaths occurred within five days of dosing in 2/10 males given the mid-dose, and 4/10 males and 3/10 females given the high dose. Deaths in these animals were preceded by lethargic behavior and at necropsy, subdural hemorrhage was revealed as the probable cause of death. The remainder of the mice recovered to healthy appearances within a week after the dosing.

Twenty mice per group (10/sex/group, CFLP strain) were injected IP with low, mid or high doses of Erwinia sp. asparaginase as described in study #11/71/D409, above. Mortality occurred within seven days after treatment in 30% of the animals dosed with the highest dose of 1,000,000 IU/kg, while only one animal died after receiving the 500,000 IU/kg dose (5% incidence of mortality). Dilation of renal tubules and subdural hemorrhage were observed microscopically in the early decedent animals. Surviving animals recovered within two weeks after the treatment, but showed lower body weights during the first week after the treatment.

Study #12/71/D410. Acute toxicity of Erwinia asparaginase (Batch 29 Jan 71).
Groups of ten rats (five males and five females) were injected IV with Erwinia asparaginase at doses of 200,000 (low), 500,000 (mid) or 1,000,000 (high) IU/kg and observed for 21 days. Death occurred within 21 hours in 5/5 males in the mid-dose and
5/5 males and 5/5 females in the high-dose groups. Deaths were preceded by tremors, ataxia, and loss of muscular co-ordination. Subdural hemorrhage and generalized congestion of blood vessels were revealed at necropsy, while signs of the recovery were visible (improvements in behavior and external appearance) in the surviving animals within one week post-treatment.

**Study #4015/71/173. Acute toxicity of Erwinia asparaginase (Batch 9 Oct 1970).** Group of ten rats were intravenously injected with *Erwinia* asparaginase at doses of 200,000 (low), 500,000 (mid), or 1,000,000 (high) IU/kg and observed for 21 days. Death occurred within 79 hours in 4/10 low dose rats and within 43 hours in 10/10 mid-dose and high dose treated animals. Early deaths were preceded by tremors, ataxia, and loss of muscular co-ordination. Subdural hemorrhage, dilation of renal tubules and depletion of lymphoid cells in the spleen were reported on microscopic pathology observations.

**Study #13/71/D411. Acute toxicity of Erwinia asparaginase (Batch 29 Jan 71).** Group of six rabbits (three males and three females) were intravenously injected with *Erwinia* asparaginase at doses of 5,000 (low), 10,000 (mid), or 20,000 (high) IU/kg and observed for 21 days. Deaths occurred within 18 days in 4/6 low dose rabbits, within 2 to 4 days in 5/6 mid-dose rabbits, and within 5 to 13 days in 5/6 of the high dose treated animals. Deaths were preceded by loss of appetite and ataxia. Necropsy of dead animals revealed congestion of the lungs and pale kidneys, with presence of subdural hemorrhage in two of the early decedent animals.

**Study #4017/71/175. Acute toxicity of Erwinia asparaginase (Batch 9 Oct 70).** Group of six rabbits (three males and three females) were intravenously injected with *Erwinia* asparaginase at doses of 5,000 (low), 10,000 (mid) or 20,000 (high) IU/kg and observed for 21 days. Deaths occurred within 14 days in 2/6 low dose rabbits, within 21 days in 4/6 mid-dose rabbits and within 6 days in 5/6 animals treated at the high dose. Early deaths were preceded by loss of muscular coordination, anorexia and ataxia. Necropsy of the dead animals revealed subdural hemorrhage in all early decedent animals. Recovery of the surviving animals, as judged from external appearance and behavior was apparent within two weeks, although marked weight loss of all survivors was reported during the first week. Dilation of renal tubules was seen by microscopic pathology observations in surviving rabbits at necropsy, and in the animals that died early while on the experiment.
6.2.1 Repeat-Dose Toxicity-Study 1969

Study title: L-Asparaginase short-term study in beagle dogs
Study no.: 1969
Study report location: electronic BLA submission, Module 4, Toxicology, Repeat-Dose Studies, Section 4.2.3.2

Conducting laboratory and location: 

Date of study initiation: 3 June 1969
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: MRE 604 L-Asparaginase Batch 24, April 1969 (ampoule of 2 mL containing 23,000 IU)

Key Study Findings

Daily intravenous injection of beagle dogs with L-asparaginase (Erwinia sp.) at a dose of 5,000 IU/kg/day for 28 days resulted in no deaths. Initial vomiting, abnormal quietness, loss of general condition and emesis were observed after dosing, primarily during the first week of the study. Loss of weight in male dogs and poor weight gain in female dogs was seen with the study prolongation and were associated with reduced food intake, but no abnormalities were found in post-mortem macroscopic or microscopic/histological observations of the organs, specifically the liver.

Methods

Doses: 5,000 IU/kg/day; no control treated animals in this study
Frequency of dosing: 1x daily, seven days a week for 28 days; four weeks
Route of administration: Intravenous injection
Dose volume: Not specified
Formulation/Vehicle: Not specified
Species/Strain: Pure bred beagle dogs
Number/Sex/Group: 2 F; and 2 M
Age: Not specified
Weight: Not specified
Satellite groups: None
Unique study design: Each dog was dosed twice during the predosing period with the anthelmintic piperazine
Deviation from study protocol: Not done in compliance with GLP regulations
Mortality
No deaths were recorded during the experimental period.

Clinical Signs
Vomiting and occasional shivering was observed in all animals 15 minutes to one hour after dosing. Loss of general condition, with occasional, slight ocular discharge was observed in the third week of dosing, together with thin appearance and somewhat drier coats of dogs given the L-asparaginase. Excessive salivation was observed in one female dog during the last few days of the dosing period.

Body Weights
The decreased food consumption and vomiting episodes in the L-asparaginase treated dogs had visible outcome on body weight in the male dogs on the study. Body weights for the female dogs, although lower than baseline, did not reach the proportion of weight loss exhibited for the male dogs.

Feed Consumption
Food intake was measured for seven days before the dosing period, and every day during dosing for all four animals tested. Based on the qualitative data provided, it was noted that all animals ate only about one quarter, or none of the food provided beginning on the third day of dosing. The decreased food consumption lasted for a period of three to five days. From that point on, dogs had cycles of four to five days of good appetite, followed by about three days of poor appetite and low food consumption. The diet for all animals was changed from mixed dry food to a wet food, with more improved appetite seen in females than males on the study.

Reviewer Comment: Ophthalmoscopy, ECG, hematology, clinical chemistry and urinalysis evaluations were not included in this non-GLP study.

Organ Weights
The observation that the percentage of liver and kidney weights were increased (as a total observed organ weights) is difficult to interpret since there were only four animals tested and there were no control animals on the study.
Histopathology

Reviewer Comment: This study was not done under GLP regulations, therefore parameters such as Adequate Battery of tissues examined, or Peer Review were not possible to evaluate.

Histological Findings: Organs from one male and one female dog each were evaluated histologically, but found to be unremarkable.

Reviewer Comment: Special evaluations, toxicokinetic and stability and homogeneity data were not provided for this non-GLP toxicity study.

6.2.2 Repeat-Dose Toxicity-Study

Study title: Toxicity studies on Enwinia carotovora L-Asparaginase batch 8/17/69 (NCS 109229) following daily I.V. administration to Rhesus monkeys

Study no.: (b)(4) - DPH-71-02

Study report location: (b)(4) - DPH-71-02

Conducting laboratory and location: (b)(4)

Date of study report: 26 December 1969

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: Enwinia carotovora batch 8/17/69; NCS 109229

Key Study Findings

This study was done in one Rhesus monkey to evaluate the effects of intravenous administration of 1,000 IU/kg of L-asparaginase, derived from Er. carotovora. There were no toxicities reported for this study, no significant changes in food consumption or body weight. The sponsor reports that this treatment did not produce diabetogenic changes or fatty liver, which had previously been reported for L-asparaginase produced from E. coli.

There were no significant changes in bromosulphthalein (BSP) clearance, prothrombin time, clotting time, fibrinogen level, blood sugar, serum protein, cholesterol, SGOT, SGPT, alkaline phosphatase, BUN, B lipoprotein, creatinine, bilirubin, amylase, lipase, electrolytes, hematology or urinary parameters.
Methods

- **Doses**: 1,000 IU
- **Frequency of dosing**: 1X day for 5 days
- **Route of administration**: IV
- **Dose volume**: 0.5 ml/kg
- **Formulation/Vehicle**: 2,000 IU/ml in saline
- **Species/Strain**: Rhesus monkey (*Macaca mulatta*)
- **Number/Sex/Group**: 1 male monkey
- **Age**: Not specified
- **Weight**: 2.96 kg
- **Satellite groups**: None
- **Unique study design**: None
- **Deviation from study protocol**: Not conducted in compliance with the GLP regulations (study predated GLP)

**Observations and Results**

Records of daily observations for this animal under the protocol were submitted. No evidence of toxicity was observed and there were no deaths. Measurements of weight, observations of clinical signs, feed consumption, diabetes or hepatotoxicity did not show any changes from normal.

**Reviewer Comment**: There were no ophthalmoscopy or ECG parameters evaluated for this study.

**Hematology and Clinical Chemistry**: measured values did not show any aberrations.

**Histopathology**: reports show all organs examined as normal.
6.2.3 Repeat-Dose Toxicity-Study - DPH-71-1

Reviewer Comment: This study was jointly reviewed by Drs. Dubravka Kufrin and Anne M. Pilaro.

Study title: Comparative Toxicity of I.V. administration of *Erwinia carotovora* L-asparaginase and *E.coli* L-asparaginase to Rhesus Monkeys

Study no.: DPH-71-1

Study report location: electronic BLA submission, Module 4, Toxicology, Repeat-Dose Studies, Section 4.2.3.2

Conducting laboratory and location: [Redacted]

Date of study report: July 29, 1970

GLP compliance: Not in compliance with GLP (precedes GLP regulations)

QA statement: No

Drug, lot #, and % purity: L-asparaginase from *Erwinia carotovora*, MRE, Lot 24/4/69, NCS 106877; and L-asparaginase from *E. coli*, Merck, Sharp & Dohme, C-7941 Lot No, 0965-HLS-2-037, NSC 109229

Key Study Findings

Rhesus monkeys treated IV daily for five days with L-asparaginase from *Er. carotovora* (10,000, 2,000 or 1,000 IU/kg/day), or L-asparaginase from *E. coli* (1,000 IU/kg/day) showed body weight losses, decreased food consumption, anemia, transient decreases in white blood cell counts, and increases in fatty liver and impairment of BSP clearance, when compared to control animals treated IV daily for 5 days with an equal volume of saline. The hepatic findings occurred more frequently and at higher severity in the monkeys treated with L-asparaginase derived from *E. coli*, as compared to those animals treated with the enzyme derived from *Er. carotovora*.

Methods

Doses: 10,000 (high dose), 2,000 (mid dose), or 1,000 (low dose) IU/kg/day of *Erwinia*-derived asparaginase; 1000 IU/kg/day of *E. coli* derived asparaginase, or saline (vehicle) control

Frequency of dosing: Once daily X 5 days

Route of administration: IV

Dose volume: 1.25 ml/kg (high dose), 1 ml/kg (mid-dose), 0.5 ml/kg (low dose)

Formulation/Vehicle: L-asparaginase in 0.9% sodium chloride/0.9% sodium chloride

Species/Strain: Rhesus monkey
1 male monkey/high dose; 1 female/mid-dose, 3 females/low dose given Erwinia L-asparaginase; 3 monkeys (2 male, 1 female)/low dose given E. coli L-asparaginase, 2 male monkeys/saline control

Age: Not specified
Weight: F: 3.9-4.58 g; M: 4.41-5.21 g
Satellite groups: None
Unique study design: Group treated with E. coli L-asparaginase as comparator

Glucose tolerance testing: blood samples taken from all 3 animals in the low dose Erwinia L-asparaginase group and the one high dose monkey at baseline and within one hour after L-asparaginase dosing (both listed as time 0), and at 0.5, 1, 2, and 3 hours after IV glucose loading with 1 gm/kg glucose on Study Days 0, 2 and 5

Bromosulphthalein (BSP) clearance was measured for all animals on Study Days 3 and 5 after L-asparaginase dosing and on Study Day 8 (recovery day 3), as an indication of hepatic function

Oil red O staining of liver sections to evaluate fatty liver development was performed.

Hepatic lipid profiles were performed on extracts of frozen liver samples obtained at necropsy from all low-dose animals treated with either isolate of L-asparaginase, and from one saline control monkey.

Deviation from study protocol: Not in compliance with GLP (precedes GLP regulations).

Blood samples for toxicokinetic evaluation of plasma/serum enzyme levels were obtained from the one animal in the 2,000 IU/kg/day dose group, but were apparently not evaluated.

Observations and Results

Mortality: None.

Body Weights and Feed Consumption

Significant body weight losses occurred in 1/2 control monkeys (maximal weight loss, 14.2% from baseline), 1/3 of the low dose Erwinia-isolated and 3/3 E. coli-derived
asparaginase treated monkeys (maximal weight loss 8.3%, and 13.5%, 11.6%, and 18.1% from baseline, respectively), and the one high dose *Er. carotovora* asparaginase-treated monkey (maximum weight loss, 6.6% from baseline), and were associated with qualitative decreases in food consumption.

**Reviewer Comment:** There were no observations of ophthalmoscopy or ECG performed for this study.

**Hematology and Clinical Chemistry**

Anemia was observed in the majority of the animals treated with either L-asparaginase enzyme, and was attributed to the repeated bleedings for test purposes. A 60% decrease in total WBC count as compared to baseline was noted for the high dose *Erwinia* L-asparaginase treated animal on Study Day 5, which remained approximately 50% of baseline at Study Day 8. Approximate 30% decreases in WBC counts from baseline were noted for the mid-dose animal at these same two time points. There were no consistent changes for the animals dosed with 1,000 IU/kg/day *Erwinia* L-asparaginase. An approximate 50% decrease in total WBC was also noted at Study Days 5 and 8 in 1/3 monkeys (animal #268M) dosed with 1,000 IU/kg/day *E. coli* L-asparaginase.

No remarkable changes were seen in the platelet or reticulocyte counts, BUN, prothrombin time, blood glucose, SGOT, SGPT, alkaline phosphatase, total bilirubin, calcium, sodium, potassium, serum proteins, cholesterol, serum triglycerides or serum amylase in any of the dose groups. Sporadic elevations in serum triglycerides and phospholipids, and elevated total hepatic lipids corresponding microscopically to fatty liver were observed in monkeys (see below) in both L-asparaginase dose groups; however, the findings were much more severe in the monkeys treated with the *E. coli*-derived asparaginase. The results of the hepatic lipid profiles are presented in the table below, which was excerpted from the final study report.
TABLE 1  -  Hepatic Lipid Profiles of Monkeys Treated with L-Asparaginase from Erwinia carotovora. L-Asparaginase from E. coli

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Total Lipid</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>261F</td>
<td>71</td>
<td>29</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>271F</td>
<td>61</td>
<td>34</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>274F</td>
<td>62</td>
<td>32</td>
<td>4</td>
<td>20</td>
</tr>
</tbody>
</table>

Monkeys Treated with L-Asparaginase from Erwinia carotovora,
NSC 106977, I.V., 1,000 I.U./kg/day x 5

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Total Lipid</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
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</thead>
<tbody>
<tr>
<td>268F</td>
<td>189</td>
<td>46</td>
<td>80</td>
<td>58</td>
</tr>
<tr>
<td>272M</td>
<td>154</td>
<td>35</td>
<td>54</td>
<td>46</td>
</tr>
<tr>
<td>279F</td>
<td>260</td>
<td>30</td>
<td>152</td>
<td>50</td>
</tr>
</tbody>
</table>

Saline Control - equivalent to 1,000 I.U./kg/day x 5, I.V.

257M  | 62          | 31          | 4             | 20           |

Reviewer Comment: No evaluations of toxicokinetics or anti-asparaginase antibody levels were performed for this study. The study report states that blood samples were obtained from the one monkey treated with 2,000 IU/kg of Erwinia-derived L-asparaginase on Study Days 0, 2 and 5. However, there are no data included in the study report that confirm that these samples were ever analyzed.

Urinalysis

A single instance of transient ketonuria was observed in the one high dose monkey on Study Day 5 after dosing. Transient proteinuria (1+ severity) was also observed on Study Day 5 in this animal and in monkey #261F in the low dose Erwinia-asparaginase treated group. An additional monkey in this same low dose group exhibited a single incidence of glucosuria (reported as 0.1%) on Study Day 5, which the contracting laboratory attributed to the glucose loading for the glucose tolerance test. Proteinuria (1+ severity) was also reported on Study Days -1 and 8 for monkey #268M and on Study Days -4, 5 and 9 for monkey #279F in the group treated with 1,000 IU/kg/day E. coli-derived L-asparaginase, and on Study Day -3 for animal # 257M in the saline control group.

Gross Pathology and Histopathology

There were no remarkable changes observed on gross pathologic evaluation of any of the tissues examined from any of the monkeys treated with Er. carotovora-derived L-asparaginase. Mild fatty infiltration of the liver (2+ severity) was observed microscopically after Oil Red O staining in 1/3 animals treated with 1,000 IU/kg/day Erwinia-obtained L-asparaginase. Fat staining was noted microscopically in the central and midzonal regions of the liver lobules as coarsely granular, intracytoplasmic droplets, and as large fatty globules present in the Kupffer cells. A similar degree of fatty
infiltration was observed in the one animal each treated with 2,000 or 10,000 IU/kg/day. The high dose animal also showed a moderate degree of intracytoplasmic vacuolization of hepatocytes on hematoxylin and eosin staining. An additional animal in the low dose group had fatty staining present only in the Kupffer cells. Both control monkeys also showed diffuse, fine intracytoplasmic vacuolization of the liver cells. All other tissues examined had no remarkable findings.

At necropsy, the livers of all three monkeys dosed with 1,000 IU/kg/day E. coli-isolated L-asparaginase appeared fatty on gross pathologic examination. Histologically, 2/3 animals showed diffuse intracytoplasmic vacuolization in the liver on hematoxylin and eosin staining; the third animal in this dose group had marked (4+ severity) vacuolization present. Fatty infiltration of the liver (4+ severity) was observed microscopically in all three animals in this dose group after Oil Red O staining. There were no remarkable findings in any of the other tissues examined.

**Special Evaluations**
**Glucose Tolerance**
Serum glucose levels were slightly elevated 0.5 and 1 hour after glucose loading on Study Days 1, 2 and 5 in the one monkey treated with 10,000 IU/kg/day Erwinia L-asparaginase and at 0.5 hour in the one monkey in the mid-dose group and 2/3 low dose monkeys, but returned to baseline values at the 2 hour time point on each day. Similar increases in serum glucose levels were noted for all 3 monkeys treated with L-asparaginase derived from E. coli and for the saline control animals. These findings were not considered evidence of impaired glucose tolerance or a diabetogenic effect of L-asparaginase treatment.

**BSP Clearance**
BSP clearance was reported as "normal" for both saline control treated monkeys and in the one monkey treated with 10,000 IU/kg/day Erwinia-derived L-asparaginase on Study Days 3 and 5 of dosing, and on Study Day 8 (day 3 of recovery). In the one mid-dose monkey, BSP clearance was reported as normal on Study Day 3 and as "moderate retention" on Study Day 5, and as "slight elevation in BSP clearance" on Study Day 6 and "elevated" on Study Day 8. BSP clearance was reported as "normal" for all 3 monkeys treated with 1,000 IU/kg/day Erwinia-derived L-asparaginase.

BSP clearance was impaired in the monkeys treated with 1,000 IU/kg/day E. coli-derived L-asparaginase. In one monkey, BSP clearance was reported as "normal" on Study Day 3, and as "BSP retention" on Study Days 5 and 8. For the second animal, BSP clearance was reported as "upper limit of normal" on Study Day 3, and as "BSP retention" day 5 and 8 and for the third animal, BSP clearance was reported as "normal" on Study Day 3, "borderline elevation" on Study Day 5, and "BSP retention" on Study Day 8.
Reviewer Comment: Numerical values for the BSP clearance were not included in the study report; therefore, the degree of impairment of BSP clearance in these animals cannot be reliably quantitated.

6.2.4 Repeat-Dose Toxicity-Study [b][4] DPH-69-00

Study title: Toxicity studies on Erwinia carotovora L-asparaginase batch 8/17/69 (NSC 109229) following daily IV administration to rhesus monkeys

Study no.: [b][4] DPH-69-00

Study report location: electronic BLA submission, Module 4, Toxicology, Repeat-Dose Studies, Section 4.2.3.2

Conducting laboratory and location: [b][4]

Date of study initiation: 26 December 1969

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: Erwinia carotovora batch 8/17/69 (L-car); NCS 109229

Key Study Findings- No overt toxic signs or changes in the tested monkeys were observed.

Methods

Doses: 1,000 IU/kg/day

Frequency of dosing: 1xd/5 days

Route of administration: IV

Dose volume: 0.5 ml/kg

Formulation/Vehicle: 2,000 IU/cc in saline/saline

Species/Strain: Rhesus monkey (Macaca mulatta)

Number/Sex/Group: 2 monkeys tested, total

Age: Not specified

Weight: 3.71 lbs

Satellite groups: None

Unique study design: No control animals on the experiment

Deviation from study protocol: Preceded GLP regulations

Observations and Results

Two Rhesus monkeys were intravenously administered 1,000 IU/kg x 5 days of L-car with no overt toxic signs noted, and no changes in food consumption or body weight. In this experiment, fatty liver was not found on microscopic evaluation, and blood results did not show diabetogenic increases in glucose tolerance. BSP clearance, prothrombin
time, clotting time, fibrinogen level, blood sugar, serum proteins, cholesterol, SGOT, SGPT, alkaline phosphatase, BUN, b lipoprotein, creatinine, bilirubin, amylases, lipase, electrolytes, hematology or urinary constituents were considered normal.

6.2.5 Repeat-Dose Toxicity-Study

Study title: The toxicity of L-asparaginase from *Erwinia carotovora* (RSC 106977) in rabbits

Study no.: (b)[(4)]-DPH-72-02
Study report location: electronic BLA submission, Module 4, Toxicology, Repeat-Dose Studies, Section 4.2.3.2
Conducting laboratory and location: 
Date of study initiation: 24 June 1971
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: *Erwinia carotovora* batch 10/9/70; NSC 106977

Key Study Findings

IV administration of *Er. carotovora* L-asparaginase to groups of five rabbits in doses of 5,000 (high), 2,000 (mid) and 1,000 (low dose) as a single injection, or 1,000 IU/kg administered daily five subsequent days resulted in deaths in 5/5 high dose (23-36 hrs post-dose), and 2/5 mid-dose (33-46 hrs post-dose) treated animals. In the animals treated with a single dose of 5,000 IU/kg, antemortem weight loss, muscle weakness, ataxia, muscle tremors and convulsions were noted, and were associated with hypocalcaemia, hyperphosphatemia and an increase in oxyphil cells in the parathyroid glands histologically. Hyperglycemia and glycosuria and in one animal, ketonuria, occurred in rabbits given 1,000 IU/kg/day for 5 days. Reversible increases in BUN, creatinine and SGPT occurred in some of the animals given 2,000 IU or 1,000 IU/kg for a single dose, and in the group given 5 daily injections at the low dose. Decreases in the erythroid elements and a shift to the right in the WBC differential (except in animals dosed with 1,000 IU/kg/day x 5) were observed in some of the animals at all dose levels. Cytoplasmic vacuolization of the hepatic cells, suggestive of fat infiltration, was noted in the majority of the animals treated with a single injection of *Erwinia* L-asparaginase at the mid- and low doses, and was also seen in one of five rabbits treated with low dose for five consecutive days.
Methods

Doses: 5,000 (high dose), 2,000 (mid dose), and 1,000 (low dose) IU/kg/day
Frequency of dosing: Once daily/only; for the low dose, one additional group was given daily injections X 5 days
Route of administration: IV
Dose volume: 0.5 ml/kg (high dose), 0.2 (mid dose), 0.1 ml/kg (low dose)
Formulation/Vehicle: L-asparaginase in 0.9% sodium chloride/ 0.9% sodium chloride
Species/Strain: Rabbits/not specified
Number/Sex/Group: 5/high dose, 5/mid dose, 5/low dose given once, and 5/low dose given five consecutive days
Age: Not specified
Weight: F: 3.02-2.85g; M: 3.61-3.27g
Satellite groups: None
Unique study design: None
Deviation from study protocol: Preceded GLP regulations

Mortality

Intravenous administration of *Enwinia carotovora* L-asparaginase to groups of five rabbits in doses of 5,000 (high), 2,000 (mid) and 1,000 (low dose) as a single injection, or 1,000 IU/kg administered daily five subsequent days resulted in deaths in 5/5 high dose (23-36 hrs post-dose), and 2/5 mid-dose (between 33 and 46 hrs post-dose) treated animals.

Clinical Signs and Body Weights

In the animals treated with 5,000 IU/kg for one day, weight loss, muscle weakness, ataxia, muscle tremors and convulsions were noted, and were associated with hypocalcaemia, hyperphosphatemia and a corresponding increase in oxyphil cells of the parathyroid glands in 5/5 high dosed rabbits on microscopic evaluation. In the surviving animals, there were no remarkable effects of L-asparaginase treatment on body weights over the duration of the study.

Reviewer Comment: Feed consumption, ophthalmoscopy and ECG were not specifically measured in study.

Hematology, Clinical Chemistry and Urinalysis

Hyperglycemia and glycosuria, and in one animal ketonuria occurred in animals given 1,000 IU/kg/day for 5 days. Reversible increases in BUN, creatinine and SGPT occurred in some of the animals given 2,000 IU or 1,000 IU/kg for a single dose, or 1,000
IU/kg/day for 5 daily injections. Decrease in the erythroid elements and a shift to the right in the WBC differential (except in animals dosed with 1,000 IU/kg/day x 5) were observed in some of the animals at all dose levels.

**Gross Pathology**

The cerebrum of one female animal given the high dose had vessels that appeared slightly congested, with signs of meningo-encephalitis observed histopathologically. One female rabbit given the high dose had severe pulmonary congestion and edema.

**Organ Weights** - measures were not noted.

**Histopathology**

Adequate Battery - Yes.

Peer Review - Not disclosed in the study.

Histological Findings: Calcification of epithelium of proximal tubules and calcium in the lumen of kidney tubules were observed in 3/5 high dosed rabbits, and 3+ increase in oxyphil cells were present in the parathyroid of 5/5 high dosed rabbits. Four mid-dose treated rabbits had 2+ vacuolization of liver cells; two mid-dose male rabbits showed tuberculoid granulomatous meningo-encephalitis in cerebrum and cerebellum, with meningo-encephalitis in midbrain. All findings were reported by the contract laboratory as attributable to the drug.

One male rabbit given 5 daily doses of 1,000 IU/kg/day L-asparaginase had mild intracytoplasmic vacuolization of liver cells, occasional eosinophils in the sinusoids of the liver, and 1+ hypoplasia of the bone marrow. The same animal had lipemic plasma from day 8 on to the end of the study (Day 31). Cytoplasmic vacuolization of the hepatic cells, suggestive of fat infiltration, was noted in the majority of the animals treated with the mid- and low doses, and the same finding was seen in one of five rabbits treated with 1,000 IU/kg/dose for five consecutive days.

**Special Evaluation** - None.

**Reviewer Comment:** Toxicokinetics, and stability and homogeneity of the test article were not specifically evaluated for this study.
7  Genetic Toxicology

No genetic toxicology studies have been conducted, as per ICH S6 Preclinical Safety Evaluations of Biotechnology-Derived Pharmaceuticals.

8  Carcinogenicity

No carcinogenicity studies have been conducted as per ICH S6 Preclinical Safety Evaluations of Biotechnology-Derived Pharmaceuticals.

9  Reproductive and Developmental Toxicology

No reproductive or developmental toxicology studies have been submitted. See reviewer comment above, for description of postmarketing requirements.

11  Integrated Summary and Safety Evaluation

Asparaginases have been used for the treatment of Acute Lymphoblastic Leukemia (ALL) since the 1970’s. Given the characterization of the clinical safety of these enzymes, the Agency did not require additional nonclinical studies to support the submission of the BLA. Thus, the nonclinical program submitted consists of a review of previously conducted toxicology studies on Erwinia sp. asparaginases, generally from studies conducted in the 1960’s and 1970’s, combined with a review of the open literature on Erwinia sp. asparaginase pharmacology and activity studies.

The nonclinical studies conducted on Erwinia sp. asparaginases predated GLP regulations, are not GLP compliant, and at this stage have been used only for evaluation of the initial clinical application of Erwinaze™. Together with the known clinical safety record, the nonclinical studies submitted are adequate to conclude that Erwinaze™ is reasonably safe for use in ALL patients who are hypersensitive to asparaginases isolated from E. coli, or pegylated asparaginase products.

12  Appendix/Attachments

Appendix 1 (Table 4.) shows the results of the Asparaginase pyrogen test in rabbits (Study # 031-665-6881).
### TABLE 4  PYROGEN TEST REPORT

<table>
<thead>
<tr>
<th>Preparation: Asparaginase</th>
<th>Batch or Sample Number</th>
<th>Sub batch B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogen Test Number: 1</td>
<td>Date of Test 7.10.71</td>
<td>Operator:</td>
</tr>
<tr>
<td>Dilution: 2 ampoules dissolved in 3 ml saline</td>
<td>Dose Administered: 1 ml/animal</td>
<td></td>
</tr>
</tbody>
</table>

**Mode of administration, IV through marginal ear vein**

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Number</th>
<th>5236</th>
<th>5237</th>
<th>5239</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>2.90</td>
<td>1.90</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td>Lead No.</td>
<td>1.00</td>
<td>2.00</td>
<td>4.00</td>
<td></td>
</tr>
</tbody>
</table>

| Date of last pyrogen test: | 7.10.71 | 7.10.71 | 7.10.71 |
| Date of trial test: | 5.10.71 | 5.10.71 | 5.10.71 |

**Maximum rise or fall in trial tests:**

| + | 0.10 | + | 0.05 | + | 0.04 |

| Have all preparations tested in the last three weeks passed | YES | YES | YES |

<table>
<thead>
<tr>
<th>Lead Temperatures</th>
<th>Contact Period</th>
<th>After Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>(min.)</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>180</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Injectable</th>
<th>38.90</th>
<th>38.70</th>
<th>38.70</th>
<th>38.70</th>
<th>38.90</th>
<th>38.90</th>
<th>38.90</th>
</tr>
</thead>
<tbody>
<tr>
<td>(min.)</td>
<td>38.70</td>
<td>38.85</td>
<td>38.85</td>
<td>38.85</td>
<td>38.90</td>
<td>38.90</td>
<td>38.90</td>
</tr>
</tbody>
</table>

| Maximum     | 39.05 | 39.00 | 39.00 | 39.00 |
| Mean Initial| 38.86 | 39.00 | 39.53 |

| Response    | +0.19 | +0.20 | +0.17 |

| Summed response in present test | 0.56 |

<table>
<thead>
<tr>
<th>Result of Test</th>
<th>PASS</th>
<th>FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASS</td>
<td>3</td>
<td>&gt;1.15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&gt;2.65</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>&gt;4.35</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>&gt;6.60</td>
</tr>
</tbody>
</table>

| Worked: | (0)(4) | Worked: | (0)(4) |

31
Table 3, below, shows the results of the analytical testing performed on the Drug Product Batches.

**Table 3: Testing Performed on Drug Product Batches CAMR 138 and CAMR 144**
- According to the Requirements of Section 3.2.P.5.1

<table>
<thead>
<tr>
<th>Test</th>
<th>Specification</th>
<th>CAMR 138</th>
<th>CAMR 144</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appearance</strong></td>
<td>Solid, white cake; USP &lt;631&gt;</td>
<td>CONFORMS*</td>
<td>CONFORMS</td>
</tr>
<tr>
<td><strong>Particulate Matter</strong></td>
<td>≤ 6,000 particles/vial ≥10 μm ≤ 600 particles/vial ≥25 μm: USP &lt;788&gt;</td>
<td>Test not available</td>
<td>≥10 μm; 355/vial ≥25 μm; 7/vial</td>
</tr>
<tr>
<td><strong>Content Uniformity</strong></td>
<td>± 15% of label; USP &lt;905&gt;</td>
<td>Test not available</td>
<td>7.9%</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>6.8 ± 0.4; USP &lt;791&gt;</td>
<td>Test not available</td>
<td>6.8</td>
</tr>
<tr>
<td><strong>Protein Concentration (A₂₈₀)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SEC-HPLC</strong></td>
<td>Monomer: ≥90.0%</td>
<td>95.0*</td>
<td>96.7</td>
</tr>
<tr>
<td></td>
<td>Dimer: ≤8.0%</td>
<td>4.5*</td>
<td>None Reported</td>
</tr>
<tr>
<td><strong>RP-HPLC</strong></td>
<td>Main Peak ≥84.0%</td>
<td>91.0*</td>
<td>94.2</td>
</tr>
<tr>
<td><strong>IEX-HPLC</strong></td>
<td>Main Peak ≥67.0%</td>
<td>79.2*</td>
<td>86.4</td>
</tr>
<tr>
<td><strong>SDS-PAGE</strong></td>
<td>Comparable to ERS</td>
<td>Comparable to ERS*#</td>
<td>Comparable to ERS</td>
</tr>
<tr>
<td><strong>Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Specific Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Enzyme Kinetics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glucose Content</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sodium Chloride Content</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Endotoxin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sterility</strong></td>
<td>Complies with USP &lt;71&gt;</td>
<td>Pass</td>
<td>Sterile/Pass</td>
</tr>
</tbody>
</table>

Notes:
* - For all tests presented in Table 3 CAMR 144 testing was performed at release. For CAMR 138, the new methods were not yet qualified at the time of batch release. The new methods were qualified at the 12 month stability time point, except K<sub>0</sub>, where the earliest data set is at 24 months (see also Report Q07-10-507). All data obtained from material other than at release is marked with an asterisk.
# - SDS-PAGE run as an identity test only, prior to development of the method as a limit test for impurities.
Table 1, below, show the analytical data for the historical reference standards of *Erwinia* L-asparaginase

<table>
<thead>
<tr>
<th>Test</th>
<th>Specification</th>
<th>128</th>
<th>138</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Containers should be of uniform appearance with no fractures. Contents should be a white pellet with no foreign matter. Crimping should be sound and uniform.</td>
<td>PASS</td>
<td>PASS</td>
</tr>
<tr>
<td>Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinetic Assay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particulate Matter</td>
<td>Conforms to Ph. Eur. 2.9.19 using Light Obscuration Particle Count Test.</td>
<td>PASS</td>
<td>PASS</td>
</tr>
<tr>
<td>Content Uniformity</td>
<td>85%-115% from target volume</td>
<td>PASS</td>
<td>PASS</td>
</tr>
<tr>
<td>Reconstitution</td>
<td>Reconstitution with 2 mL of 0.9% NaCl injection BP; dissolution should be complete within 2 min to give a clear solution</td>
<td>PASS</td>
<td>PASS</td>
</tr>
<tr>
<td>pH</td>
<td>pH 6.0 to pH 7.5 as per Ph. Eur. 2.2.3</td>
<td>7.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Glucose Content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Chloride Content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endotoxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogenicity</td>
<td>Must not exceed the level approved by the Eur. Ph. &lt;2.6.1&gt; with a test dose of 2,000 U/kg</td>
<td>PASS</td>
<td>PASS</td>
</tr>
<tr>
<td>Sterility</td>
<td>Complies with the requirements of the Eur. Ph. &lt;2.6.1&gt; with a test dose of 2,000 U/kg</td>
<td>PASS</td>
<td>PASS</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>Should be greater than 95% by cellulose acetate membrane electrophoresis (CAME) on Cellgel at pH 10.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PAGE and CAME</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal Toxicity</td>
<td>Complies with requirement of the Eur. Ph. &lt;2.6.1&gt; dose (general test): 2,000 U/mouse</td>
<td>PASS</td>
<td>PASS</td>
</tr>
<tr>
<td>Identity (Ouchterlony)</td>
<td>Immunologically identical to ERS</td>
<td>PASS</td>
<td>PASS</td>
</tr>
<tr>
<td>Leak Test</td>
<td>No evidence of any leakage into vial. Freeze-dried cake remains intact</td>
<td>PASS</td>
<td>PASS</td>
</tr>
</tbody>
</table>
PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement

STN BLA Number: 125359/0  Applicant: EUSA Pharma (USA), Stamp Date: Sept 8, 2010
(IND 290)  Inc.
Drug Name: Erwinaze  BLA Type: C

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

<table>
<thead>
<tr>
<th>Content Parameter</th>
<th>Yes</th>
<th>No</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?</td>
<td></td>
<td>X</td>
<td>Study reports in this section are scanned, PDF copies of original typewritten reports from the 1970s, and are challenging to read in electronic format. The sponsor will be requested to provide legible copies (e.g., paper copy) for review.</td>
</tr>
<tr>
<td>2 Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?</td>
<td></td>
<td>X</td>
<td>All required pharm/tox studies are submitted, with the exception of teratogenicity, effects on fertility and perinatal development and reproductive toxicity (DART). The sponsor has been informed that the full range of DART studies are needed for the BLA review process, and that it will be acceptable to conduct these studies as post-marketing requirements. Carcinogenicity and mutagenicity studies have not been requested as per the ICH S9 and ICH S6 guidelines.</td>
</tr>
<tr>
<td>3 Is the pharmacology/toxicology section legible so that substantive review can begin?</td>
<td></td>
<td>X</td>
<td>Study reports in this section are scanned, PDF copies of original typewritten reports from the 1970s, and are challenging to read in electronic format. The sponsor will be requested to provide legible copies (e.g., paper copy) for review.</td>
</tr>
<tr>
<td>4 Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?</td>
<td></td>
<td>X</td>
<td>All required pharm/tox studies are submitted, with the exception of teratogenicity, effects on fertility and perinatal development and reproductive toxicity (DART). The sponsor has been informed that the full range of DART studies are needed for the BLA review process, and that it will be acceptable to conduct these studies as post-marketing requirements. Carcinogenicity and mutagenicity studies have not been requested as per the ICH S9 and ICH S6 guidelines.</td>
</tr>
<tr>
<td>5 If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).</td>
<td></td>
<td>X</td>
<td>See item 6, below.</td>
</tr>
<tr>
<td>6 Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?</td>
<td></td>
<td>X</td>
<td>Submitted single and repeat dose studies were done by IP or IV route of administration and none of the studies were done by IM route that is proposed by the sponsor. Based on clinical IM route.</td>
</tr>
<tr>
<td>Content Parameter</td>
<td>Yes</td>
<td>No</td>
<td>Comment</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>7 Has the applicant submitted a statement(s) that all of the pivotal pharm/tox</td>
<td></td>
<td>X</td>
<td>administration of Erwinaze outside of USA, additional animal studies done by IM route were not needed.</td>
</tr>
<tr>
<td>studies have been performed in accordance with the GLP regulations (21 CFR 58)</td>
<td></td>
<td></td>
<td>*The pharmacology/toxicology part of the drug development predated GLP regulations; therefore, none of pharm/tox studies submitted are GLP compliant.</td>
</tr>
<tr>
<td>or an explanation for any significant deviations?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8 Has the applicant submitted all special Studies/data requested by the Division</td>
<td></td>
<td></td>
<td>Not applicable (NA)</td>
</tr>
<tr>
<td>during pre-submission discussions?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>9 Are the proposed labeling sections relative to pharmacology/toxicology</td>
<td></td>
<td>X</td>
<td>Data from DART studies are required to address sections 8.1 and 13.1 of the label.</td>
</tr>
<tr>
<td>appropriate (including human dose multiples expressed in either mg/m2 or</td>
<td></td>
<td></td>
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<td>comparative serum/plasma levels) and in accordance with 201.57?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10 Have any impurity – etc. issues been addressed? (New toxicity studies may</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>not be needed.)</td>
<td></td>
<td></td>
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<tr>
<td>11 Has the applicant addressed any abuse potential issues in the submission?</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>12 If this NDA/BLA is to support an Rx to OTC switch, have all relevant studies</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>submitted?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE?** Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

No RTF issues.

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

1. The electronic scanned copies of the nonclinical study reports (Study numbers [b][d] DPH-71-02 (first page only), [b][d] DPH-69-00, [b][d] DPH-71-01) are not legible. Please provide paper copies of these study reports, and ensure that all critical information

File name: 5_Pharacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908
PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement

including dosing, clinical and histopathology findings and any available toxicokinetic
data are clearly presented and legible, to facilitate review.

2. FDA will consider the conduct of the developmental and reproductive toxicity studies a
postmarketing requirement for an eventual approval of Erwinaze. In your resubmission,
please provide your plans and a timeline for a conduct of these studies as a formal
postmarketing requirement.

Dubravka Kufrin, Ph.D.  Date
Reviewing Pharmacologist

Anne M. Pilaro, Ph.D.  Date
Team Leader/Supervisor