

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125359Orig1s000

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

Clinical Pharmacology Review-Addendum

BLA: 125359	Amendment Submission Date: 9/15/2011 BLA Submission Date: 9/8/2010 and 2/2/2011
Brand Name	Erwinaze®
Generic Name	Erwinia L-asparaginase
Submission Type; Code	NME
Formulation; Strength(s)	Supplied as a sterile, lyophilized powder in vials, 10,000 International Units/vial
Proposed Indication	As a component of a multi-agent chemotherapeutic regimen for the treatment of (b) (4) patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to <i>E. coli</i> derived asparaginase
Dosing Regimen	25,000 International Units/m ² administered intramuscularly three times a week (Monday/Wednesday/Friday) for six doses to replace each dose of pegaspargase
Relevant IND(s)	BB-IND 290
PDUFA Date	8/2/2011
Sponsor	EUSA
Clinical Division	Division of Oncology Products 2 (DOP2)
OCP Division	Clinical Pharmacology V (DCP5)
Primary Reviewer	Jun Yang, Ph.D.
Team Leader	Hong Zhao, Ph.D.

OVERALL SUMMARY

The original BLA submission contains a clinical pharmacokinetic (PK) and pharmacodynamic (PD) study report (COG AALL07P2) in support of the proposed use of Erwinaze in patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to (b) (4) *E.Coli* derived asparaginase. Study COG AALL07P2 was a single-arm, multi-center, open-label, safety and clinical pharmacology trial conducted in 59 patients treated under the front line cooperative group ALL protocols who were unable to continue to receive pegaspargase due to hypersensitivity reactions. Patients received Erwinaze 25,000 International Units (IU)/m² x 6 doses intramuscularly on a Monday, Wednesday, and Friday schedule as a replacement for each scheduled dose of pegaspargase remaining on their original treatment protocol. The primary clinical endpoint was trough serum asparaginase activity. The objective of the treatment is to obtain asparaginase trough activity of ≥ 0.1 IU/mL.

The clinical samples were analyzed by an academic laboratory and FDA's data verification inspection identified major issues/deficiencies with the sample handling and assay performance, rendering the analytical results for both the serum asparaginase activity and plasma asparagine concentrations unreliable (see original BLA review, dated 25 May 2011). Subsequently, the FDA agreed with the Sponsor's proposal to use the frozen immunogenicity samples collected at pre-dose 4 in Course 1 to test the trough level of asparaginase activity in a CRO facility using a validated assay. On September 15, 2011, EUSA submitted the asparaginase activity results together with the assay validation report. The tested patient samples at 48 hour (n=35) and at 72 hour (n=13) on Day 8 (pre-Dose 4) in Course 1 of treatment all (100 %) met the pre-specified criterion of ≥ 0.1 IU/mL trough level asparaginase activity.

RECOMMENDATION

From a Clinical Pharmacology perspective, the asparaginase activity results from patients in the clinical trial meet the prespecified clinical endpoint and support the approval of this application.

Signatures:

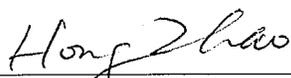


10/21/2011

Jun Yang, Ph.D.

Clinical Pharmacology Reviewer, Biologic Product Team

Division of Clinical Pharmacology V

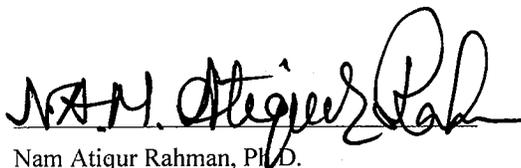


10/21/2011

Hong Zhao, Ph.D.

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10/24/2011

Nam Atiqur Rahman, Ph.D.

Division Director, Division of Clinical Pharmacology V

CLINICAL PHARMACOLOGY FINDINGS

The clinical portion of the rolling BLA 125,359 was initially submitted on 8 September 2010 and the CMC portion was submitted on 1 November 2010. The original submission contains a clinical pharmacokinetic (PK) and pharmacodynamic (PD) study report (COG AALL07P2) in support of the proposed use of Erwinaze in patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to (b) (4) *E. Coli* derived asparaginase. Study COG AALL07P2 was a single-arm, multi-center, open-label, safety and clinical pharmacology trial conducted in 59 patients treated under the front line cooperative group ALL protocols who were unable to continue to receive pegaspargase due to hypersensitivity reactions. Patients received Erwinaze 25,000 IU/m² x 6 doses intramuscularly on a Monday, Wednesday, and Friday schedule as a replacement for each scheduled dose of pegaspargase remaining on their original treatment protocol. The primary and secondary clinical endpoints were trough serum asparaginase activity and plasma asparagine concentration.

The clinical samples were analyzed by an academic laboratory at the (b) (4). (b) (4) DSI audited the laboratory during (b) (4). FDA's data verification inspection identified major issues/deficiencies with the sample handling and assay performance of the serum asparaginase (See detail in Appendices 1). At a face to face meeting between the FDA and EUSA held on June 23, 2011, FDA informed the applicant that the asparaginase activity results would not be accepted to support approval because of the inspection findings. However, FDA did not issue a CR letter on the PDUFA day (August 2, 2011); instead, FDA agreed with the Sponsor's proposal to use the frozen immunogenicity samples stored for analysis to determine the trough level of asparaginase activity by a Contract Research Organization, (b) (4). The objective would be to assess the primary clinical endpoint, the trough asparaginase activity levels ≥ 0.1 IU/mL.

Per agreement between the FDA and EUSA, the immunogenicity samples collected at 48 hours (Dosing started on Wednesday or Friday) and at 72 hours (dosing started on Monday) pre-dose 4 on Day 8 in Course 1 will be tested for asparaginase enzyme activity by (b) (4). As part of the AALL07P2 study, the remaining immunogenicity samples collected during Course 1 and all other Courses will be analyzed once the anti-product antibody assays are validated. According to the sponsor, these samples were stored at (b) (4) 1 (b) (4)

Asparaginase activity was determined in serum samples from 35 patients with 48 hour trough samples and 13 patients with 72 hour trough samples on Day 8 (pre-Dose 4) in Course 1 of treatment (See Appendices 3). Of this population, the results of all patient samples tested met the criterion of ≥ 0.1 IU/mL trough level of asparaginase activity. Since a trough asparaginase activity ≥ 0.4 IU/mL has been also used for another asparaginase product activity assessment, the proportion of patients having a trough asparaginase activity ≥ 0.1 IU/mL and ≥ 0.4 IU/mL are shown below.

Trough Time	≥ 0.1 IU/mL	≥ 0.4 IU/mL
48 hours (n=35)	35/35 (100 %)	28/35 (80 %)
72 hours (n=13)	13/13 (100 %)	5/13 (39 %)

The FDA Division of Bioequivalence and GLP Compliance (DBGC) audited bioanalytical portions of the study conducted at (b) (4) on (b) (4). According to the DBGC report (Appendices 4), there were no objectionable findings, and no Form FDA 483 was issued.

CONCLUSION

The tested patient trough asparaginase samples (N=48) at pre-dose 4 in Course 1, both the 48-hour samples and 72-hour samples, all meet the prespecified clinical endpoint (≥ 0.1 IU/mL).

ASPARAGINASE ASSAY VALIDATION REPORT

For the assay validation, *E. coli* asparaginase was used as the standard and serum was used as the sample matrix. The validation protocol was submitted to the FDA on August 04, 2011. The FDA has reviewed this protocol and accepted it with the caveat that it be expanded to include robustness (See Appendices 2). The principle of this enzymatic assay is that aspartic acid formed from deamination of asparagine via asparaginase catalysis reacts with α -ketoglutaric acid, yielding oxaloacetic acid and glutamic acid, which oxidizes reduced β -nicotinamide adenine dinucleotide in the presence of malic dehydrogenase, resulting in a decrease in absorbance at 340 nm. The upper and lower limits of quantitation (ULOQ and LLOQ) were determined to be 12.9 mIU/mL and 150 mIU/mL, respectively. The limit of quantitation (LOQ) was determined to be 6.4 mIU/mL.

Plate Uniformity

To assess the consistency of results across all positions on a plate, serum spiked with *E. coli* L-Asparaginase at 75 mIU/mL was assayed in all 96 wells of a plate. The average, standard deviation, and relative standard deviation (%RSD) of V_{max} was calculated for all wells on the plate meeting system suitability (the overall average V_{max} , "OAV"), and compared to the averages of each row, column, and section of the plate as detailed in the Validation Protocol. The results are summarized in Table 1.

Table 1. Summary of Validation Acceptance Criteria and Results for Plate Uniformity

Validation Parameter	Acceptance Criterion	Validation Results	Conclusion
Number of valid wells per plate	91/96 (>95%)	94/96 (98%)	Passed
Overall average V_{max} (OAV)	N/A	-9.1 (mAU/min)	N/A
Average V_{max} for each well	Difference from OAV \leq 15% for > 95% of valid wells	Difference from OAV \leq 15% for 92/94 wells (98%)	Passed
Average V_{max} for each row	Difference from OAV \leq 20%	Difference from OAV \leq 15% for all 8 rows	Passed
Average V_{max} for each column	Difference from OAV \leq 20%	Difference from OAV \leq 15% for all 12 columns	Passed
Average V_{max} for each section	Difference from OAV \leq 20%	Difference from OAV \leq 20% for all 6 sections	Passed

Selectivity in Human Serum

Twelve individual, independent blank serum samples were spiked with *E. coli* L-Asparaginase or Erwinaze at 37.5 mIU/mL and analyzed in duplicate together with the matched paired 'unspiked' blank matrix samples (i.e. the same serum sample without spike). All 12 of the 'unspiked' serum samples had activities below the LLOQ (12.9 mIU/mL). All of the samples spiked with Erwinaze met all assay criteria, and acceptable recovery was obtained (ranged from 83.8 to 103.8 %).

Dilutional Linearity

To evaluate the dilutional linearity of the assay, samples were prepared in normal serum spiked with *E. coli* L-Asparaginase or Erwinaze at 1000 mIU/mL. These samples were diluted 2-fold down to 256-fold (3.9 mIU/mL), and the serial dilutions assayed in duplicate. The dilutional

linearity of *E. coli* L-Asparaginase and Erwinaze was assessed by comparing the experimentally determined concentrations to their respective nominal values (%RE). The criteria are that the dilution-corrected back-calculated analyte concentration for samples > 2*LLOQ should be within $\pm 20\%$ of nominal (1000 mIU/mL), and for samples < 2*LLOQ, the concentration should be within $\pm 25\%$ of nominal. For *E. coli* L-Asparaginase, acceptable dilutional linearity was observed from 250 mIU/mL to 15.6 mIU/mL, equivalent to a 64-fold dilution of the starting material, and for Erwinaze the acceptable range was from 250 mIU/mL to 3.9 mIU/mL, equivalent to a 256-fold dilution of the starting material.

Intra-assay and Inter-assay Precision and Accuracy of Standard Curves

For assessing the intra-assay precision and accuracy, eight replicate sets of standards were included on a single plate. For assessing the inter-assay precision and accuracy, duplicate sets of standards were included in a six separate plates which were assayed on different days. The precision of the curves was evaluated by determining the %RSD of the Vmax values and the back-calculated enzyme activity at each concentration of standard (1.6 to 150 mIU/mL), and the accuracy was evaluated by calculating the corresponding relative errors (%RE). The results are summarized in Table 2.

Table 2. Intra-assay and Inter-assay Precision and Accuracy of Standard Curves.

		Concentrations	Precision or Accuracy	Acceptance Criterion
Intra-assay	Precision of Vmax (%RSD)	LLOQ (12.86 mIU/mL)	4.4	$\leq 20\%$
		Above LLOQ	≤ 12.4	$\leq 15\%$
	Precision (%RSD)	LLOQ (12.86 mIU/mL)	10.0	$\leq 25\%$
		Above LLOQ	≤ 12.0	$\leq 20\%$
	Accuracy (%RE)	LLOQ (12.86 mIU/mL)	2.0	$\leq 30\%$
		Above LLOQ	± 4.2	$\leq 25\%$
Inter-assay	Precision of Vmax (%RSD)	LLOQ (12.86 mIU/mL)	6.8	$\leq 20\%$
		Above LLOQ	≤ 5.5	$\leq 15\%$
	Precision (%RSD)	LLOQ (12.86 mIU/mL)	3.4	$\leq 25\%$
		Above LLOQ	≤ 3.4	$\leq 20\%$
	Accuracy (%RE)	LLOQ (12.86 mIU/mL)	1.3	$\leq 30\%$
		Above LLOQ	-1.8 to 1.5	$\leq 25\%$

Intra-assay and Inter-assay Precision and Accuracy of QC Samples

Intra-assay precision was evaluated by performing an assay with six replicates of the QC samples (37.5, 75, and 115 mIU/mL) on a single plate (n=6). Inter-assay precision was evaluated by including duplicate sets of QC samples on a six separate plates (n=6) which were assayed on different days. The results of precision (%RSD) and accuracy (%RE) are summarized in Table 3.

Table 3. Intra-assay and Inter-assay Precision and Accuracy of QC Samples

		QC Samples	115 mIU/mL	75 mIU/mL	37.5 mIU/mL	Acceptance Criterion
Intra- assay	Precision of Vmax	E. coli Asparaginase %RSD	4.26	3.37	3.78	≤ 15%
		Erwinaze %RSD	2.36	1.53	4.83	≤ 15%
	Precision of QC samples	E. coli Asparaginase %RSD	4.99	4.05	4.88	≤ 20%
		Erwinaze %RSD	2.79	1.85	6.35	≤ 20%
	Precision of QC samples	E. coli Asparaginase %RE	-7.08	-6.26	-6.39	≤ 25%
		Erwinaze %RE	-11.07	-9.04	-11.89	≤ 25%
Inter- assay	Precision of Vmax	E. coli Asparaginase %RSD	3.90	4.93	7.88	≤ 15%
		Erwinaze %RSD	1.53	4.17	5.11	≤ 15%
	Precision of QC samples	E. coli Asparaginase %RSD	4.33	4.42	15.15	≤ 20%
		Erwinaze %RSD	2.77	3.57	5.51	≤ 20%
	Precision of QC samples	E. coli Asparaginase %RE	-4.70	-4.18	-1.75	≤ 25%
		Erwinaze %RE	-11.34	-10.45	-10.53	≤ 25%

Stability Test

Short Term "Bench" Stability of Critical Reagents: The critical reagents in this study are the *E. coli* standard, the Erwinia asparaginase quality control (QC) solutions (37.5, 75 and 115 mIU/mL), and the assay enzyme mixture. The 'bench' condition for the standards and QC solutions is room temperature (~ 22°C) and the 'bench' condition for the enzyme mixture is 37°C. Each reagent was kept at their bench condition for 6 hrs to bracket the longest potential time for use in the assay. After the 6 hr incubation, an assay was performed using the 'bench' held reagents alongside the same critical reagents prepared freshly at the time of test. The short term stability was evaluated by comparing the experimentally determined concentrations of the QC to their respective nominal values (%RE) as shown in Table 4.

Table 4. Short Term Stability of Critical Reagents Under "Bench" Conditions

Stds&QC	Enz mix	nominal: Asnase	115 mIU/mL		75 mIU/mL		37.5 mIU/mL	
			meas.	%RE	meas.	%RE	meas.	%RE
fresh	fresh	E. coli	118	2.5	73.1	-2.8	33.6	-10.5
		Erwin	111	-3.9	80.7	7.7	43.4	15.6
fresh	bench	E. coli	123	6.7	75.6	0.8	33.3	-11.2
		Erwin	121	5.5	91.0	21.4	46.0	22.6
bench	fresh	E. coli	107	-6.9	73.1	-2.5	31.4	-16.4
		Erwin	101	-11.9	65.7	-12.3	31.2	-16.9
bench	bench	E. coli	115	0.3	73.5	-2.1	33.5	-10.8
		Erwin	106	-7.4	76.0	1.3	33.3	-11.3
Criterion: % RE < 25%			Conclusion: All Passed					

* average of 2 wells (the other 2 replicate wells failed system suitability criteria for r and duplicates so were invalid)

Short Term Stability of L-Asparaginase and Erwinaze in Human Serum: The short term stability of *E. coli* L-Asparaginase and Erwinaze in human serum was evaluated by preparing samples of each enzyme at high (115 mIU/mL), mid (75mIU/mL), and low (37.5 mIU/mL) QC

concentrations. The samples were then kept at 2-8 °C for 24 hr, then four replicates of each sample were assayed along with freshly prepared standards and QC samples. The results are summarized in Table 5.

Table 5. Short Term Stability of L-Asparaginase and Erwinaze (24 hr at 2-8 °C)

	115 mIU/mL	75 mIU/mL	37.5 mIU/mL	Acceptance Criterion
E. coli Asparaginase %RE	-11.9	-9.07	-10.65	≤ 25%
Erwinaze %RE	-13.47	-12.44	-12.31	≤ 25%

Freeze-Thaw Stability of L-Asparaginase and Erwinaze in Human Serum: The freeze-thaw (FT) stability of *E. coli* L-Asparaginase and Erwinaze in human serum was evaluated by preparing two sets of samples of each enzyme (in serum) at the high (115 mIU/mL), mid (75 mIU/mL), and low (37.5 mIU/mL) QC concentrations. The samples were then subjected to either 1, 2, or 3 cycles of freezing and thawing. A freeze-thaw cycle consisted of placing the samples nominally at ~ -65°C overnight, and thawing them at room temperature for 3 hours prior to refreezing. Samples subjected to freeze and thaw were assayed along with freshly prepared standards and QC samples. The results are summarized in Table 6.

Table 6. Freeze-Thaw Stability of L-Asparaginase and Erwinaze in Human Serum

		115 mIU/mL	75 mIU/mL	37.5 mIU/mL	Acceptance Criterion
E. coli Asparaginase %RE	1 FT	-15.2	-15.5	-10.0	≤ 25%
	2 FT	-17.4	-14.4	-19.5	≤ 25%
	3 FT	-15.6	-9.1	-17.7	≤ 25%
Erwinaze %RE	1 FT	-14.8	-13.8	-12.1	≤ 25%
	2 FT	-18.1	-17.3	-18.5	≤ 25%
	3 FT	-18.3	-14.0	-15.7	≤ 25%

Long-term Stability of Samples: According to the sponsor, experiments will be detailed in a Study Protocol Amendment and appended to the validation report when data are available. According to the DBGC report, 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 (See Appendices 4).

Robustness

As requested by the FDA (refer to "Response to EUSA Pharma information requests" dated 8/16/2011), experiments were done to evaluate the Robustness of the assay. The parameters evaluated included the effects on the assay of varying the pH, temperature, and concentration of reagents.

Effect of pH: To assess the effect of variation of pH on the assay, the enzyme mix was prepared at pH 8.2 and 8.7 (the target condition is pH 8.45). A full set of standards and QC samples were assayed with each of the mixtures. The results were evaluated by comparing

the experimentally determined concentrations of the QC's to their respective nominal values (%RE) at pH 8.7, both the *E.coli* and Erwinaze QCs met the criterion that the measured concentrations should be within $\pm 25\%$ of their respective nominal concentrations (%RE $\sim 25\%$). At pH 8.2, however, the *E. coli* QC samples met the criteria, whereas the Erwinaze QC samples did not.

Effect of Temperature: To assess the effect of reaction temperature on the assay, the assay was conducted at 32°C and 42°C (the target condition is 37°C). The reagent (enzyme mixture) and plate pre-incubations were done at these temperatures, as well as the plate reading. A full set of standards and QC samples then were assayed at each temperature. The effect of reaction temperature was evaluated by comparing the experimentally determined concentrations of the QC's to their respective nominal values (%RE). At 32°C, both the *E. coli* and Erwinaze QC met the criterion that the measured concentrations should be within $\pm 25\%$ of their respective nominal concentrations (%RE $\leq 25\%$). At 42°C, however, the *E. coli* QC samples met the criteria, whereas the Erwinaze QC samples did not.

Effect of variations in critical reagents: To assess the effect of variations of critical reagents on the assay, the enzyme mix were prepared with all components at concentrations 20% higher and 20% lower than specified in the SOP. A full set of standards and QC samples were then be assayed with each mixture. The effect of critical reagent concentration was evaluated by comparing the experimentally determined concentrations of the QC's to their respective nominal values (%RE). Under conditions of both higher or lower reagent concentrations, all *E. coli* and Erwinaze QC samples met the criterion that the measured values should be within $\pm 25\%$ of their respective nominal values (%RE $\leq 25\%$).

Based on the evaluation of the data in this validation study, it appears that the method has the required attributes to make it suitable for its intended use.

APPENDICES 1.

FDA's data verification inspection identified the following major issues/deficiencies with the serum asparaginase activity sample handling and serum asparaginase assay performance and these issues/deficiencies render the PK results unreliable.

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:

<u>Subject. #</u>	<u>Course #</u>	<u>Sample #</u>
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

2. Failure to exclude serum samples from clinical sites received in the thawed state.
3. Records of freezer temperatures for storage of asparaginase samples were not retrievable in an auditable form.
4. Failure to document the times when samples were removed from frozen storage for analysis.
5. Failure to adequately document preparation and storage of asparaginase stock solutions.
6. Failure to adjust nominal asparaginase concentrations in calibrator and quality control solutions for the actual content of L-asparaginase commercial vials.
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.

The sponsor had responded to FDA concerns for each item. However, items 5 and 6 remain unresolved and make the PK results unreliable. This supports the FDA request for a new clinical trial.

FDA's data verification inspection identified the following major issues/deficiencies with the plasma asparagine sample handling and plasma asparagine assay performance. These issues/deficiencies render the PD results unreliable.

1. 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.
2. Failure to reject chromatograms when no asparagine internal standard was detected or when peaks could not be accurately integrated.
3. Failure to exclude plasma samples from clinical sites which were unacidified or were received in the thawed state.
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.
 - 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.

c) Records of freezer temperatures for storage of asparagine samples were not retrievable in an auditable form. The alarm system for temperatures outside -70 DC to -90 DC 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.

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).

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

f) The times when samples were removed from frozen storage for analysis were not recorded.

5. Between-run accuracy and precision for the asparagine assay were not evaluated.

6. Failure to evaluate the variability in recovery of asparagine in more than one plasma sample in a run.

7. Failure to evaluate the stability of asparagine in stock solutions or extracts.

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

9. Failure to verify (by balance printer or witness) the weights of asparagine used for calibrator and QC stock solutions.

APPENDICES 2. Email Communication on (b) (4) Proposed Assay for Asparaginase

From: Zhao, Hong (CDER)
Sent: Monday, August 15, 2011 4:37 PM
To: Skelly, Michael F; Beaucage, Serge
Cc: Laughner, Erik; Cherney, Barry; Spiridonov, Nikolay; Yang, Jun
Subject: RE: STN 125359 (EUSA Pharma): Asparaginase Activity Validation for new testing site; (b) (4)

I noticed the wider limits that the sponsor proposed for the assay acceptance, but thought those might be the best they could do. I am glad to see CMC review comments addressing this issue. Let's hear the sponsor's justification.

Thanks to Mike for compiling these comments together. Jun and I have no edits.

Hong

From: Skelly, Michael F
Sent: Monday, August 15, 2011 3:56 PM
To: Beaucage, Serge
Cc: Cherney, Barry; Spiridonov, Nikolay; Laughner, Erik; Zhao, Hong (CDER); Yang, Jun
Subject: RE: STN 125359 (EUSA Pharma): Asparaginase Activity Validation for new testing site; (b) (4)

Does anyone object to combining the texts from Serge (today 3:38 p.m.) and from Hong (Friday 4:26 p.m.), or wish to add/subtract/change? /MFS

We have reviewed your Draft 163 and found that your proposed method outline and reporting formats reasonable. However, we request that the following information also be provided: a record or statement of the complete temperature history for the serum samples, including the records mentioned in sections 3.6 , 3.2.2, and 3.3.3, or min/max records, in addition to the statements of continuous dry ice during shipping. If there were accidents or deviations in samples' storage and handling, or if spurious asparaginase values are encountered, please address these matters with fact-based reasoning.

We have the following comments on the (b) (4) validation protocol and SOP on the Asparaginase Activity assay:

Part 58 (GLP) and the 2001 Guidance on Bioanalytical Method Validation don't apply to this work, but we do not object to your using these references to outline validation and analysis plans, and to establish a quality model. We do not have a specific regulation or guidance to provide details for analytical conduct. We intend to authenticate records and assess fundamental data integrity, in evaluating your measurements of a surrogate endpoint to an efficacy study.

We acknowledge that the reagents/reactants, and the calibrators /quality control sample concentrations are approximately the same as the reference studies, so we expect the results to be comparable to the work of Asselin et al. and others.

We recommend avoiding plasticware and water treated with diethylpyrocarbonate (DEPC), a known inhibitor of asparaginase [Bagert-U and Roehm-KH, BBA 999:36-41, 1989] and mitochondrial MDH [Anderton-BH and Rabin-BR, Eur J. Biochem. 15:568-573, 1970].

In regard to the validation protocol for the quantitation of the enzymatic activity of L-Asparaginase in human serum, the validation parameters for selectivity, precision, accuracy, limits of quantitation, linearity, and stability were evaluated and the following limits were proposed:

- a. The RSDs for intra-assay precision and accuracy of the calibration curve in terms of L-asparaginase concentrations should be $\leq 20\%$ to $\leq 25\%$ and $\leq 25\%$ to $\leq 30\%$, respectively.
- b. The RSDs for inter-assay precision and accuracy of the calibration curve in terms of L-asparaginase concentrations should be $\leq 20\%$ to $\leq 25\%$ and $\leq 25\%$ to $\leq 30\%$, respectively.
- c. The RSDs for intra-assay precision and accuracy of QC L-asparaginase samples over the range of concentration tested should be $\leq 20\%$ to $\leq 25\%$.
- d. The RSDs for intra-assay precision and accuracy of QC L-asparaginase samples over the range of concentration tested should be $\leq 20\%$ to $\leq 25\%$.
- e. The % RSD for precision at ULOQ and LLOQ should be $\pm 20\%$ and $\pm 25\%$, respectively. The % RSD for accuracy at ULOQ and LLOQ should be $\pm 20\%$ and $\pm 25\%$, respectively.
- f. The acceptance criterion for the short term stability of *E. coli* L-Asparaginase and Erwinaze at 2-8°C for 24 hr is that the average (mean) values for the activities at each of QC concentration tested should be within $\pm 25\%$ of their respective nominal values.
- g. The proposed acceptance criterion for freeze-thaw stability of *E. coli* L-Asparaginase and Erwinaze is that the average (mean) values for the activities at each of QC concentration tested should be within $\pm 25\%$ of their respective nominal values.

The proposed limits for all the above parameters appears excessively broad given that these limits allow the determination of L-asparaginase activity values in human plasma to differ by 50% at the extremes of these limits. Please tighten these limits based on the analytical capabilities of the assay or provide a justification, supported by data, demonstrating that these limits are acceptable for each validation parameter.

You have proposed to determine L-Asparaginase V_{max} in the samples by fitting the data to a 4-parameter equation using the parameters derived from the fit of the standard curve. However, enzyme kinetic assays are typically carried out by measuring the product at an initial rate that is still linear. While it is possible to measure the complete reaction curve and fit this data to a non linear rate equation, we are not sure that this approach using a complex sample as human serum is the optimal approach. Please provide a justification why this is the optimal approach.

The validation protocol does not provide for evaluation of the assay robustness to variability in pH, reaction temperature, and volume/activity of critical reagents, such as asparaginase standards and enzyme/substrate mix. Please consider including these parameters in your validation protocol to better assess the robustness of the assay. Please also provide a description of the procedures and criteria used for the qualification of new batches of critical reagents that are required for performing the assay.

From: Beaucage, Serge
Sent: Monday, August 15, 2011 3:38 PM
To: Skelly, Michael F
Cc: Cherney, Barry; Spiridonov, Nikolay; Laughner, Erik; Zhao, Hong (CDER); Yang, Jun
Subject: STN 125359 (EUSA Pharma): Asparaginase Activity Validation for new testing site; (b) (4)

Michael,
Please find attached our comments regarding the validation of asparagine activity at the new test site.

Serge

<< File: Validation of asparaginase assay in human plasma .doc >>

Appendices 3.

Table 4: Specimens Representing the 48 Hour Post-Dose 3 Trough

Sample ID (BPC Receiving Code)	(b) (4) Sample ID	Patient ID no.	Treatment Start	Erwinase Dosing Information				Collection Of AB02 Samples		Time after Dose (hr)	Asparaginase Activity (IU/mL)
				Course	Dose	Date	Hour	Date	Hour		
2008-10-P0678	3	785414	W	1	3	09-Mar-09	12:45	11-Mar-09	12:57	48.2	0.35
2008-12-P0224	4	786636	F	1	3	18-Mar-09	11:15	20-Mar-09	11:15	48.0	0.97
2008-12-P0725	6	787136	F	1	3	24-Jun-09	12:15	26-Jun-09	12:10	47.9	0.43
2008-12-P0726	7	787137	W	1	3	13-Apr-09	10:52	15-Apr-09	10:52	48.0	1.84
2009-01-P0616	9	787929	F	1	3	01-Jul-09	15:20	03-Jul-09	15:47	48.5	0.24
2009-01-P0655	10	787968	F	1	3	22-Jul-09	13:15	24-Jul-09	13:04	47.8	0.65
2009-02-P0123	13	788317	W	1	3	18-May-09	14:32	20-May-09	15:00	48.5	0.26
2009-03-P0438	15	789461	W	1	3	14-Sep-09	12:50	16-Sep-09	12:45	47.9	0.71
2009-03-P0774	17	789794	W	1	3	06-Jul-09	12:10	08-Jul-09	11:10	47.0	0.33
2009-03-P1016	18	790037	W	1	3	27-Jul-09	10:30	29-Jul-09	10:15	47.8	0.67
2009-03-P1026	19	790047	W	1	3	29-Jun-09	11:45	01-Jul-09	10:03	46.3	1.58
2009-04-P0527	20	790620	W	1	3	16-Nov-09	14:15	18-Nov-09	14:25	48.2	1.14
2009-04-P0677	21	790770	W	1	3	11-May-09	12:57	13-May-09	11:22	46.4	0.25
2009-04-P0853	22	790945	W	1	3	14-Sep-09	11:14	16-Sep-09	11:14	48.0	1.35
2009-05-P0166	24	791109	W	1	3	10-Aug-09	14:15	12-Aug-09	14:11	47.9	0.76
2009-05-P0252	25	791194	F	1	3	16-Sep-09	11:50	18-Sep-09	09:25	45.6	0.97
2009-05-P0831	27	791771	F	1	3	05-Aug-09	14:49	07-Aug-09	14:49	48.0	0.50
2009-07-P0221	29	793008	F	1	3	18-Nov-09	11:53	20-Nov-09	13:00	49.1	0.59
2009-07-P0583	30	793370	F	1	3	13-Jan-10	12:50	15-Jan-10	12:20	47.5	0.52
2009-07-P0638	31	793425	M	1	3	24-Oct-09	10:30	26-Oct-09	10:30	48.0	0.70

Sample ID (BPC Receiving Code)	(b) (4) Sample ID	Patient ID no.	Treatment Start	Erwinase Dosing Information				Collection Of AB02 Samples		Time after Dose (hr)	Asparaginase Activity (IU/mL)
				Course	Dose	Date	Hour	Date	Hour		
2009-07-P0848	32	793635	F	1	3	07-Apr-10	13:00	09-Apr-10	12:23	47.4	0.64
2009-08-P0219	33	794013	F	1	3	09-Dec-09	12:25	11-Dec-09	12:30	48.1	0.99
2009-08-P0307	34	794101	W	1	3	07-Dec-09	11:00	09-Dec-09	10:30	47.5	1.36
2009-08-P0371	35	794165	F	1	3	27-Jan-10	12:55	29-Jan-10	10:35	45.7	0.57
2009-09-P0181	37	794765	W	1	3	07-Dec-09	13:35	09-Dec-09	13:53	48.3	0.25
2009-09-P0382	38	794966	W	1	3	15-Mar-10	12:00	17-Mar-10	10:50	46.8	0.74
2009-09-P0655	39	795239	W	1	3	25-Jan-10	10:45	27-Jan-10	09:15	46.5	0.35
2009-09-P0672	40	795256	W	1	3	18-Jan-10	14:30	20-Jan-10	09:00	42.5	0.87
2009-10-P0275	41	795635	W	1	3	07-Dec-09	12:00	09-Dec-09	10:45	46.8	0.41
2009-10-P0565	43	795925	W	1	3	18-Jan-10	12:29	20-Jan-10	08:30	44.0	0.48
2009-11-P0019	44	796268	F	1	3	14-Apr-10	10:20	16-Apr-10	10:10	47.8	0.40
2009-11-P0699	45	796945	W	1	3	29-Mar-10	10:30	31-Mar-10	10:45	48.3	0.58
2009-11-P0832	46	797078	W	1	3	22-Feb-10	10:26	24-Feb-10	09:47	47.4	1.21
2010-01-P0140	47	798278	F	1	3	31-Mar-10	10:20	02-Apr-10	09:05	46.8	0.95
2010-01-P0708	48	798844	F	1	3	31-Mar-10	13:30	02-Apr-10	10:30	45.0	0.78
Total Samples:	35										%Percent > 0.10 IU/mL: 100%

Table 5: Specimens Representing the 72 Hour Post-Dose 3 Trough

Sample ID (BPC Receiving Code)	Sample ID	Patient ID no.	Treatment Start	Erwinase Dosing Information				Collection Of ABO2 Samples		Time after Dose (hr)	Asparaginase Activity (IU/mL)
				Course	Dose	Date	Hour	Date	Hour		
2008-10-P0277	1	785017	M	1	3	20-Mar-09	13:15	23-Mar-09	13:15	72.0	0.48
2008-10-P0295	2	785035	M	1	3	27-Feb-09	12:00	02-Mar-09	11:20	71.3	0.24
2008-12-P0662	5	787073	M	1	3	03-Apr-09	13:45	06-Apr-09	14:15	72.5	0.80
2008-12-P0811	8	787221	M	1	3	26-Jun-09	14:30	29-Jun-09	09:45	67.3	0.11
2009-01-P0694	11	788007	M	1	3	17-Apr-09	12:20	20-Apr-09	11:00	70.7	0.62
2009-02-P0055	12	788249	M	1	3	24-Jul-09	14:30	27-Jul-09	14:50	72.3	0.21
2009-02-P0700	14	788890	M	1	3	05-Jun-09	11:30	08-Jun-09	09:44	70.2	0.22
2009-03-P0722	16	789742	M	1	3	17-Jul-09	10:30	20-Jul-09	09:36	71.1	0.31
2009-05-P0085	23	791030	M	1	3	31-Jul-09	12:11	03-Aug-09	12:14	72.1	0.28
2009-05-P0508	26	791449	M	1	3	17-Jul-09	15:00	20-Jul-09	12:53	69.9	0.70
2009-07-P0010	28	792798	M	1	3	16-Oct-09	12:40	19-Oct-09	12:40	72.0	0.19
2009-09-P0085	36	794669	M	1	3	11-Dec-09	11:30	14-Dec-09	10:10	70.7	0.52
2009-10-P0278	42	795638	W	1	3	08-Feb-10	13:30	11-Feb-10	11:00	69.5	0.26
Total Samples:	13									Percent > 0.10 IU/mL: 100%	

Clinical Pharmacology Review

BLA: 125359	Submission Date(s): 9/8/2010
Brand Name	Erwinaze®
Generic Name	Erwinia L-asparaginase
Submission Type; Code	NME
Formulation; Strength(s)	Supplied as a sterile, lyophilized powder in vials, 10,000 International Units/vial
Proposed Indication	As a component of a multi-agent chemotherapeutic regimen for the treatment of (b) (4) patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to <i>E. coli</i> derived asparaginase
Dosing Regimen	25,000 International Units/m ² administered intramuscularly three times a week (Monday/Wednesday/Friday) for six doses to replace each dose of pegaspargase
Relevant IND(s)	BB-IND 290
PDUFA Date	8/2/2011
Sponsor	EUSA
Clinical Division	Biologic Oncology Products
OCP Division	Clinical Pharmacology V
Primary Reviewer	Jun Yang, Ph.D.
Team Leader	Hong Zhao, Ph.D.

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1 EXECUTIVE SUMMARY

The purpose of this new BLA application is to obtain the FDA's approval on the use of Erwinaze in patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to ^{(b) (4)} *E. Coli* derived asparaginase. The current submission contains a clinical pharmacokinetic (PK) and pharmacodynamic (PD) study report (COG AALL07P2) in support of the proposed use. Study COG AALL07P2 was a single-arm, multi-center, open-label, safety and clinical pharmacology trial conducted in 59 patients treated on front line cooperative group ALL protocols who were unable to continue to receive pegaspargase due to hypersensitivity reactions. Patients received Erwinaze 25,000 international units/m² x 6 doses intramuscularly on a Monday, Wednesday, and Friday schedule as a replacement for each scheduled dose of pegaspargase remaining on their original treatment protocol. The primary endpoint of this study was the proportion of patients with the 48-hour trough serum asparaginase activity ≥ 0.1 IU/mL. The secondary endpoints included safety profile and asparagine depletion. Clinical efficacy was not assessed in this study. The current submission does not include the immunogenicity results because the validated assays have been under development by the sponsor. FDA's data verification inspection identified major issues/deficiencies with the sample handling and assay performance of the serum asparaginase and plasma asparagine assays. For the serum asparaginase activity assay, deficiencies include: 1) the failure to adequately document preparation and storage of asparaginase stock solutions; 2) failure to adjust nominal asparaginase concentrations in calibrator and quality control solutions for the actual content of L-asparaginase commercial vials; 3) incomplete documentation of sample storage and handling conditions and stability during these conditions, failure to reject analytical run #480 when one of the three quality control samples failed the acceptance criterion; 4) failure to exclude serum samples from clinical sites received in the thawed state. For the plasma asparagine assay, deficiencies include: 1) the failure to reject analytical runs when the quality control samples failed the acceptance criterion; 2) failure to reject chromatograms when no asparagine internal standard was detected or peaks could not be accurately integrated; 3) failure to exclude plasma samples from clinical sites which were unacidified or received in the thawed state; 4) failure to demonstrate stability of samples under the conditions of the study. These bioanalytical deficiencies/issues render the analytical results for both the serum asparaginase activity and plasma asparagine concentrations unreliable. The deficiency of necessary long-term freezer stability and freezer-thaw stability data for both assays as well as the large number of plasma asparagine samples that were either received thawed, collected without acid preservative, or with documented delays between sample collection and acidification, prohibit the re-assay of frozen serum (asparaginase) and plasma (asparagine) samples

1.1 RECOMMENDATIONS

The submitted clinical pharmacology information does not support the approval of Erwinaze because the submitted pharmacokinetic and pharmacodynamic results from Trial COG AALL07P2 are not reliable due to inappropriate handling of patient samples and deficiencies with the bioanalytical process identified during inspection and review. The Office of Clinical Pharmacology recommends that a new clinical trial should be conducted in a minimum of 24

evaluable patients to provide reliable results on the primary clinical endpoint (serum asparaginase activity) for two treatment cycles. Immunogenicity samples should be collected from all patients participating in the new trial. In addition, please provide details of the bioanalytical assay methodology and experimental procedures prior to the start of the new trial for FDA review.

The sponsor is requested to submit an immunogenicity study report including testing results for patient samples and validation of the immunogenicity assays.

Signatures:

 5/25/2011

Jun Yang., Ph.D.

Clinical Pharmacology Reviewer, Biologic Product Team

Division of Clinical Pharmacology V

 5/25/2011

Hong Zhao, Ph.D.

Team Leader, Biologic Product Team

Division of Clinical Pharmacology V

 Deputy Director, DCP5 for

Nam Atiqur Rahman, Ph.D.

Division Director, Division of Clinical Pharmacology V

1.2 SUMMARY OF CLINICAL PHARMACOLOGY FINDINGS

Erwinia asparaginase (Crisantaspase, Erwinaze), derived from *Erwinia chrysanthemi*, is licensed in European and Canada but has never been commercially available in the US. The clinical study report (#AALL07P2 Study) includes pharmacokinetics (PK) and pharmacodynamics (PD) of Erwinaze in acute lymphoblastic leukemia (ALL) patients who have developed hypersensitivity to (b) (4) *E. Coli* derived asparaginase. For replacement of pegaspargase, the recommended dose is 25,000 International Units (IU)/m² administered intramuscularly three times a week (Monday/Wednesday/Friday) for six doses to replace each dose of pegaspargase. A primary objective of this study was to determine whether the 48-hour trough serum asparaginase activity was ≥ 0.1 IU/mL. This study was powered to test the hypothesis target level in 70% of patients against an alternative of meeting this trough threshold activity in 50% of patients. Efficacy was not assessed in this study. Due to major deficiencies in patient sample handling and in determination of asparaginase activity and plasma asparagine level, the submitted PK and PD results are not reliable.

Pharmacokinetic and Pharmacodynamic Findings: The sponsor reported that the serum trough concentrations of *Erwinia* L-asparaginase were determined in 53 ALL patients aged ≥ 2 year to ≤ 18 years. Following administration of ERWINAZE 25,000 IU/m² x 6 doses intramuscularly on a Monday, Wednesday, and Friday schedule, 81% to 100% of patients in course 1, 82% to 100% of patients in course 2, and 90% to 100% of patients in course 3 achieved steady state serum trough asparaginase concentrations ≥ 0.1 IU/mL at 48-hour post dose. At 72-hour post dose, 82% to 93% of patients in course 1, 75% to 83% of patients in course 2, and 75% to 100% of patients in course 3 achieved steady state serum trough asparaginase concentrations ≥ 0.1 IU/mL.

The trough plasma asparagine levels were measured during the first three courses at either 48- or 72-hours post-dose in 47 patients receiving six doses of ERWINAZE per treatment course. The sponsor reported that among evaluable patients, 98% (n=47) of the patients in course 1; 97% (n=31) of the patients in course 2, and 100% (n=22) of the patients in course 3 achieved the clinically desired level of plasma asparagine depletion of < 0.4 μ g/mL.

However, FDA's data verification inspection identified several major issues/deficiencies with the sample handling and assay performance of the serum asparaginase and plasma asparagine assays. For the serum asparaginase activity assay, deficiencies include the failure to adequately document preparation and storage of asparaginase stock solutions, failure to adjust nominal asparaginase concentrations in calibrator and quality control solutions for the actual content of L-asparaginase commercial vials, failure to reject analytical run #480 when one of the three quality control samples failed the acceptance criterion, and failure to exclude serum samples from clinical sites received in the thawed state. For the plasma asparagine assay, deficiencies include the failure to reject analytical runs when the quality control samples failed the acceptance criterion, failure to reject chromatograms when no asparagine internal standard was detected or peaks could not be accurately integrated, failure to exclude plasma samples from clinical sites which were unacidified or received in the thawed state, and failure to demonstrate stability of samples under the conditions of the study. These bioanalytical deficiencies/issues render the analytical results for both the serum asparaginase activity and plasma asparagine concentrations unreliable.

Immunogenicity: Immunogenicity samples at predose were collected at pre-courses, on Days 8 and 22 (Course 1) and on Days 6 and 15 (subsequent Courses). This study report does not contain the antibody results because the immunogenicity assays have not been adequately validated. The sponsor states that the samples remain frozen and will be analyzed when the assays are validated.

QT/QTc Evaluation: Electrocardiograms (ECGs) were performed at prior to dose 1, and at 1 hour following dose 6 in Course 1 only. No patient had a QTc interval >500 msec at the pre-dose 1. At 1 hour after dose 6, 1 patient had both a >500 msec value and a change in QTc \geq 60 msec, 2 patients had a QTc interval >500 msec, and 2 different patients had a change in QTc \geq 60 msec. FDA QT-IRT review suggests that no language related to QT liability is included in the future labeling due to study limitations (See Attachment for QT-IRT review).

Conclusion: Overall, unacceptable sample handling and unreliable assay methodology preclude acceptability of the Clinical Pharmacology information presented in this BLA. A new clinical trial should be conducted to provide reliable results of serum asparaginase activity data and to support the approval of this application.

2 QUESTION BASED REVIEW

2.1 GENERAL ATTRIBUTES

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?

ERWINAZE contains an enzyme L-asparaginase derived from *Erwinia chrysanthemi*. L-asparaginase is a tetrameric enzyme consisting of four identical subunits, each having a molecular weight of about 35 kDa; its activity is expressed in terms of International Units (IU) according to the rules of the International Union of Biochemistry.

ERWINAZE is supplied as a sterile, lyophilized, white powder in vials. Each vial contains 10,000 IU of *Erwinia* L-asparaginase, and the following inactive ingredients: glucose monohydrate (5.0 mg), sodium chloride (0.5 mg).

2.1.2 What are the proposed mechanisms of action and therapeutic indications?

Mechanism of Action: ERWINAZE (*Erwinia* L-asparaginase) is an asparagine specific enzyme. The leukemic cells depend on an exogenous source of the amino acid asparagine for their protein metabolism and survival. According to the sponsor, the mechanism of action of ERWINAZE is thought to be based on the inability of leukemic cells to synthesize asparagine due to lack of asparagine synthetase activity.

Proposed Indication: ERWINAZE is indicated as a component of a multi-agent chemotherapeutic regimen for the treatment of ^{(b) (4)} patients with acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to *E. coli* derived asparaginase

2.2 GENERAL CLINICAL PHARMACOLOGY

2.2.1 What are the design features of the clinical studies used to support dosing or claims?

This supplemental BLA contains a clinical study report AALL07P2. Efficacy was not assessed in this study; however, pharmacokinetics (PK), pharmacodynamics (PD), and safety parameters were measured. Under the Erwinaze IND, a compassionate use protocol was begun to enable US patients to get the medication outside of the Children's Oncology Group (COG) AALL07P2 PK/PD study. This trial was called the Erwinaze Master Treatment Protocol (EMTP). The safety portion of this BLA consists of information from both the COG AALL07P2 study and the EMTP study.

STUDY AALL07P2

Study Title: Pharmacology and Toxicity of *Erwinia* Asparaginase Following Allergy to Pegaspargase in Treatment of Children with Acute Lymphoblastic Leukemia (ALL).

Study Design: This study was to assess PK, PD and related toxicities of Erwinaze used as replacement therapy for pegaspargase dosing. Patients received Erwinaze 25,000 IU/m² x 6 doses intramuscularly (IM) on a Monday/Wednesday/Friday, Wednesday/Friday/Monday or Friday/Monday/Wednesday schedule as a replacement for each scheduled dose of pegaspargase remaining on the original treatment protocol. All patients received at least 1 Course of treatment. A total enrollment of 59 children was achieved, 58 of whom received Erwinaze. Fifty-three patients were evaluable for the primary endpoint based on availability of PK samples in Course 1. The median age was 10 years (2 to 18 years), 59% were male, 78% were White, 10% were Black/African American, 5% were Asian, and 5% were Hispanic or Latino. This study was powered to test the hypothesis target level in 70% of patients against an alternative of meeting this trough threshold activity in 50% of patients.

For inclusion in the study, subjects were required to fulfill all of the following key criteria:

- Patients were enrolled on a frontline Children's Oncology Group (COG) ALL treatment study at a participating institution;
- Patients must have had \geq Grade 2 hypersensitivity reaction to pegaspargase, according to Common Toxicity Criteria for Adverse Event (CTCAE, version 3.0);
- Patients must have had 1 or more Courses of asparaginase remaining in their treatment protocol.

The primary objectives of this study were to

- determine if the 48 hour trough serum asparaginase activity was \geq 0.1 IU/mL.
- to determine the frequency of asparaginase-related toxicity following Erwinaze treatment..
- to characterize the PK of Erwinaze in children with ALL who are hypersensitive to pegaspargase.

The secondary objectives of this study were to:

- informally compare serum asparaginase activity and plasma asparagine concentration between patients treated with Erwinaze in this trial versus historical controls treated with pegaspargase on Children's Cancer Group (CCG) Studies-1962 and 1961
- determine serum asparaginase activity at 72 hours post-dose.
- determine the presence of anti-*Erwinia* asparaginase antibodies in children treated with a Course(s) of Erwinaze following a clinical hypersensitivity to pegaspargase.
- determine if plasma asparagine was adequately depleted (Day 12 [pre-dose 6] if the Course of Erwinaze was started on Monday; Day 13 [pre-dose 6] if the Course of Erwinaze was started on Wednesday or Friday) in a subset of patients.

2.2.2 *What are the study endpoints, and how are they measured in clinical pharmacology and clinical studies?*

The pharmacology studies of CCG Studies 1941, 1962 and 1961 helped establish the following expected PK parameters for pegaspargase:

1. Therapeutic depletion of asparagine requires an asparagine concentration \leq 3 μ M.
2. Adequate depletion of asparagine is achieved with serum L-asparaginase activity levels \geq 0.1 IU/mL.

According to the sponsor, a relatively high dose of *Erwinia* L-asparaginase at 25,000 IU/m² should be given every 2-3 days, in order to achieve the target of L-asparaginase activity and asparagine depletion.

PK Assessment: The PK measurement was pre-specified as the primary endpoint (48-hour trough serum asparaginase activity ≥ 0.1 IU/mL). In Study COG AALL07P2, the PK parameter (asparaginase activity) was measured at 12 different time points during the 1st Course of treatment. In subsequent Courses of the treatment, blood samples were collected at 3 different time points. The time points varied slightly depending on whether the patient started on Monday, Wednesday, or Friday. The 48-hour data used were from post dose 5 (all Courses), if the Course started on Monday; from post dose 4 (Course 1) and post dose 6 (Courses 2 and 3), if the Course started on Wednesday; and from post dose 5 (all Courses) and post dose 6 (Courses 2 and 3), if the course started on Friday. The 72-hour data used were from pre-dose 4 (Course 1) and post dose 6 (Courses 2 and 3), if the Course started on Monday; from pre-dose 6 (Course 1) and post dose 5 (Courses 2 and 3), if the Course started on Wednesday; and pre-dose 5 (Course 1), if the Course started on Friday. For cohort started on Friday in Course 2, no 72-hour post asparaginase activity was assessed.

PD Assessment: In Study COG AALL07P2, the PD endpoint (plasma asparagine level) was measured at 5 different time points during Course 1 of treatment. In subsequent Courses of treatment, blood samples were collected at 3 different time points. The time points varied slightly depending on whether the patient started Erwinaze treatment on Monday, Wednesday, or Friday. As a secondary objective, plasma asparagine depletion, defined as a trough plasma asparagine level $\leq 3\mu\text{M}$ (0.396 $\mu\text{g/mL}$), was determined for all subjects who had PD data for Day 12 if course started on Monday and Day 13 if the course started on Wednesday or Friday.

2.2.3 Do the PK meet the primary endpoint criteria?

PK measurements were taken in a total of 53 patients. The sponsor provided a summary of PK results in Table 1. Based on the sponsor's report, following administration of ERWINAZE 25,000 IU/m² x 6 doses intramuscularly on a Monday, Wednesday, and Friday schedule, 81% to 100% of patients in course 1, 82% to 100% of patients in course 2, and 90% to 100% of patients in course 3 achieved steady state serum trough asparaginase concentrations ≥ 0.1 IU/mL at 48-hour post dose. At 72-hour post dose, 82% to 93% of patients in course 1, 75% to 83% of patients in course 2, and 75% to 100% of patients in course 3 achieved steady state serum trough asparaginase concentrations ≥ 0.1 IU/mL.

Table 1. Proportion of Patients with Trough Serum Asparaginase Activity Level ≥ 0.1 IU/mL.

Treatment Regime	Timepoint ^a	n/N	% Attaining ≥ 0.1 IU/mL
Course 1			
Monday/Wednesday/Friday	48 hours	13/16	81.3%
	72 hours	13/15	86.7%
Wednesday/Friday/Monday	48 hours	22/22	100.0%
	72 hours	18/22	81.8%
Friday/Monday/Wednesday	48 hours	14/15	93.3%
	72 hours	14/15	93.3%
Total	48 hours	49/53	92.5%
	72 hours	45/52	86.5%
Course 2			
Monday/Wednesday/Friday	48 hours	15/16	93.8%
	72 hours	11/14	78.6%
Wednesday/Friday/Monday	48 hours	9/11	81.8%
	72 hours	9/12	75.0%
Friday/Monday/Wednesday ^b	48 hours	8/8	100.0%
	48 hours	5/6	83.3%
Total	48 hours	37/41	90.2%
	72 hours	20/26	76.9%
Course 3			
Monday/Wednesday/Friday	48 hours	8/8	100.0%
	72 hours	6/8	75.0%
Wednesday/Friday/Monday	48 hours	7/7	100.0%
	72 hours	7/7	100.0%
Friday/Monday/Wednesday ^b	48 hours	10/10	100.0%
	48 hours	9/10	90.0%
Total	48 hours	34/35	97.1%
	72 hours	13/15	86.7%

^a The 48-hour data used were from post dose 5 (all courses), if the course started on Monday; from post dose 4 (course 1) and post dose 6 (courses 2 and 3), if the course started on Wednesday; and from post dose 5 (all courses) and post dose 6 (courses 2 and 3), if the course started on Friday. The 72-hour data used were from pre-dose 4 (course 1) and post dose 6 (courses 2 and 3), if the course started on Monday; from pre-dose 6 (course 1) and post dose 5 (courses 2 and 3), if the course started on Wednesday; and pre-dose 5 (course 1), if the course started on Friday.

^b For this cohort, no 72 post Erwinaze L-asparaginase activity was assessed in courses other than Course 1.

However, FDA's data verification inspection identified major issues/deficiencies with the serum asparaginase activity sample handling and serum asparaginase assay performance. The serum asparaginase activity assay deficiencies include the failure to adequately document preparation and storage of asparaginase stock solutions, failure to adjust nominal asparaginase concentrations in calibrator and quality control solutions for the actual content of L-asparaginase commercial vials, failure to reject analytical run #480 when one of the three quality control samples failed the acceptance criterion, and failure to exclude serum samples from clinical sites received in the thawed state. These issues/deficiencies render the PK results unreliable; therefore, these results cannot be used to support the approval of this application.

2.2.4 Do the PD results meet the pre-defined criteria?

The trough plasma asparagine levels were measured during the first three courses at either 48- or 72-hours post-dose in 47 patients receiving six doses of ERWINAZE per treatment course. The sponsor reported results on proportion of patients having attaining the pre-defined secondary endpoint are shown in Table 2. According to the sponsor, among evaluable patients, 98% (n=47) of the patients in course 1; 97% (n=31) of the patients in course 2, and 100% (n=22) of the

patients in course 3 achieved the clinically desired level of trough plasma asparagine level $\leq 3\mu\text{M}$ (0.396 $\mu\text{g/mL}$).

Table 2. Percentage of Subjects Achieving Plasma Asparagine Depletion.

Treatment Regime	n/N	% Achieving Asparagine Depletion
Course 1		
Monday/Wednesday/Friday	13/14	92.9%
Wednesday/Friday/Monday	21/21	100%
Friday/Monday/Wednesday	12/12	100%
Total	46/47	97.9%
Course 2		
Monday/Wednesday/Friday	14/15	93.3%
Wednesday/Friday/Monday	10/10	100%
Friday/Monday/Wednesday	6/6	100%
Total	30/31	96.8%
Course 3		
Monday/Wednesday/Friday	7/7	100%
Wednesday/Friday/Monday	7/7	100%
Friday/Monday/Wednesday	8/8	100%
Total	22/22	100%

Depletion counts are the number of subjects with a trough plasma asparagine level $\leq 3\mu\text{M}$ (0.396 $\mu\text{g/mL}$).

Data used for adequate depletion are from Day 12 if course was started on Monday and from Day 13 if started on Wednesday or Friday.

Cross-reference: 5.3.4.2 Table 14.2.4.2

To date, the biggest challenge of measuring asparagine level is the instability of asparagine due to *ex vivo* metabolism. FDA's data verification inspection identified major issues/deficiencies with the plasma asparagine sample handling and plasma asparagine assay performance (See Attachment for FDA DSI memorandum to DBOP). The asparagine assay deficiencies include the failure to reject analytical runs when the quality control samples failed the acceptance criterion, failure to reject chromatograms when no asparagine internal standard was detected or peaks could not be accurately integrated, failure to exclude plasma samples from clinical sites which were unacidified or received in the thawed state, and failure to demonstrate stability of samples under the conditions of the study. These issues/deficiencies render the PD results unreliable; therefore, the PD results cannot be used to support the approval of this application

2.2.5 What are the immunogenicity incidences for Erwinaze?

There is a potential for immunogenicity with therapeutic proteins such as ERWINAZE. Blood samples for determining anti-Erwinaze and neutralizing antibody formation were collected in Study COG AALL07P2. During Course 1, blood samples were taken pre-course and on Day 8 and Day 22. During subsequent Courses, pre-course, Day 6 and Day 15 samples were collected. Because the immunogenicity assays have not been adequately validated, the patient samples remain frozen and will be analyzed when the assays are validated.

2.2.6 What are the QT/QTc results?

Electrocardiograms (ECGs) were performed to assess the possible effect of Erwinaze on QTc (QT interval transformed/normalized into a heart rate corrected value) at Course 1 only. ECGs were performed at prior to Erwinaze dose 1, and at 1 hour following Erwinaze dose 6.

The sponsor reported that no patient had a QTc interval >500 msec at the pre-dose 1 time point. At 1 hour following dose 6, 1 patient had both a >500 msec value and a change in QTc ≥ 60 msec, 2 patients had a QTc interval >500 msec 1 hour after dose 6, and 2 different patients had a change in QTc ≥ 60 msec. Due to the study design limitations, FDA QT-IRT team suggests that no language related to QT liability is included in the future labeling (See Attachment for QT-IRT review).

2.3 ANALYTICAL SECTION

2.3.1 What bioanalytical methods are used to assess therapeutic protein concentrations? Briefly describe the methods and summarize the assay performance?

Asparaginase Activity: A validated enzymatic assay for determining the activity of L-asparaginase in human serum was included in the current submission. The principle of this enzymatic assay is that aspartic acid formed from deamination of asparagine via asparaginase catalysis reacts with α -ketoglutaric acid, yielding oxaloacetic acid which oxidizes reduced β -nicotinamide adenine dinucleotide in the presence of malic dehydrogenase, resulting in a decrease in absorbance at 340 nm.

According to the sponsor, data used for determining the between-day accuracy and precision of the analytical method for measuring asparaginase activity in 11 calibration standards and quality control solutions of the enzyme in human serum prepared and assayed during a period of 4 weeks. The lowest concentration included in the standard curve, 0.025 IU/mL was measured with a between-day accuracy of 102.5% and a precision of 4.6%. The results for all other concentrations included in the calibration curve (ranged from 0.025-0.250 IU/mL) and quality control (QC) samples (nominal enzyme activities of 0.035, 0.120, and 0.220 IU/mL) were shown in Table 3.

Within-day accuracy and precision was determined separately for both preparations of the enzyme by spiking normal human serum with working solutions to provide activities comparable to the quality control samples. The added activities of the enzyme for these solutions, based upon the assayed activity of the working solutions, were 0.038, 0.114 and 0.208 IU/mL for *Erwinia* asparaginase. Five sets of these solutions were made and each solution was assayed once together with a set of calibration standards and quality control solutions during the course of a single day. The accuracy of the assay for *Erwinia* asparaginase solutions were shown in Table 3.

The assay validation results also suggested that serum samples containing *E coli* asparaginase can be thawed up to 3 times for reanalysis without a 10% loss in enzymatic activity, whereas serum samples containing *Erwinia* asparaginase should not be assayed after being thawed more than twice. Serum samples containing either *E coli* or *Erwinia* asparaginase may be stored frozen at -80°C for at least three months prior to analysis. The stock solution of *E coli* asparaginase was

stable for 72 h when stored in a refrigerator and the *Erwinia* asparaginase stock solution was stable for 48 h after reconstitution.

Table 3. Inter- and Intra-day Accuracy and Precision of Assay for Asparaginase activity.

Asparaginase		Calibration Standards	Serum Samples
		Inter-day	intra-day
Standard Samples (0.025-0.250 IU/mL)	Accuracy	95.3-103.7%	*
	Precision	2.1-8.2%	*
QC samples (low, medium, high levels)	Accuracy	98.2-104.7%	79.8-94.6%
	Precision	1.8-5.6%	2.4-6.9%

*, not provided.

However, FDA's data verification inspection identified the following major issues/deficiencies with the serum asparaginase activity sample handling and serum asparaginase assay performance and these issues/deficiencies render the PK results unreliable.

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:

<u>Subject #</u>	<u>Course #</u>	<u>Sample #</u>
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

2. Failure to exclude serum samples from clinical sites received in the thawed state.
3. Records of freezer temperatures for storage of asparaginase samples were not retrievable in an auditable form.
4. Failure to document the times when samples were removed from frozen storage for analysis.
5. Failure to adequately document preparation and storage of asparaginase stock solutions.
6. Failure to adjust nominal asparaginase concentrations in calibrator and quality control solutions for the actual content of L-asparaginase commercial vials.
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.

The sponsor had responded to FDA concerns for each item. However, items 5 and 6 remain unresolved and make the PK results unreliable. This supports the FDA request for a new clinical trial.

Asparagine: There was no assay validation report except a final study report for the asparagine assay in this BLA package. Asparagine in blood specimens is subject to rapid and extensive ex-

vivo metabolism by the presence of asparaginase. Consequently, asparagine cannot be accurately measured in serum or plasma samples in the absence of procedures to rapidly and completely inhibit the catalytic activity of asparaginase. According to the sponsor, there is no available inhibitor to stop or inhibit this enzymatic reaction at this moment. The sponsor stated that their developed acidification method may be used to help stop the *ex-vivo* degradation. However, the process requires rapid plasma sample preparation and acidification (e.g., within 5 minutes).

According to the sponsor, only a small proportion of the clinical samples could be extracted, centrifuged and acidified within 10 minutes. LC/MS assay was developed to measure plasma level of asparagine. The lowest concentration included in the standard curves, 0.10 µg/mL (0.76 µM) was measured with a between-day accuracy of 98.2% and a precision of 19.7%. Within-day accuracy and precision were also determined to be 85.6% and 14.3% at concentration of 0.10 µg/mL. Results for all other concentrations included in the calibration curve (ranged from 0.25-50.0 µg/mL) were shown in Table 4. The accuracy and precision of the assay for measuring asparagine in plasma was also determined by analyzing human donor plasma with added concentrations of reference asparagine of 0.0, 2.0, 8.0 and 40.0 µg/mL in triplicate on three different days. During the three days, the concentration of endogenous asparagine ranged from 4.90 ± 0.32 µg/mL to 5.51 ± 0.15 µg/mL (37.1-41.7 µM) with an overall mean ± SD of 5.15 ± 0.10 µg/mL (CV, 1.94%). The within-day and between-day accuracy and precision of the assay were shown in Table 4.

Table 4. Inter- and Intra-day Accuracy and Precision of Assay for Asparagine Level.

Asparagine		Calibration Standards		Plasma Samples	
		Inter-day	intra-day	Inter-day	intra-day
Standard Samples (0.25-50 IU/mL)	Accuracy	95.8-107.2%	*	*	*
	Precision	1.5-13.2%	*	*	*
QC samples (low, medium, high levels)	Accuracy	*	*	89.5-98.5%	85.8-106.4%
	Precision	*	*	3.7-9.3%	0.8-30.3%

*, not provided.

FDA's data verification inspection identified the following major issues/deficiencies with the plasma asparagine sample handling and plasma asparagine assay performance. These issues/deficiencies render the PD results unreliable.

1. 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.
2. Failure to reject chromatograms when no asparagine internal standard was detected or when peaks could not be accurately integrated.
3. Failure to exclude plasma samples from clinical sites which were unacidified or were received in the thawed state.
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.
 - 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.
 - c) Records of freezer temperatures for storage of asparagine samples were not retrievable in an auditable form. The alarm system for temperatures outside -70 DC to -90 DC 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.
 - 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).
 - e) The effectiveness of hydrochloric acid in preserving asparagine in plasma was tested only for E. coli asparaginase, not for Erwinia asparaginase.
 - f) The times when samples were removed from frozen storage for analysis were not recorded.
5. Between-run accuracy and precision for the asparagine assay were not evaluated.
 6. Failure to evaluate the variability in recovery of asparagine in more than one plasma sample in a run.
 7. Failure to evaluate the stability of asparagine in stock solutions or extracts.
 8. Only a single stock solution of asparagine was used for both calibrators and QC samples, rather than independently-prepared stock solutions, in both prestudy validation and within-study conduct.
 9. Failure to verify (by balance printer or witness) the weights of asparagine used for calibrator and QC stock solutions.

Clinical Pharmacology Comments

The submitted clinical pharmacology information does not support the approval of Erwinaze because the submitted pharmacokinetic and pharmacodynamic results from Trial COG AALL07P2 are not reliable due to inappropriate handling of patient samples and deficiencies with the bioanalytical process identified during inspection and review.

Recommendation

The Office of Clinical Pharmacology recommends that a new clinical trial should be conducted in a minimum of 24 evaluable patients to provide reliable results on the primary clinical endpoint (serum asparaginase activity) for two treatment cycles. Immunogenicity samples should be collected from all patients participating in the new trial. In addition, please provide details of the bioanalytical assay methodology and experimental procedures prior to the start of the new trial for FDA review.

Jun Yang, Ph.D.
Reviewer
CDER/OTS/OCP/DCP5

Hong Zhao, Ph.D.
Team Leader
CDER/OTS/OCP/DCP5

Office of Clinical Pharmacology

General Information About the Submission

	Information		Information
NDA/BLA Number	125359/0	Brand Name	Erwinaze
OCP Division (I, II, III, IV, V)	V	Generic Name	Erwinia L-asparaginase
Medical Division	DBOP	Drug Class	Biologics
OCP Reviewer	Jun Yang, Ph.D.	Indication(s)	Acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to (b) (4) (b) (4) <i>E. coli</i> derived asparaginase
OCP Team Leader	Hong Zhao, Ph.D.	Dosage Form	10,000 IU/vial
Pharmacometrics Reviewer	Jun Yang, Ph.D.	Dosing Regimen	25,000 IU/m ² IM three times a week for two weeks
Date of Submission	11/1/2010	Route of Administration	Intramuscular (IM) Injection
Estimated Due Date of OCP Review	4/10/2011	Sponsor	EUSA Pharma
Medical Division Due Date	4/12/2011	Priority Classification	Priority
PDUFA Due Date	5/3/2011		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x	██████		
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -	█	█		
HEALTHY VOLUNTEERS-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:	x			
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				

Subpopulation studies -					
	ethnicity:				
	gender:				
	pediatrics:				
	geriatrics:				
	renal impairment:				
	hepatic impairment:				
PD -					
	Phase 2:				
	Phase 3:	x	1		
PK/PD -					
	Phase 1 and/or 2, proof of concept:				
	Phase 3 clinical trial:				
Population Analyses -					
	Data rich:				
	Data sparse:				
II. Biopharmaceutics					
Absolute bioavailability					
Relative bioavailability -					
	solution as reference:				
	alternate formulation as reference:				
Bioequivalence studies -					
	traditional design; single / multi dose:				
	replicate design; single / multi dose:				
Food-drug interaction studies					
Bio-waiver request based on BCS					
BCS class					
Dissolution study to evaluate alcohol induced dose-dumping					
III. Other CPB Studies					
Genotype/phenotype studies					
Chronopharmacokinetics					
Pediatric development plan					
Literature References					
Total Number of Studies			2		

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			x	
2	Has the applicant provided metabolism and drug-drug interaction information?		x		Not required
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?			x	
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	x			
5	Has a rationale for dose selection been submitted?	x			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	x			

7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	x			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?	x			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?		x		
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?		x		
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?		x		
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?		x		
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?		x		

Reviewer's Comments

-Immunogenicity results were not available and will be pending on a validated assay.

-Need to consult QT-IRT review team.

-Clinical review team requested the sponsor to clarify the actual dose for each patient.

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

Yes

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

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

Reviewing Clinical Pharmacologist

Date

Team Leader/Supervisor

Date

Attachment 1. FDA QT-IRT Review

Date: April 5, 2011

From: CDER DCRP QT Interdisciplinary Review Team

Through: Norman Stockbridge, M.D., Ph.D. 
Division Director
Division of Cardiovascular and Renal Products /CDER

To: Erik Laughner
Regulatory Project Manager
Division of Biologic Oncology Products

Subject: QT-IRT Consult to BLA 102359

**Interdisciplinary Review Team for QT Studies Consultation:
QT Assessment Review**

BLA	125359
Brand Name	Erwinaze
Generic Name	<i>Erwinia</i> L-asparaginase
Sponsor	EUSA Pharma (USA) Inc.
Indication	ERWINAZE (<i>Erwinia</i> L-asparaginase) is indicated as a component of a multiagent chemotherapeutic regimen for the treatment of patients with: • Acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to (b) (4) <i>E. coli</i> derived asparaginase (b) (4)
Dosage Form	Erwinaze solution given as intramuscular (IM) injection
Drug Class	Therapeutic Protein
Therapeutic Dosing Regimen	25,000 IU/m ² IM three times a week for two weeks to replace each dose of peg-asparaginase or each course of asparaginase treatment
Duration of Therapeutic Use	Acute or Chronic
Maximum Tolerated Dose	Not Known
Submission Number and Date	September 9, 2010
Review Division	DBOP/HFD 150

1 SUMMARY

1.1 OVERALL SUMMARY OF FINDINGS

No definitive conclusions can be made regarding QTc effects due to Erwinaze from this study since this assessment involved single post-treatment, locally read ECGs collected at 1 hour post-dose 6 with categorical analysis only. The sponsor did not report the actual numeric values for QTc but number of patients who had absolute QTc ≥ 500 ms and change from baseline QTc ≥ 60 ms were reported. Therefore it is infeasible to derive the mean QT effect or to explore concentration-QT relationships following treatment with Erwinaze. In the absence of comparator arm data, the significance of the findings in categorical analysis is unclear.

ECG evaluations were conducted as part of the open-label study to evaluate pharmacology and toxicity of *erwinia* asparaginase following allergy to pegaspargase in treatment of children with acute lymphoblastic leukemia (ALL). In this study, 58 evaluable patients received 25,000 IU/m² IM for various courses (one course defined as three times a week for two weeks). During Course 1 only, 2 ECGs were performed at each of the following time points: 1) prior to Erwinaze dose 1, and 2) 1 hour following Erwinaze dose 6. Categorical analysis results are summarized in Table 1.

Table 1: Categorical QTc Interval Changes from Baseline – Safety Population

Parameter/Course	Time	QTc >500 ms	Change in QTc ≥ 60 ms
QT interval (sec)/1	Pre-dose (N=57)	0 (0%)	-
	1 h post dose (N=56)	3 (5.4%)	3 (5.4%)

The study was only conducted at the therapeutic dose level (25,000 IU/m²), which appears to be adequate.

1.2 QT INTERDISCIPLINARY REVIEW TEAM'S COMMENTS

- Typically, for monoclonal antibodies and similar large proteins we ask for an integrated cardiac safety analysis and categorical ECG values *from the entire clinical program*. If ECGs have not been collected, we have agreed in similar circumstances that an ECG sub-study with intensive PK and time-matched ECGs and central over-read may be an alternative assessment. However, the division has communicated to the sponsor that the categorical assessments conducted in this study would be sufficient (pre-BLA clinical meeting minutes, September 30, 2009). Since the division is more familiar with the safety profile of this product and other asparaginase preparations, and are in a better position to decide on feasibility issues related to conducting an ECG-substudy in this patient population, we defer to the division regarding further regulatory action related to QT assessment.

2 PROPOSED LABEL

Sponsor's proposes the following language under section 12.4 (Cardiac Repolarization) of the proposed label:

(b) (4)

QT-IRT Comments: Due to the study limitations we suggest that no language related to QT liability is included in the PI.

3 BACKGROUND

3.1 PRODUCT INFORMATION

Erwinaze contains the purified enzyme L-asparagine amidohydrolase (L-asparaginase) derived in crystalline form from the non-human (plant) pathogen *Erwinia chrysanthemi* (nee carotovora). The enzyme is also isolated from a variety of other sources (yeast, animal cells, fungi). Its molecular weight is about 135,000 daltons and is composed of four subunits (tetramers), each subunit having a molecular weight of about 35,000 daltons.

3.2 MARKET APPROVAL STATUS

In the 50 years since its discovery, L-asparaginase has become a vital component of multi-agent chemotherapy for childhood ALL. In the US, there are 2 asparaginase preparations approved by the FDA and available for clinical use: 1) native *E. coli* (Elspar®); and 2) pegaspargase (Oncaspar®), a modified (pegylated) form of the *E. coli* enzyme. A third preparation, *Erwinia* asparaginase (Crisantaspase, Erwinaze), derived from *Erwinia chrysanthemi*, is licensed in some countries in Europe and in Canada but is not and never has been commercially available in the US. Prior to 2002, it was available to patients with hypersensitivity to the *E. coli* derived preparations on a named patient basis. Because of manufacturing difficulties, the product was removed from the market, worldwide in 2003. In 2004, the issues were resolved and Erwinaze was again made available. EUSA Pharma is pursuing marketing approval in the USA.

3.3 PRECLINICAL INFORMATION

Non-Clinical studies per S7B guidelines were not conducted.

3.4 PREVIOUS CLINICAL EXPERIENCE

Source: Summary of Clinical Safety, eCTD 2.7.4

A total of 627 subjects received at least 1 dose of Erwinaze in the AALL07P2 and EMTP studies and were integrated across the clinical program. There were no deaths in the AALL07P2 study. There were six deaths during the EMTP study after informed consent was signed, one of which occurred one year after the patient completed treatment, patient ERW6012. One patient had Grade 5 Cardiac LV diastolic dysfunction/infection/disease

progression, another from intracranial hemorrhage and four were attributed to disease progression. ECGs were not routinely collected in the clinical program.

3.5 CLINICAL PHARMACOLOGY

Appendix 6.1 summarizes the key features of erwinaze's clinical pharmacology.

4 SPONSOR'S SUBMISSION

4.1 OVERVIEW

The QT-IRT did not review the protocol prior to conducting this study. The sponsor submitted the study report AALL07P2, including electronic datasets. PDF files of ECGs were sent for review.

4.2 QT STUDY

4.2.1 Title

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

4.2.2 Protocol Number

COG AALL07P2

4.2.3 Study Dates

February 23, 2009-April 30, 2010

4.2.4 Objectives

The primary objectives of this study were to determine if the 48-hour trough serum asparaginase activity was ≥ 0.1 IU/mL; to determine the frequency of L-asparaginase-related toxicity following Erwinaze treatment; and to characterize the pharmacokinetics (PK) of Erwinaze in children with acute lymphoblastic leukaemia (ALL) who are hypersensitive to pegylated asparaginase (pegaspargase).

4.2.5 Study Description

4.2.5.1 Design

This was an open-label study was to assess PK and pharmacodynamics (PD) and related toxicities of Erwinaze used as replacement therapy for pegaspargase dosing. Patients received Erwinaze 25,000 IU/m² x 6 doses intramuscularly (IM) on a Monday/Wednesday/Friday, Wednesday/Friday/Monday or Friday/Monday/Wednesday schedule as a replacement for each scheduled dose of pegaspargase remaining on the original treatment protocol.

4.2.5.2 Sponsor's Justification for Doses

"*Erwinia* L-asparaginase has been in clinical use for 25 years in both the US and abroad. Clinical trials with Erwinaze delivered intramuscularly (IM) have been conducted with

different doses and schedules in comparisons with standard therapies including *E. coli* and pegaspargase. Based on the results of numerous studies done to determine the depletion of asparagine, the rate of disease free-survival during Induction and Re-Induction, and the incidence of hypersensitivity reaction to asparaginase, an estimate of equivalent doses of the different preparations of asparaginase (*Erwinia*, *E. coli*, *E. coli* pegylated) has been proposed for complete asparagine depletion for a time period of 2 weeks that included: *E. coli* 6,000 to 10,000 IU/m² every 2 to 3 days; Erwinaze 20,000 to 25,000 IU/m² every 2 to 3 days; and pegasparaginase 2,500 to 3,500 IU/m² every 2 weeks."

Reviewer's Comment: The study was only conducted at the therapeutic dose level (25,000 IU/m²), which appears to be reasonable. No information regarding the effect of intrinsic and extrinsic factors on the PK of the drug is available. Thus, whether or not the dose is appropriate to cover increase in exposures that may result from effect of intrinsic and extrinsic factors is not known.

4.2.5.3 Instructions with Regard to Meals

Reviewer's comments: The effect of food is not applicable since erwinaze is administered via intramuscular injection.

4.2.5.4 ECG and PK Assessments

"Two 12-lead ECGs were obtained during Course 1 only, one prior to Erwinaze dose 1, and one 1 hour following Erwinaze dose 6."

(Source: PK/PD Study in Patients with ALL: COG AALL07P2, pg 42)

"Pharmacokinetic studies were performed after the first or second dose of Erwinaze (depending on the first day of treatment) and then at steady state in all patients during the first course of therapy. The time intervals for blood sampling (for each sampling, 2 mL of blood was drawn) included pre-dose, 2-hour, and 24-hour samples as well as 48 hour and 72-hour trough samples. Additionally, a pre-Course 1 sample and Day 15 and Day 22 samples were assayed. In subsequent courses, studies performed included pre-course, 48-hour, and Day 15 trough samples."

(Source: PK/PD Study in Patients with ALL: COG AALL07P2, pg 41)

Reviewer's Comment: The t_{max} of the compound is not reported and thus it cannot be determined if one sample (2 h post-dose) collected in each patient would capture the t_{max}.

4.2.6 ECG Collection

Single Electrocardiograms (ECGs) were performed to assess the possible effect of Erwinaze on QTc (QT interval transformed/normalized into a heart rate corrected value). During Course 1 only, 2 ECGs were performed at each of the following time points: 1) prior to Erwinaze dose 1, and 2) 1 hour following Erwinaze dose 6.

If the QTc value was ≥ 550 ms or there was an increase in the QTc of ≥ 100 ms from baseline, Erwinaze was to be discontinued and the patient was considered to be off

protocol therapy. ECG and electrolyte measurements were to be performed 3 times per week post QTc prolongation, until the QTc fell below 460 ms.

If the QTc value was ≥ 500 ms (and < 550 ms), or there was an increase from baseline of ≥ 60 ms (but < 100 ms), to a QTc value ≥ 460 ms, then the ECG was to be repeated within 48 hours to confirm the QTc prolongation. If the repeat ECG again met the criteria, Erwinaze was to be discontinued and the patient was considered to be off protocol therapy. ECG and electrolyte measurements were to be performed 3 times per week post QTc prolongation until the QTc fell below 460 ms.

4.2.7 Sponsor's Results

4.2.7.1 Study Subjects

59 patients 1-30 years of age with ALL and \geq Grade 2 hypersensitivity reaction (Common Terminology Criteria for Adverse Events [CTCAE] version 3.0) to pegaspargase were enrolled. Of the 59 patients included, 58 patients initiated protocol therapy; 10 (17.2%) prematurely discontinued the study. Four (6.9%) patients discontinued the study early due to AEs (\geq Grade 2 allergy/hypersensitivity to Erwinaze), 2 (3.4%) patients withdrew consent, and 2 (3.4%) patients were discontinued at the physician's discretion. One (1.7%) patient discontinued the study early due to progressive disease, and 1 (1.7%) patient discontinued due to 'other' (confirmation of the patient's ineligibility was not confirmed by the COG study team until after the patient received the first dose of Erwinaze). The sponsor reports no discontinuation from treatment due to QT prolongation.

4.2.7.2 Statistical Analyses

Sponsor conducted a categorical analysis and reported the number and percent of patients with a QTc interval increase ≥ 500 ms and a change ≥ 60 ms from baseline.

"QTc through ECG was only assessed in Course 1. No patient had a QTc interval > 500 msec at the pre-dose 1 time point. One patient had both a > 500 msec value and a change in QTc ≥ 60 msec. Two patients had a QTc interval > 500 msec 1 hour after dose 6, and 2 different patients had a change in QTc ≥ 60 msec."

Table 2: QTc Interval and Changes from Baseline – Safety Population

Parameter/Course n (%)	Dose	QTc > 500 msec N=58	Change in QTc ≥ 60 msec N=58
QT interval (sec)/1	Pre-dose 1	0 (0)	
	1 hr post-dose 6	3 (5.2)	3 (5.2)

Percentages were calculated from the number of patients dosed.

(Source: PK/PD Study in Patients with ALL: COG AALL07P2, Table 12.11, Pg 95)

Reviewer's Comments: Sponsor did not report the absolute QTc values for these patients. The above numbers were verified from the adsl.xpt dataset using QTC500D1, QTC500D6

and QTC60D1 variables. However, pre-dose $QTc \geq 500$ ms, post-dose $QTc \geq 500$ ms and change in $QTc > 60$ ms were evaluated for 57, 56 and 56 patients, respectively. Thus, percent of patients with $QTc \geq 500$ ms or change in $QTc \geq 60$ sec are 5.4% (3/56).

4.2.7.3 Safety Analysis

No deaths occurred in the study. The sponsor reported no cardiac AEs.

4.2.7.4 Clinical Pharmacology

4.2.7.4.1 Pharmacokinetic Analysis

The PK of erwinaze was measured in form of asparaginase activity. Sparse PK samples with one sample collected at 2 h post dose and trough samples were collected in order to assess asparaginase activity. For details refer to section 11.3.1 of the study report (Source: PK/PD Study in Patients with ALL: COG AALL07P2, pg 57).

4.2.7.4.2 Exposure-Response Analysis

No exposure-response analysis was conducted by the sponsor.

5 REVIEWERS' ASSESSMENT

5.1 CLINICAL PHARMACOLOGY ASSESSMENT

Not applicable. Sponsor did not report the actual numeric QTc values.

5.2 CLINICAL ASSESSMENTS

5.2.1 Safety assessments

None of the events identified to be of clinical importance per the ICH E 14 guidelines i.e. syncope, seizure, significant ventricular arrhythmias or sudden cardiac death occurred in this study. As reported earlier, 3 subjects had an absolute increase in $QTcF$ over 500 ms post-treatment and a change from baseline $QTcF$ greater than 60 ms. In the absence of data from an active comparator, these changes are hard to interpret in this patient population.

5.2.2 ECG assessments

PDF copies of the single ECGs, of poor quality, were available for review. On review of a subset, 40-ms markings are not visible in several ECGs making assessment of interpretation quality difficult –see example below. However since these are PDF copies, no judgment can be made regarding the original ECGs. Overall ECGs were possibly adequate for categorical assessment but not for any quantification.

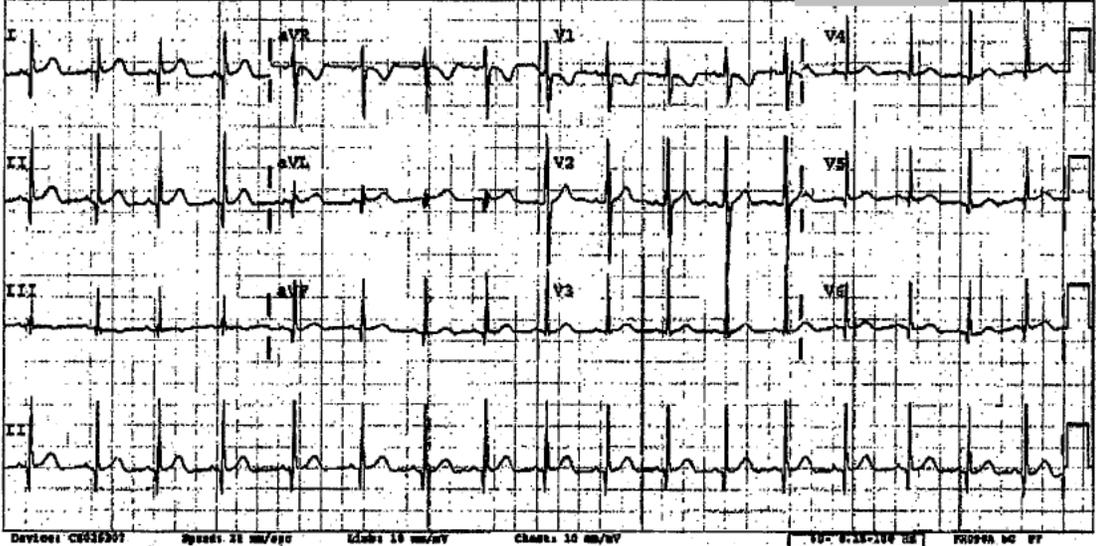
COG # 791194

post dose 6

X001081811
DOB: (b) (6) 3 Years Female 23-Sep-2009 13:04:48

Dept: Med/Surg
Room: 701 END
Opnt: JMS/MSDC

XR 102 [PRP] (b) (4) ----- PEDIATRIC ECG INTERPRETATION -----
PR 118
QRSD 48
QT 318
QTc 412
Accounts: (b) (4)
-- AXES --
P 59
QRS 58
T 22
Order #: K09K09930009
Site ID: K0009934542
Standard 12
Requested by: (b) (4)
Previous ECG: 06-Sep-2009 12:47:58 - No Severity Confirmed
Mortum Healthcare System - Kousik Children (11-40-04) Continued On Behalf Of: (b) (4) 6-Sep-2009 15:13:06



5.2.3 PR and QRS Interval
Effects on these intervals were not reported.

6 APPENDIX

6.1 HIGHLIGHTS OF CLINICAL PHARMACOLOGY

Highlights of Clinical Pharmacology STN 125359 (ERWINAZE)

Therapeutic dose	25,000 IU/m ²	
Maximum tolerated dose	This was not done	
Principal adverse events	Hypersensitivity Reaction, Pancreatitis, Hyperglycemia, thrombo-embolic disorders, liver dysfunction	
Maximum dose tested	Single Dose	No done
	Multiple Dose	25,000 IU/m ² on a Monday/Wednesday/Friday schedule for 2 weeks to replace every dose of Oncaspar
Exposures Achieved at Maximum Tested Dose	Single Dose	Not Done
	Multiple Dose	Not Done
Range of linear PK	Not Done	
Accumulation at steady state	Not Done	
Metabolites	Not Known	
Absorption	Absolute/Relative Bioavailability	Not done
	Tmax	Not done
Distribution	Vd/F or Vd	Not done
	% bound	Not done
Elimination	Route	• Liver
	Terminal t _{1/2}	Not Known
	CL/F or CL	Not Know
Intrinsic Factors	Age	Not Known
	Sex	Not Known
	Race	Not Known
	Hepatic & Renal Impairment	Not Known
Extrinsic Factors	Drug interactions	Not Done
	Food Effects	Given only IM
Expected High Clinical Exposure Scenario	None Done	

Attachment 2. FDA DSI Comments

MEMORANDUM

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

DATE: March 23, 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: Inspectional findings at (b)(4)
(b)(4), BLA 125359, *Erwinia* L-Asparaginase,
sponsored by EUSA Pharma (USA), Inc.

The following is a summary of inspectional findings at the
(b)(4)
(b)(4) both under the supervision of
(b)(4) concerning COG Study AALL07P2 of BLA
125359:

Findings Specific for the Asparaginase Assay:

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:

<u>Subject #</u>	<u>Course #</u>	<u>Sample #</u>
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

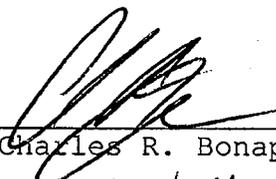
2. Failure to exclude serum samples from clinical sites received in the thawed state.
3. Records of freezer temperatures for storage of asparaginase samples were not retrievable in an auditable form.
4. Failure to document the times when samples were removed from frozen storage for analysis.
5. Failure to adequately document preparation and storage of asparaginase stock solutions.
6. Failure to adjust nominal asparaginase concentrations in calibrator and quality control solutions for the actual content of L-asparaginase commercial vials.
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.

Findings Specific for the Asparagine Assay:

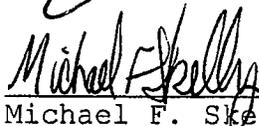
1. Failure to reject analytical runs on 3/9/10, 3/11/10, 3/15/10, 3/25/10, 3/30/10, 4/2/10, 4/8/10, 4/15/10, and 12/8/10 when the quality control (QC) samples failed the acceptance criterion at one or two of the three QC concentrations.
2. Failure to reject chromatograms when no asparagine internal standard was detected or when peaks could not be accurately integrated.

3. Failure to exclude plasma samples from clinical sites which were unacidified or were received in the thawed state.
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.
 - 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.
 - c) 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.
 - 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).
 - e) The effectiveness of hydrochloric acid in preserving asparagine in plasma was tested only for *E. coli* asparaginase, not for *Erwinia* asparaginase.
 - f) The times when samples were removed from frozen storage for analysis were not recorded.
5. Between-run accuracy and precision for the asparagine assay were not evaluated.
6. Failure to evaluate the variability in recovery of asparagine in more than one plasma sample in a run.

7. Failure to evaluate the stability of asparagine in stock solutions or extracts.
8. 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.
9. Failure to verify (by balance printer or witness) the weights of asparagine used for calibrator and QC stock solutions.



Charles R. Bonapace, Pharm.D.



Michael F. Skelly, Ph.D.

cc:
DSI/GLPBB/Haidar/Yau/Lee/Dejernet/CF
DBOP/Dinndorf/Demko/Laughner
DCP5/Yang/Zhao/Booth
OPS/OBP/DTP/Beaucage
ORA/HFR-NE250/Noe/Murphy
OC/DSI/Ball/Viswanathan
OC/Cooper
Draft: MFS 3/23/11
Draft: CRB 3/23/11
Edit: MKY 3/24/11
DSI: 6168
O:\BIOEQUIV\EIRCOVER\125359
FACTS: 1252967

Email:
CDER DSI PM TRACK

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA/BLA or Supplement**

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	125359/0	Brand Name	Erwinaze
OCP Division (I, II, III, IV, V)	V	Generic Name	Erwinia L-asparaginase
Medical Division	DBOP	Drug Class	Biologics
OCP Reviewer	Jun Yang, Ph.D.	Indication(s)	Acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to (b) (4) (b) (4) <i>E. coli</i> derived asparaginase
OCP Team Leader	Hong Zhao, Ph.D.	Dosage Form	10,000 IU/vial
Pharmacometrics Reviewer	Jun Yang, Ph.D.	Dosing Regimen	25,000 IU/m ² IM three times a week for two weeks
Date of Submission	11/1/2010	Route of Administration	Intramuscular (IM) Injection
Estimated Due Date of OCP Review	4/10/2011	Sponsor	EUSA Pharma
Medical Division Due Date	4/12/2011	Priority Classification	Priority
PDUFA Due Date	5/3/2011		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:	x			
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD -				
Phase 2:				
Phase 3:	x	1		
PK/PD -				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies				
Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		2		

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			x	
2	Has the applicant provided metabolism and drug-drug interaction information?		x		Not required
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?			x	
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	x			
5	Has a rationale for dose selection been submitted?	x			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	x			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	x			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?	x			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?		x		
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?		x		
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?		x		
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?		x		
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?		x		

Reviewer's Comments

- Immunogenicity results were not available and will be pending on a validated assay.
- Need to consult QT-IRT review team.
- Clinical review team requested the sponsor to clarify the actual dose for each patient.

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

Yes

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

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



Reviewing Clinical Pharmacologist 12/16/2010
Date



Team Leader/Supervisor 12/16/10
Date

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

Date: April 6, 2011

From: CDER DCRP QT Interdisciplinary Review Team

Through: Norman Stockbridge, M.D., Ph.D. 
Division Director
Division of Cardiovascular and Renal Products /CDER

To: Erik Laughner
Regulatory Project Manager
Division of Biologic Oncology Products

Subject: QT-IRT Consult to BLA 125359

**Interdisciplinary Review Team for QT Studies Consultation:
QT Assessment Review**

BLA	125359
Brand Name	Erwinaze
Generic Name	<i>Erwinia</i> L-asparaginase
Sponsor	EUSA Pharma (USA) Inc.
Indication	ERWINAZE (<i>Erwinia</i> L-asparaginase) is indicated as a component of a multiagent chemotherapeutic regimen for the treatment of patients with: • Acute lymphoblastic leukemia (ALL) who have developed hypersensitivity to (b) (4) <i>E. coli</i> derived asparaginase • (b) (4)
Dosage Form	Erwinaze solution given as intramuscular (IM) injection
Drug Class	Therapeutic Protein
Therapeutic Dosing Regimen	25,000 IU/m ² IM three times a week for two weeks to replace each dose of peg-asparaginase or each course of asparaginase treatment
Duration of Therapeutic Use	Acute or Chronic
Maximum Tolerated Dose	Not Known
Submission Number and Date	September 9, 2010
Review Division	DBOP/HFD 150

1 SUMMARY

1.1 OVERALL SUMMARY OF FINDINGS

No definitive conclusions can be made regarding QTc effects due to Erwinaze from this study since this assessment involved single post-treatment, locally read ECGs collected at 1 hour post-dose 6 with categorical analysis only. The sponsor did not report the actual numeric values for QTc but number of patients who had absolute QTc ≥ 500 ms and change from baseline QTc ≥ 60 ms were reported. Therefore it is infeasible to derive the mean QT effect or to explore concentration-QT relationships following treatment with Erwinaze. In the absence of comparator arm data, the significance of the findings in categorical analysis is unclear.

ECG evaluations were conducted as part of the open-label study to evaluate pharmacology and toxicity of *erwinia* asparaginase following allergy to pegaspargase in treatment of children with acute lymphoblastic leukemia (ALL). In this study, 58 evaluable patients received 25,000 IU/m² IM for various courses (one course defined as three times a week for two weeks). During Course 1 only, 2 ECGs were performed at each of the following time points: 1) prior to Erwinaze dose 1, and 2) 1 hour following Erwinaze dose 6. Categorical analysis results are summarized in Table 1.

Table 1: Categorical QTc Interval Changes from Baseline – Safety Population

Parameter/Course	Time	QTc >500 ms	Change in QTc ≥ 60 ms
QT interval (sec)/1	Pre-dose (N=57)	0 (0%)	-
	1 h post dose (N=56)	3 (5.4%)	3 (5.4%)

The study was only conducted at the therapeutic dose level (25,000 IU/m²), which appears to be adequate.

1.2 QT INTERDISCIPLINARY REVIEW TEAM'S COMMENTS

- Typically, for monoclonal antibodies and similar large proteins we ask for an integrated cardiac safety analysis and categorical ECG values *from the entire clinical program*. If ECGs have not been collected, we have agreed in similar circumstances that an ECG sub-study with intensive PK and time-matched ECGs and central over-read may be an alternative assessment. However, the division has communicated to the sponsor that the categorical assessments conducted in this study would be sufficient (pre-BLA clinical meeting minutes, September 30, 2009). Since the division is more familiar with the safety profile of this product and other asparaginase preparations, and are in a better position to decide on feasibility issues related to conducting an ECG-substudy in this patient population, we defer to the division regarding further regulatory action related to QT assessment.

2 PROPOSED LABEL

Sponsor's proposes the following language under section 12.4 (Cardiac Repolarization) of the proposed label:



QT-IRT Comments: Due to the study limitations we suggest that no language related to QT liability is included in the PI.

3 BACKGROUND

3.1 PRODUCT INFORMATION

Erwinaze contains the purified enzyme L-asparagine amidohydrolase (L-asparaginase) derived in crystalline form from the non-human (plant) pathogen *Erwinia chrysanthemi* (nee carotovora). The enzyme is also isolated from a variety of other sources (yeast, animal cells, fungi). Its molecular weight is about 135,000 daltons and is composed of four subunits (tetramers), each subunit having a molecular weight of about 35,000 daltons.

3.2 MARKET APPROVAL STATUS

In the 50 years since its discovery, L-asparaginase has become a vital component of multi-agent chemotherapy for childhood ALL. In the US, there are 2 asparaginase preparations approved by the FDA and available for clinical use: 1) native *E. coli* (Elspar®); and 2) pegaspargase (Oncaspar®), a modified (pegylated) form of the *E. coli* enzyme. A third preparation, *Erwinia* asparaginase (Crisantaspase, Erwinaze), derived from *Erwinia chrysanthemi*, is licensed in some countries in Europe and in Canada but is not and never has been commercially available in the US. Prior to 2002, it was available to patients with hypersensitivity to the *E. coli* derived preparations on a named patient basis. Because of manufacturing difficulties, the product was removed from the market, worldwide in 2003. In 2004, the issues were resolved and Erwinaze was again made available. EUSA Pharma is pursuing marketing approval in the USA.

3.3 PRECLINICAL INFORMATION

Non-Clinical studies per S7B guidelines were not conducted.

3.4 PREVIOUS CLINICAL EXPERIENCE

Source: Summary of Clinical Safety, eCTD 2.7.4

A total of 627 subjects received at least 1 dose of Erwinaze in the AALL07P2 and EMTP studies and were integrated across the clinical program. There were no deaths in the AALL07P2 study. There were six deaths during the EMTP study after informed consent was signed, one of which occurred one year after the patient completed treatment, patient ERW6012. One patient had Grade 5 Cardiac LV diastolic dysfunction/infection/disease

progression, another from intracranial hemorrhage and four were attributed to disease progression. ECGs were not routinely collected in the clinical program.

3.5 CLINICAL PHARMACOLOGY

Appendix 6.1 summarizes the key features of erwinaze's clinical pharmacology.

4 SPONSOR'S SUBMISSION

4.1 OVERVIEW

The QT-IRT did not review the protocol prior to conducting this study. The sponsor submitted the study report AALL07P2, including electronic datasets. PDF files of ECGs were sent for review.

4.2 QT STUDY

4.2.1 Title

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

4.2.2 Protocol Number

COG AALL07P2

4.2.3 Study Dates

February 23, 2009-April 30, 2010

4.2.4 Objectives

The primary objectives of this study were to determine if the 48-hour trough serum asparaginase activity was ≥ 0.1 IU/mL; to determine the frequency of L-asparaginase-related toxicity following Erwinaze treatment; and to characterize the pharmacokinetics (PK) of Erwinaze in children with acute lymphoblastic leukaemia (ALL) who are hypersensitive to pegylated asparaginase (pegaspargase).

4.2.5 Study Description

4.2.5.1 Design

This was an open-label study was to assess PK and pharmacodynamics (PD) and related toxicities of Erwinaze used as replacement therapy for pegaspargase dosing. Patients received Erwinaze 25,000 IU/m² x 6 doses intramuscularly (IM) on a Monday/Wednesday/Friday, Wednesday/Friday/Monday or Friday/Monday/Wednesday schedule as a replacement for each scheduled dose of pegaspargase remaining on the original treatment protocol.

4.2.5.2 Sponsor's Justification for Doses

"*Erwinia* L-asparaginase has been in clinical use for 25 years in both the US and abroad. Clinical trials with Erwinaze delivered intramuscularly (IM) have been conducted with

different doses and schedules in comparisons with standard therapies including *E. coli* and pegaspargase. Based on the results of numerous studies done to determine the depletion of asparagine, the rate of disease free-survival during Induction and Re-Induction, and the incidence of hypersensitivity reaction to asparaginase, an estimate of equivalent doses of the different preparations of asparaginase (*Erwinia*, *E. coli*, *E. coli* pegylated) has been proposed for complete asparagine depletion for a time period of 2 weeks that included: *E. coli* 6,000 to 10,000 IU/m² every 2 to 3 days; Erwinaze 20,000 to 25,000 IU/m² every 2 to 3 days; and pegasparginase 2,500 to 3,500 IU/m² every 2 weeks.”

Reviewer’s Comment: The study was only conducted at the therapeutic dose level (25,000 IU/m²), which appears to be reasonable. No information regarding the effect of intrinsic and extrinsic factors on the PK of the drug is available. Thus, whether or not the dose is appropriate to cover increase in exposures that may result from effect of intrinsic and extrinsic factors is not known.

4.2.5.3 Instructions with Regard to Meals

Reviewer’s comments: The effect of food is not applicable since erwinaze is administered via intramuscular injection.

4.2.5.4 ECG and PK Assessments

“Two 12-lead ECGs were obtained during Course 1 only, one prior to Erwinaze dose 1, and one 1 hour following Erwinaze dose 6.”

(Source: PK/PD Study in Patients with ALL: COG AALL07P2, pg 42)

“Pharmacokinetic studies were performed after the first or second dose of Erwinaze (depending on the first day of treatment) and then at steady state in all patients during the first course of therapy. The time intervals for blood sampling (for each sampling, 2 mL of blood was drawn) included pre-dose, 2-hour, and 24-hour samples as well as 48 hour and 72-hour trough samples. Additionally, a pre-Course 1 sample and Day 15 and Day 22 samples were assayed. In subsequent courses, studies performed included pre-course, 48-hour, and Day 15 trough samples.”

(Source: PK/PD Study in Patients with ALL: COG AALL07P2, pg 41)

Reviewer’s Comment: The t_{max} of the compound is not reported and thus it cannot be determined if one sample (2 h post-dose) collected in each patient would capture the t_{max} .

4.2.6 ECG Collection

Single Electrocardiograms (ECGs) were performed to assess the possible effect of Erwinaze on QTc (QT interval transformed/normalized into a heart rate corrected value). During Course 1 only, 2 ECGs were performed at each of the following time points: 1) prior to Erwinaze dose 1, and 2) 1 hour following Erwinaze dose 6.

If the QTc value was ≥ 550 ms or there was an increase in the QTc of ≥ 100 ms from baseline, Erwinaze was to be discontinued and the patient was considered to be off

protocol therapy. ECG and electrolyte measurements were to be performed 3 times per week post QTc prolongation, until the QTc fell below 460 ms.

If the QTc value was ≥ 500 ms (and < 550 ms), or there was an increase from baseline of ≥ 60 ms (but < 100 ms), to a QTc value ≥ 460 ms, then the ECG was to be repeated within 48 hours to confirm the QTc prolongation. If the repeat ECG again met the criteria, Erwinaze was to be discontinued and the patient was considered to be off protocol therapy. ECG and electrolyte measurements were to be performed 3 times per week post QTc prolongation until the QTc fell below 460 ms.

4.2.7 Sponsor’s Results

4.2.7.1 Study Subjects

59 patients 1-30 years of age with ALL and \geq Grade 2 hypersensitivity reaction (Common Terminology Criteria for Adverse Events [CTCAE] version 3.0) to pegaspargase were enrolled. Of the 59 patients included, 58 patients initiated protocol therapy; 10 (17.2%) prematurely discontinued the study. Four (6.9%) patients discontinued the study early due to AEs (\geq Grade 2 allergy/hypersensitivity to Erwinaze), 2 (3.4%) patients withdrew consent, and 2 (3.4%) patients were discontinued at the physician’s discretion. One (1.7%) patient discontinued the study early due to progressive disease, and 1 (1.7%) patient discontinued due to ‘other’ (confirmation of the patient’s ineligibility was not confirmed by the COG study team until after the patient received the first dose of Erwinaze). The sponsor reports no discontinuation from treatment due to QT prolongation.

4.2.7.2 Statistical Analyses

Sponsor conducted a categorical analysis and reported the number and percent of patients with a QTc interval increase ≥ 500 ms and a change ≥ 60 ms from baseline.

“QTc through ECG was only assessed in Course 1. No patient had a QTc interval > 500 msec at the pre-dose 1 time point. One patient had both a > 500 msec value and a change in QTc ≥ 60 msec. Two patients had a QTc interval > 500 msec 1 hour after dose 6, and 2 different patients had a change in QTc ≥ 60 msec.”

Table 2: QTc Interval and Changes from Baseline – Safety Population

Parameter/Course n (%)	Dose	QTc > 500 msec N=58	Change in QTc ≥ 60 msec N=58
QT interval (sec)/1	Pre-dose 1	0 (0)	
	1 hr post-dose 6	3 (5.2)	3 (5.2)

Percentages were calculated from the number of patients dosed.

(Source: PK/PD Study in Patients with ALL: COG AALL07P2, Table 12.11, Pg 95)

Reviewer’s Comments: Sponsor did not report the absolute QTc values for these patients. The above numbers were verified from the adsl.xpt dataset using QTC500D1, QTC500D6

and QTC60D1 variables. However, pre-dose QTc \geq 500 ms, post-dose QTc \geq 500 ms and change in QTc >60 ms were evaluated for 57, 56 and 56 patients, respectively. Thus, percent of patients with QTc \geq 500 ms or change in QTc \geq 60 sec are 5.4% (3/56).

4.2.7.3 Safety Analysis

No deaths occurred in the study. The sponsor reported no cardiac AEs.

4.2.7.4 Clinical Pharmacology

4.2.7.4.1 Pharmacokinetic Analysis

The PK of erwinaze was measured in form of asparaginase activity. Sparse PK samples with one sample collected at 2 h post dose and trough samples were collected in order to assess asparaginase activity. For details refer to section 11.3.1 of the study report (*Source: PK/PD Study in Patients with ALL: COG AALL07P2, pg 57*).

4.2.7.4.2 Exposure-Response Analysis

No exposure-response analysis was conducted by the sponsor.

5 REVIEWERS' ASSESSMENT

5.1 CLINICAL PHARMACOLOGY ASSESSMENT

Not applicable. Sponsor did not report the actual numeric QTc values.

5.2 CLINICAL ASSESSMENTS

5.2.1 Safety assessments

None of the events identified to be of clinical importance per the ICH E 14 guidelines i.e. syncope, seizure, significant ventricular arrhythmias or sudden cardiac death occurred in this study. As reported earlier, 3 subjects had an absolute increase in QTcF over 500 ms post-treatment and a change from baseline QTcF greater than 60 ms. In the absence of data from an active comparator, these changes are hard to interpret in this patient population.

5.2.2 ECG assessments

PDF copies of the single ECGs, of poor quality, were available for review. On review of a subset, 40-ms markings are not visible in several ECGs making assessment of interpretation quality difficult –see example below. However since these are PDF copies, no judgment can be made regarding the original ECGs. Overall ECGs were possibly adequate for categorical assessment but not for any quantification.

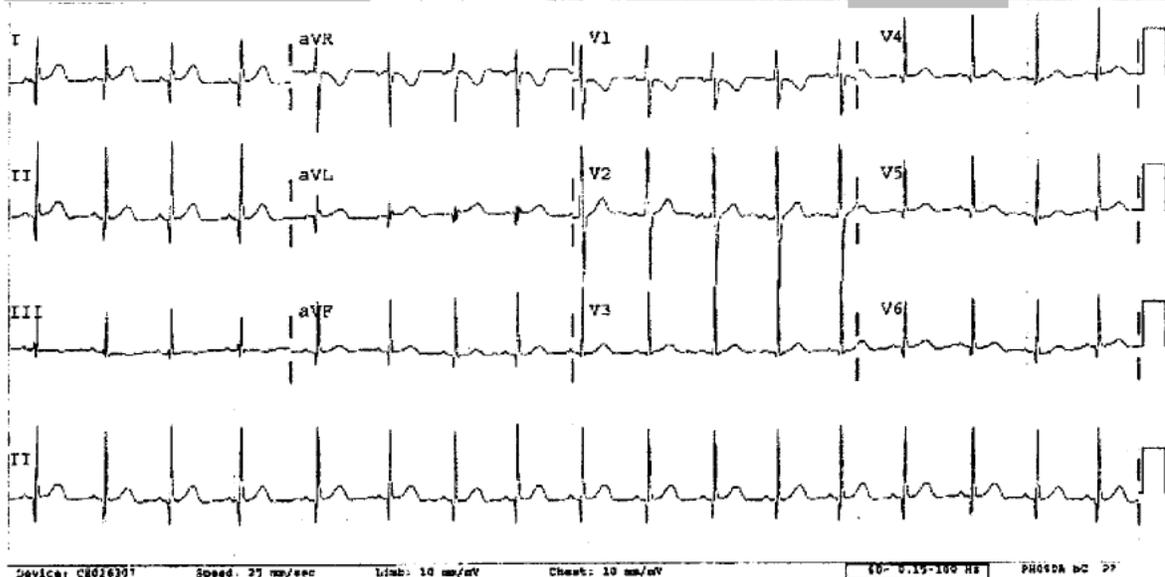
COG # 791194 post close to
 X081DR1511 23-Sep-2009 13:04:48
 DOB: (b) (6) Female Dept: Med/Surg
 Room: 703
 Oper: (b) (4)

HR 102 (PEDI) PEDIATRIC ECG INTERPRETATION
 (SR) SINUS RHYTHM Account# (b) (6)

PA 116
 QRS 68
 QT 316
 QTc 412

-- AXYS --
 P 55
 QRS 55
 T 22

Order #: K09K0923009
 Rec ID: R0007334568
 Standard 12
 Recorded By: (b) (4)
 Previous ECG: 06-Sep-2009 12:47:59 - No Severity Confirmed
 Confirmed On Behalf Of: (b) (4) 24-Sep-2009 13:12:08



5.2.3 PR and QRS Interval

Effects on these intervals were not reported.

6 APPENDIX

6.1 HIGHLIGHTS OF CLINICAL PHARMACOLOGY

Highlights of Clinical Pharmacology STN 125359 (ERWINAZE)

Therapeutic dose	25,000 IU/m ²	
Maximum tolerated dose	This was not done	
Principal adverse events	Hypersensitivity Reaction, Pancreatitis, Hyperglycemia, thrombo-embolic disorders, liver dysfunction	
Maximum dose tested	Single Dose	No done
	Multiple Dose	25,000 IU/m ² on a Monday/Wednesday/Friday schedule for 2 weeks to replace every dose of Oncaspar
Exposures Achieved at Maximum Tested Dose	Single Dose	Not Done
	Multiple Dose	Not Done
Range of linear PK	Not Done	
Accumulation at steady state	Not Done	
Metabolites	Not Known	
Absorption	Absolute/Relative Bioavailability	Not done
	T _{max}	Not done
Distribution	V _d /F or V _d	Not done
	% bound	Not done
Elimination	Route	• Liver
	Terminal t _{1/2}	Not Known
	CL/F or CL	Not Know
Intrinsic Factors	Age	Not Known
	Sex	Not Known
	Race	Not Known
	Hepatic & Renal Impairment	Not Known
Extrinsic Factors	Drug interactions	Not Done
	Food Effects	Given only IM
Expected High Clinical Exposure Scenario	None Done	