Erwinia Asparaginase Achieves Therapeutic Activity after Pegaspargase Allergy: A Report from the Children’s Oncology Group

Wanda L. Salzer, MD\textsuperscript{1}, Barbara Asselin MD\textsuperscript{2}, Jeffrey G. Supko, Ph.D.\textsuperscript{3}, Meenakshi Devidas, PhD\textsuperscript{4}, Nicole A. Kaiser, RPh\textsuperscript{5}, Paul Plourde, MD\textsuperscript{6}, Naomi J. Winick, MD\textsuperscript{7}, Gregory H. Reaman, MD\textsuperscript{8}, Elizabeth Raetz, MD\textsuperscript{9}, William L. Carroll, MD\textsuperscript{10}, Stephen P Hunger, MD\textsuperscript{5,11}

\textsuperscript{1}U.S. Army Medical Research and Materiel Command, Fort Detrick, MD, USA
\textsuperscript{2}Department of Pediatrics, Univ of Rochester School of Medicine, Golisano Children's Hospital at URMC, Rochester, NY, USA
\textsuperscript{3}Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
\textsuperscript{4}Department of Biostatistics, Colleges of Medicine Public Health & Health Professions, University of Florida, Gainesville, FL, USA
\textsuperscript{5}Children’s Hospital Colorado, Aurora, CO, USA
\textsuperscript{6}EUSA Pharma, Langhorne, PA, USA
\textsuperscript{7}Division of Pediatric Hematology/Oncology, University of Texas Southwestern School of Medicine, Dallas, TX, USA
\textsuperscript{8}Department of Hematology-Oncology, Children’s National Medical Center, Washington, DC, USA
\textsuperscript{9}New York University Langone Medical Center, New York, NY, USA
\textsuperscript{10}New York University Cancer Institute, New York, NY, USA
\textsuperscript{11}Pediatric Heme/Onc/BMT, University of Colorado School of Medicine, Aurora, CO, USA
Address for correspondence and reprint requests

Wanda L. Salzer, M.D.

U.S. Army Medical Research and Materiel Command

Combat Casualty Care Research Program

504 Scott St

Ft. Detrick, MD 21702-5012

Phone: 301-619-5308

E-mail: wanda.l.salzer.mil@mail.mil

Running Title: Erwinia Asparaginase After Pegaspargase Allergy

Key words: ALL, asparaginase, chemotherapy, pharmacokinetics, clinical trials
**Key Points:**

Following allergy to pegasparagase, *Erwinia* asparaginase 25,000-IU/m² x 6 doses IM M/W/F can be substituted for a single dose of pegasparagase.

*Erwinia* asparaginase was granted approval by the FDA in November 2011, for use in patients with allergic reactions to *E. coli*-derived asparaginase.

**Abstract**

Children’s Oncology Group AALL07P2 tested whether substitution of *Erwinia* asparaginase 25,000-IU/m² for 6 doses intramuscularly (IM) given Monday/Wednesday/Friday to children and young adults with acute lymphoblastic leukemia (ALL) and clinical allergy to pegasparagase would provide a 48-hour nadir serum asparaginase activity (NSAA) ≥0.10-IU/mL in at least 70% of patients. AALL07P2 enrolled 55 eligible/evaluable patients. NSAA ≥0.1-IU/mL was achieved in 38/41 patients (92.7%) with samples meeting acceptability criteria 48-hours after dosing and in 38/43 patients (88.4%) 72-hours after dosing during course 1. Among acceptable samples obtained during all therapy courses, 95.8% (252/263) of 48-hour samples and 84.5% (125/148) of 72-hour samples had NSAA ≥0.10-IU/mL. Pharmacokinetic parameters were estimated by fitting the serum asparaginase activity-time course for all 6 doses given during course 1 to a one compartment open model with first order absorption. *Erwinia* asparaginase administered with this schedule achieved therapeutic NSAA at both 48- and 72-hours and was well tolerated with no reports of hemorrhage, thrombosis, or death, and few cases of grade 2-3 allergic reaction (n=6), grade 1-3 hyperglycemia (n=6), and grade 1 pancreatitis (n=1). Following allergy to pegasparagase, *Erwinia* asparaginase 25,000-IU/m² x 6 doses IM Monday/Wednesday/Friday for 2 weeks can be substituted for a single dose of pegasparagase.
Introduction

Following the first reported use of asparaginase in an 8 year old boy with relapsed acute lymphoblastic leukemia (ALL) in 1966, single agent studies in the early 1970s of asparaginase for the treatment of children diagnosed with ALL demonstrated response rates of 20-68%. Since this time, asparaginase has become an essential component of multi-agent chemotherapy for childhood ALL.

In the United States prior to November 2011, two asparaginase preparations received approval for use by the Food and Drug Administration (FDA), native E. coli asparaginase and pegaspargase. Pegaspargase has been the more commonly used product because it requires less frequent administration than E. coli asparaginase as a consequence of its longer biological half-life, and because of its lower immunogenicity. A third preparation, Erwinia asparaginase, derived from the bacterium Erwinia chrysanthemi, was not commercially available in the United States during the conduct of this study but was available on a compassionate use basis.

Pegaspargase has been the sole asparaginase preparation used in Children’s Oncology Group (COG) trials for newly diagnosed patients with ALL since 2005 (excluding infants ≤1 year old). The overall aim of COG AALL07P2, undertaken to support FDA approval of Erwinia asparaginase, was to evaluate the utility of Erwinia asparaginase as an alternative in cases of hypersensitivity to pegaspargase by determining if intramuscular (IM) administration of Erwinia asparaginase 25,000-IU/m² Monday/Wednesday/Friday (MWF) schedule for 2 weeks will achieve a nadir serum asparaginase activity (NSAA) ≥0.10-IU/mL, which has been associated with complete asparagine depletion.
Patients and Methods

Patients

Eligible patients on COG AALL07P2 were >1 to <30 years of age, currently enrolled on a frontline COG ALL treatment study, had documented $\geq$ grade 2 allergy (NCI Common Terminology Criteria 3.0) to pegaspargase, and had $\geq$1 remaining scheduled doses of pegaspargase. Patients who had previously received Erwinia asparaginase or had a history of $\geq$ grade 2 pancreatitis were excluded 24.

The study was approved by the National Cancer Institute and by Institutional Review Boards at the individual institutions prior to patient enrollment. Informed consent was obtained according to Department of Health and Human Services Guidelines and in accordance with the Declaration of Helsinki.

Treatment Plan

Erwinia asparaginase was provided by EUSA Pharma, Inc. (Langhorne, PA). Patients received Erwinia asparaginase 25,000-IU/m$^2$ x 6 doses IM on a MWF schedule for 2 weeks as a replacement for each remaining scheduled dose of pegaspargase. Because all other chemotherapy continued according to the original treatment protocol, patients were permitted to begin receiving Erwinia asparaginase on Monday, Wednesday, or Friday, so that their schedules are defined as MWF, WFM, or FMW. Adverse events (all grades) related to Erwinia asparaginase were reported according to the Common Terminology Criteria for Adverse Events version 3.0.
Determination of Serum Asparaginase Activity

Twelve blood samples (2-mL) were scheduled for collection from each patient during course 1: prior to each *Erwinia* asparaginase dose; on days 15 and 22 post-administration; and at 2- and 24-hours following doses 1 and 4 for patients beginning treatment on Monday or Wednesday or doses 2 and 5 for patients beginning treatment on Friday. Additional samples were collected before administering doses 1 and 6 and on day 15 during all subsequent courses of therapy. Blood was allowed to clot for 1-2-hours over ice before centrifuging (1,300-g, 10-min, 4°C). Serum was removed and stored at -70°C until packaged for shipment to a central laboratory for analysis.

Asparaginase activity was determined by a coupled enzymatic assay as previously reported with minor modifications. Briefly, aspartic acid formed from the asparaginase catalyzed deamination of added asparagine reacts with **α**-ketoglutaric acid in the presence of glutamic-oxaloacetic transaminase, 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. The rate of reaction at 37°C is a linear function of enzyme activity. *E. coli* asparaginase purchased from Sigma (St. Louis, MO) was used as the analytical reference standard to prepare a series of seven calibration standards in normal human serum with activities of 0.025-0.25-IU/mL. Samples with activities exceeding the upper range of the calibration curve were reanalyzed after diluting with normal human serum. Back-calculated asparaginase activities from a total of 81 calibration curves were used to assess the between day accuracy and precision of the assay during its application to samples from this study. Accuracy was 97.0% of the nominal activity and the precision was 6.0% at the 0.025 IU/mL lower limit of quantitation. Quality control samples with asparaginase in serum at activities of 0.035, 0.120,
and 0.220-IU/mL were assayed with a between-day accuracy of 99.1-102.1% and a precision of 4.7-5.8%.

**Statistical Analysis of Serum Asparaginase Activity Data**

This study was powered to test the hypothesis that the 48 hour NSAA would be $\geq 0.1$-IU/mL in at least 70% of patients versus meeting this target activity in 50% of patients. A Simon “minimax” two-stage design was used with a total sample size of 50, to distinguish between a true null response probability of 70% versus a true alternative response probability of 50% with a significance level of 5%, and 90% power. A total of 31 patients were to be accrued in the first stage. The study would be closed to further accrual if the 48 hour NSAA was $\geq 0.1$ IU/mL in $\leq 15$ patients. Otherwise, the study would continue to the second stage of accrual. A total of 19 more patients would be accrued in the second stage. Acceptance of the null hypothesis required achieving a 48 hour NSAA $\geq 0.1$ IU/mL in at least 30 of the 50 total patients.

Actual dosing and sample collection times were calculated relative to the time of the prior *Erwinia* asparaginase dose and to the initial dose for course 1. Samples obtained on the scheduled day relative to the starting dose of the course, prior to the successive dose, and within 5% of the scheduled time relative to administration of the prior dose were considered to be acceptable for assessing the primary and secondary endpoints of the clinical trial. Additional criteria for excluding assay results were: (1) failure to record the actual dosing or sample collection times; (2) the dose differed from 25,000-IU/m$^2$ by more than ±5%; (3) the prior dose was not administered on the correct day; (4) samples were thawed upon receipt from the study site.
Results from the analysis of 48 and 72-h NSAA samples found to be unacceptable for any of the above reasons were excluded from statistical analyses. The primary endpoint of the study was evaluated from a single 48-h NSAA determination for each patient during course 1, which included samples obtained before giving dose 6 for patients receiving dose 1 on a Monday or Friday and prior to dose 5 for patients beginning treatment on Wednesday. A secondary endpoint of the study was evaluated from a single 72-h NSAA determination during course 1, which included samples collected before dose 4 for patients beginning treatment on Monday, before dose 6 for patients starting on Wednesday, and before dose 5 for patients starting on Friday. Descriptive statistics for all NSAA data were calculated using Microsoft Office Excel 2003 SP3 (Microsoft Corp., Redmond, WA).

Pharmacokinetic Modeling

Serum asparaginase activity-time data for the 6 doses of IM *Erwinia* asparaginase given during the first course of therapy were fit to a one compartment open model with first-order absorption by unweighted nonlinear regression using WinNonlin Professional 5.0 (Pharsight Corp., Cary, NC). Data for each patient was initially fit to the equation,

$$A_s(t) = \frac{ka \ D}{((V/F) \times (ka - ke)))} \times [\exp(-ke \ t) - \exp(-ka \ t)]$$ (A)

with the repeated dosing option, where $A_s(t)$ is the serum asparaginase activity at time t relative to the first dose, $ka$ is the apparent rate constant for absorption of enzyme from the extravascular administration site into serum, $ke$ is the apparent rate constant for the loss of serum enzyme activity, $V/F$ is the extravascular apparent volume of distribution, and $D$ is the dose. In cases
where the estimated values of $k_a$ and $k_e$ were within 10% of each other, the data was refit using the form of the equation for which $k_a = k_e = k$, \(^{26}\)

$$A_s(t) = \frac{k \ t \ D}{(V/F)} \exp(-k \ t) \quad (B)$$

which is also included in the WinNonlin library of pharmacokinetic models. Pharmacokinetic variables (*i.e.* half-lives, apparent extravascular clearance, maximum serum asparaginase activity and time) were calculated by the program using final values of the iterated parameters (*i.e.* $V/F$, $k_a$, $k_e$). The two-tailed t-test was used to compare mean pharmacokinetic variables between groups of patients after logarithmic transformation of the data. A p-value <0.05 was the criterion for statistical significance.

**Results**

**Patient Characteristics**

Fifty-nine patients were enrolled on AALL07P2 (Figure 1). Patients were also enrolled on one of six upfront studies, AALL0232, AALL0331, AALL0434, AALL07P4, AALL0622, and AALL08P1, where planned doses of pegaspargase varied by study arm and ranged from 1-13. Fifty-eight enrolled patients received at least one dose of *Erwinia* asparaginase and are included in the safety analysis (Table 1). The mean age at study entry was 9.7 years (range 2-18) and majority were male (34 [58.6%]). Fifty-one (87.9%) patients had B-precursor ALL and 7 (12.1%) had T-ALL. All patients were within 9 months of diagnosis (50% 0-3 months, 44.8% 4-6 months, 5.2% 7-9 months). Patients received a median of 3 (range 1-5) doses of pegaspargase prior to enrollment on AALL07P2 and received a median of 3 (range 1-9) courses of *Erwinia* asparaginase. Fifty-five patients were eligible and evaluable for AALL07P2, and 44/55 (80%) patients were able to complete all remaining courses of planned asparaginase therapy.
Toxicity

Grade 2-3 allergic reaction, grade 1-3 hyperglycemia, and grade 1 pancreatitis related to *Erwinia* asparaginase were reported in 6, 6 and 1 patients, respectively (Table 2). There were no reports of hemorrhage, thrombosis, hyperlipidemia, ketoacidosis, or death.

Nadir Serum Asparaginase Activity

The primary endpoint was based upon a single 48-hour NSAA determination in 41 patients during course 1. Samples from 14 patients were excluded from statistical analysis because they were either not obtained, collected at a time differing from 48-hours by more than 5%, dosing or sample collection times were not recorded, or due to dosing inconsistencies. The median asparaginase activity 48-hours after the prior dose of *Erwinia* asparaginase was 0.684-IU/mL (range, <Limit of Quantitation (LOQ)-2.884-IU/mL) and 38 (92.7%) patients had an enzyme activity ≥0.100-IU/mL. Forty-three patients had acceptable 72-hour NSAA determinations during course 1 for assessing the secondary endpoint. The median asparaginase activity in these samples was 0.327-IU/mL (range, 0.043-1.026-IU/mL) and the enzyme activity was ≥0.100-IU/mL in samples from 38 (88.4%) of the patients.

Data pertaining to all acceptable 48-hour and 72-hour NSAA determinations for each course of therapy are summarized in Table 3. Four 48-hour NSAA samples and two 72-hour samples were scheduled for collection from each patient during course 1 regardless of the day on which therapy was initiated. During each subsequent course, single 48-hour and 72-hour NSAA samples were obtained from patients beginning therapy on Monday and Wednesday whereas two 48-hour NSAA samples were obtained from patients starting on a Friday. The number of patients with NSAA samples that met the criteria for acceptability decreased from 50/55 (90.9%)
for both trough times in course 1 to 16/24 (66.7%) for the 48-hour NSAA and 7/13 (53.8%) for the 72-hour NSAA in course 4. Overall, 94.5% of the patients had at least one acceptable NSAA sample and 69.8% of the 48-hour NSAA samples (n=263) and 72.9% (n=148) of the 72-h NSAA samples were acceptable. The median 48-hour NSAA decreased progressively from 0.715-IU/mL during course 1 to 0.418-IU/mL in course 4, with an overall median of 0.645 IU/mL for all acceptable samples collected in all courses. The median 72-hour NSAA did not show evidence of a course-dependent trend and the overall median was 0.248-IU/mL. The percentage of patients with acceptable 48-hour NSAA samples ≥0.10-IU/mL ranged from 92.0 to 100% during the first 4 courses, with 92.9-97.4% of the acceptable samples being ≥0.10-IU/mL. With regard to the 72-hour NSAA, 78.9-98.0% of the patients had samples with an enzyme activity ≥0.10-IU/mL and 78.9-88.9% of the acceptable samples were ≥0.10-IU/mL.

**Pharmacokinetics of Serum Asparaginase Activity**

Serum asparaginase activity-time data obtained during treatment with the 6 doses of *Erwinia* asparaginase given in the first course of therapy were amenable to pharmacokinetic analysis for a total of 54 patients. A sparse sampling schedule was employed in which asparaginase activity was monitored at 10 time points over a time interval of 14-days to enable patients to be treated with minimal inconvenience on an outpatient basis. Although it is not possible to estimate any pharmacokinetic parameters by non-compartmental methods of analysis, simultaneously fitting the entire data set by nonlinear regression to a one compartment open model with first-order absorption, using the repeated dosing option for the model, yielded an acceptable fit of the experimental data for all patients.
The existence of two distinct populations of patients was discerned for which the estimated values of $k_a$ and $k_e$ were either well differentiated (group A) or kinetically indistinguishable (group B). Specifically, the average $k_a/k_e$ ratio was 6.6 (range, 1.8-26.8) for 26 categorical group A patients and 0.98 (range, 0.9-1.1) for 28 group B patients when initially fit to equation A. Pharmacokinetic data for group B patients were refit to equation B. The goodness of fit of the appropriate form of the equation for the pharmacokinetic model to the observed serum asparaginase activity-time data for each patient is illustrated by the correlation plot shown in Fig. 2A. Linear regression analysis of the relationship between the observed and model-predicted asparaginase activity for the 510 acceptable data points yielded a correlation coefficient of 0.94 with a slope of 0.86 for the best-fit line.

Mean values of the parameters describing the best-fit equations and derived variables are presented separately in Table 4 for both groups of patients. The mean CL/F was not significantly different ($P=0.33$) for the two groups whereas V/F was approximately 50% greater for group A than group B ($P=0.0045$). Consequently, there was a difference of similar magnitude in the mean apparent biological half-life of serum asparaginase activity between the two groups, which was 22.1±7.7-hours for group A as compared to 12.6±2.1-hours for group B ($P<0.001$). The maximum predicted asparaginase activity for the first dose of *Erwinia* asparaginase was not significantly different ($P=0.41$) for the two pharmacokinetic groups, although the time that it occurred was earlier ($P<0.001$) for group A patients (13.0±5.3-hours) than group B (18.2±3.1-hours).

More of the patients started therapy on a Wednesday (44%) than either a Monday (26%) or Friday (28%). The mean asparaginase activity-time course for the 24 patients with a Wednesday starting day is shown in Fig. 2B. The predicted maximum asparaginase activity
achieved after each dose of *Erwinia* asparaginase was relatively constant and the mean trough asparaginase activity remained well above the 0.10-IU/mL threshold for at least 72-hours after all doses.

**Discussion**

Leukemic lymphoblasts are deficient in asparagine synthetase and are, therefore, thought to be dependent upon extracellular sources of asparagine for protein synthesis. Asparaginase is an exogenous enzyme that catalyzes the hydrolysis of asparagine to aspartic acid and ammonia. Studies both in Europe and the United States have concluded that maintaining serum asparaginase activity above 0.10-IU/mL is adequate to deplete asparagine. This therapeutic threshold was established from a number of independent investigations which revealed that the pharmacodynamic effects of asparaginase are best demonstrated by monitoring the concentration of asparagine in CSF. Accurately measuring the concentration of asparagine in either plasma or serum is complicated by the rapid *ex vivo* hydrolysis of asparagine that occurs in the presence of asparaginase, even at very low, sub-therapeutic activity levels, during the time required to harvest plasma from blood samples and inactivate the enzyme by acidification.

Asparaginase has become a critical component of multi-agent chemotherapy for the treatment of ALL. Current chemotherapy regimens for pediatric ALL typically include a post-induction intensification phase during which asparaginase is routinely administered. All COG trials for newly diagnosed patients with ALL, with the exception of infant ALL, administer pegaspargase starting during induction therapy and as the sole asparaginase product. However, allergic reactions develop in many patients treated with pegaspargase. In addition, immunologic responses to all asparaginase preparations are associated with formation of
neutralizing antibodies against the enzyme that may or may not be associated with a symptomatic allergy \(^{19,21,22,42,43}\).

The primary role of *Erwinia* asparaginase in the treatment of ALL has been to replace native *E. coli* asparaginase or pegasparagase after patients exhibit allergic reactions to either or both preparations \(^{25,44,45}\). In large randomized clinical trials, administration of *Erwinia* asparaginase once or twice a week was associated with inferior outcomes as compared to *E. coli* asparaginase \(^{40,46}\) when administered on identical dosing schedules. It is likely that the NSAA in patients receiving *Erwinia* asparaginase would not have been maintained above 0.10 IU/mL because *Erwinia* asparaginase has a biological half-life that is much shorter than *E. coli* asparaginase \(^{17,23}\). It has been suggested that treatment with lower doses of *Erwinia* asparaginase given daily or on alternating days could be more effective than higher but less frequent doses in producing complete and sustained asparagine depletion \(^{37}\). Specifically, the MWF schedule was recommended when substituting *Erwinia* asparaginase for weekly *E. coli* asparaginase \(^{47}\). The dose of *Erwinia* asparaginase is also a critical consideration, as only 33% of the patients receiving a 10,000-IU/m\(^2\) dose IM once every 3 days had mean trough levels \(\geq\) 0.10 IU/mL \(^{48}\).

The results of COG AALL07P2 showed that of the 55 eligible/evaluable patients NSAA \(\geq 0.1\)-IU/mL was achieved in 38/41 patients (92.7%) with samples meeting acceptability criteria 48-hours after dosing and hence it is concluded that > 70% of patients achieved the required trough serum asparaginase activity. Further, IM administration of *Erwinia* asparaginase 25,000-IU/m\(^2\) MWF for 2-weeks achieved serum enzyme activity \(\geq 0.10\)-IU/mL in 97.4% of the 48-hour trough samples and 84.9% of the 72-hour trough samples during the initial course of therapy. These percentages did not change significantly during successive courses of therapy, as 95.8% of all 48-hour trough samples (median activity, 0.645-IU/mL) and 84.5% of all 72-hour trough
samples (median activity, 0.248-IU/mL) had a serum enzyme activity ≥0.10-IU/mL. Moreover, the serum asparaginase activity was maintained continuously above 0.10-IU/mL for 14-days during course 1 in 34 out of 50 patients (68%). These findings are in excellent agreement with data previously reported for \textit{Erwinia} asparaginase 25,000 IU/m² IM given twice weekly at 3- and 4-day intervals to patients who experienced allergic reactions to weekly \textit{E. coli} asparaginase \cite{25}. After switching to \textit{Erwinia} asparaginase, the median 72-hour NSAA was found to be 0.247-IU/mL and the asparaginase activity was ≥0.10 IU/mL in 83% of evaluable samples.

AALL07P2 also provides the most comprehensive assessment of the pharmacokinetics of IM \textit{Erwinia} asparaginase undertaken to date. Although \textit{Erwinia} asparaginase was first introduced into clinical trials in the early 1970s \cite{49}, prior clinical pharmacokinetic studies of this preparation of the enzyme are limited to only two investigations involving relatively small patient numbers \cite{17,18}. The time course of serum asparaginase activity resulting from IM injection of a single 25,000-IU/m² dose of \textit{Erwinia} asparaginase to pediatric ALL patients was first reported by Asselin \cite{17}. They found that the mean terminal phase half-life of asparaginase activity was 15.6±3.1-hours for IM \textit{Erwinia} asparaginase in 10 patients. In our study, the overall mean apparent biological half-life of asparaginase activity for the entire group of 54 patients with evaluable course 1 pharmacokinetic data was 16.5±6.4-hours, in excellent agreement with this value.

In another study, the plasma pharmacokinetics of asparaginase activity was characterized in 13 pediatric ALL patients receiving daily doses of 30,000-IU/m² \textit{Erwinia} asparaginase given either as a 3-hour IV infusion or IM injection \cite{18}. Asparaginase activity decayed with a mean half-life of 6.4±1.9-hours when the enzyme was given by the IV route. The mean total body clearance of \textit{Erwinia} asparaginase was 0.16±0.06-liters/hour/m² and it had a mean apparent
volume of distribution of $1.35\pm0.31\text{-liters/m}^2$. The time course of asparaginase activity in plasma following IM administration was best described by a one compartment open model with first-order absorption. However, the much shorter half-life of the enzyme when given IV as compared to IM demonstrated that *Erwinia* asparaginase exhibits absorption rate-limiting pharmacokinetics, also commonly known as the “flip-flop phenomenon”, when given by the IM route. Under these circumstances, the smaller rate constant derived from the terminal region of decreasing drug levels actually corresponds to $k_a$ whereas the larger rate constant derived from the early region of increasing drug levels corresponds to $k_e^{50}$. This association is strictly true only if the ratio between the larger and small rate constant is sufficiently large (i.e., $>3$), otherwise estimated values of the rate constants do not approximate either the true $k_a$ or $k_e$.

Results obtained from our study, which involves considerably more patients than previously evaluated, confirms the absorption rate-limiting pharmacokinetics of IM *Erwinia* asparaginase. Two subpopulations of patients, each of which comprised approximately 50% of the total cohort, were identified by the relative magnitude of $k_a$ and $k_e$. Patients designated as group A had kinetically distinguishable apparent rate constants and those designated as group B did not. The mean $k_a$ for group A patients ($0.15\pm0.09\text{-hours}^{-1}$) is in very good agreement with the mean $k_e$ previously reported for the loss of asparaginase activity following IV administration of *Erwinia* asparaginase in 13 patients ($0.12\pm0.04\text{-hours}^{-1}$) $^{18}$. In this earlier study, the apparent $k_e$ for IM *Erwinia* asparaginase could only be estimated for 7 of the 16 patients evaluated (44%). It is perhaps more than coincidental that this is similar to the proportion of patients in the present study whose enzyme activity-time data were described by equation A of the pharmacokinetic model (i.e. 48%). In any event, the mean $k_e$ for group A patients ($0.031\pm0.013\text{-hours}^{-1}$) was very
similar to the apparent $k_e$ reported for the 7 patients receiving IM Erwinia asparaginase in the prior study (0.034±0.005-hours$^{-1}$).

The mean apparent half-life of serum asparaginase activity is almost two-fold shorter for patients in group B as compared to group A. Enzyme absorption from the IM injection site remains rate-limiting for group B patients because the mean apparent half-life in these patients is still 2-times larger than the mean half-life reported for IV administration of the enzyme. It follows that patient specific factors, which remain to be identified, are probably responsible for influencing the rate of absorption of the enzyme from the IM injection site into systemic circulation, resulting in the observed differences in the pharmacokinetic behavior of asparaginase activity between the two subgroups. The very low bioavailability of IM Erwinia asparaginase, which was reported to be only 27.1% on average and highly variable between patients, as indicated by a 57.7% coefficient of variation, lends additional plausibility to this hypothesis 18. Relatively minor changes in the rate or extent of absorption of the enzyme would have a marked effect on the time course of serum asparaginase activity under these circumstances. Most importantly, the effect does not appear to be clinically relevant for the Erwinia asparaginase dosing regimen evaluated in this study because serum asparaginase activity remained above the 0.10-IU/mL therapeutic threshold for up to 72-hours after dosing in 84.5% of acceptable samples.

The DFCI 95-01 and EORTC-CLG 58881 40,46, suggested that toxicities associated with Erwinia asparaginase therapy were less than those associated with E. coli asparaginase. These studies however failed to dose adjust Erwinia asparaginase based on its shorter half-life, raising concern that sub-optimal dosing may have resulted in fewer toxicities. The AALL07P2 dosing schedule of 25,000-IU/m$^2$ on a MWF schedule resulted in few clinically significant toxicities.
Because of the sample size of this study, firm conclusions cannot be made on the incidence of these targeted toxicities.

In conclusion, *Erwinia* asparaginase administered IM using the MWF AALL07P2 regimen was well tolerated and achieved a therapeutic NSAA at both 48- and 72-hours after dosing in a high percentage of patients. Although IM administration of *Erwinia* asparaginase on an every other day schedule would theoretically provide greater consistency in the NSAA, the ability to dose on the MWF schedule and maintain NSAA ≥0.10-IU/mL with a periodic 72 hour dosing interval enabled the drug to be given during normal operating hours of most outpatient clinics, thereby avoiding the problems and inconvenience associated with weekend dosing. This study provided the basis for the FDA approval, granted in November 2011, to use *Erwinia* asparaginase in patients with allergic reactions to pegasparagase, substituting a single dose of pegasparagase with *Erwinia* asparaginase 25,000-IU/m² x 6 doses IM on a MWF schedule. Future studies should investigate the pharmacokinetics and toxicity profile of IM *Erwinia* asparaginase given according to this MWF schedule in young adults who are at least 18 years old, as all patients enrolled in AALL07P2 were <18 years of age. In addition, the dose and schedule of intravenous *Erwinia* asparaginase required to achieve therapeutic NSAA on a continuous basis has yet to be established.
Acknowledgements

Supported by grants from the National Institutes of Health (grants CA13539 and CA98543) and a research agreement with Jazz Pharma, Inc. (Langhorne, PA) formerly EUSA Pharma, Inc.

SPH is the Ergen Family Chair in Pediatric Cancer.

Authorship Contributions

I: Designed research, performed research, collected data, analyzed and interpreted data, wrote manuscript:

Wanda L. Salzer, MD, Barbara Asselin MD, Jeffrey G. Supko, Ph.D., Nicole A. Kaiser, RPh, Naomi J. Winick, MD, Gregory H. Reaman, MD, Elizabeth Raetz, MD, William L. Carroll, MD, Stephen P Hunger, MD

II: Designed research, collected data, analyzed and interpreted data, performed statistical analyses, wrote manuscript:

Meenakshi Devidas PhD, Paul Plourde, MD

Disclosure of Conflicts of Interest

Paul Plourde, MD, is the Senior Vice President of Clinical Oncology for Jazz Pharmaceuticals, Inc.

The opinions and assertions contained herein are the private views of the author(s) and are not to be construed as the official policy or position of the U.S. Government, the Department of Defense, or the Department of the Air Force.
References


24. Salzer W, Asselin B, Supko J, et al. Administration of Erwinia Asparaginase Following Allergy to PEG-Asparaginase In Children and Young Adults with Acute Lymphoblastic Leukemia Treated on AALL07P2 Achieves Therapeutic Nadir Serum Asparaginase Activity: A


### Table 1. Demographic and Baseline Characteristics - Safety Population

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Safety Population, n</strong></td>
<td>58</td>
</tr>
<tr>
<td><strong>Age years</strong></td>
<td></td>
</tr>
<tr>
<td>Mean (Standard deviation)</td>
<td>9.7 (5.20)</td>
</tr>
<tr>
<td>Median</td>
<td>10.5</td>
</tr>
<tr>
<td>Min, Max</td>
<td>2, 18</td>
</tr>
<tr>
<td><strong>Gender, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34 (58.6)</td>
</tr>
<tr>
<td>Female</td>
<td>24 (41.4)</td>
</tr>
<tr>
<td><strong>Race, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>45 (77.6)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>6 (10.3)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (12.1)</td>
</tr>
<tr>
<td><strong>Ethnicity, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>20 (34.5)</td>
</tr>
<tr>
<td>Not Hispanic or Latino</td>
<td>38 (65.5)</td>
</tr>
<tr>
<td><strong>Primary Disease, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Precursor B ALL</td>
<td>51 (88%)</td>
</tr>
<tr>
<td>T Cell ALL</td>
<td>7 (12%)</td>
</tr>
<tr>
<td>Toxicity</td>
<td>N (%)</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>(n=55)</td>
</tr>
<tr>
<td><strong>Allergy</strong></td>
<td>6 (10.9%)</td>
</tr>
<tr>
<td>Grade 2, n=4; Grade 3, n=2</td>
<td></td>
</tr>
<tr>
<td><strong>Hyperglycemia</strong></td>
<td>6 (10.9%)</td>
</tr>
<tr>
<td>Grade 1, n=3; Grade 2, n=2; Grade 3, n=1</td>
<td></td>
</tr>
<tr>
<td><strong>Pancreatitis</strong></td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>Grade 1, n=1</td>
<td></td>
</tr>
<tr>
<td><strong>Hemorrhage/Thrombosis (Grade 3-4)</strong></td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3. Summary statistics for 48 and 72 hour NSAA data for each course of therapy

<table>
<thead>
<tr>
<th>Trough time (h)</th>
<th>Cycle no.</th>
<th>Acceptable data</th>
<th>NSAA (IU/mL)</th>
<th>NSAA ≥ 0.10 IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Patients No. (%)</td>
<td>Samples No. (%)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>48</td>
<td>1</td>
<td>50 (90.9)</td>
<td>155 (72.4)</td>
<td>0.715 (&lt;LOQ, 2.884)</td>
</tr>
<tr>
<td>48</td>
<td>2</td>
<td>25 (58.1)</td>
<td>28 (56.0)</td>
<td>0.654 (&lt;LOQ, 1.294)</td>
</tr>
<tr>
<td>48</td>
<td>3</td>
<td>29 (90.6)</td>
<td>37 (88.1)</td>
<td>0.551 (0.099, 1.429)</td>
</tr>
<tr>
<td>48</td>
<td>4</td>
<td>16 (66.7)</td>
<td>24 (70.6)</td>
<td>0.418 (0.080, 1.635)</td>
</tr>
<tr>
<td>48</td>
<td>≥5</td>
<td>12 (75.0)</td>
<td>19 (51.4)</td>
<td>0.669 (&lt;LOQ, 1.114)</td>
</tr>
<tr>
<td>48</td>
<td>All</td>
<td>52 (94.5)</td>
<td>263 (69.8)</td>
<td>0.645 (&lt;LOQ, 2.884)</td>
</tr>
<tr>
<td>72</td>
<td>1</td>
<td>50 (90.9)</td>
<td>86 (79.6)</td>
<td>0.251 (0.043, 1.612)</td>
</tr>
<tr>
<td>72</td>
<td>2</td>
<td>19 (57.6)</td>
<td>19 (57.6)</td>
<td>0.248 (&lt;LOQ, 0.873)</td>
</tr>
<tr>
<td>72</td>
<td>3</td>
<td>18 (90.0)</td>
<td>18 (90.0)</td>
<td>0.163 (0.030, 0.741)</td>
</tr>
<tr>
<td>72</td>
<td>4</td>
<td>7 (53.8)</td>
<td>7 (53.8)</td>
<td>0.475 (&lt;LOQ, 0.622)</td>
</tr>
<tr>
<td>72</td>
<td>≥5</td>
<td>11 (78.6)</td>
<td>18 (62.1)</td>
<td>0.245 (0.087, 0.717)</td>
</tr>
<tr>
<td>72</td>
<td>All</td>
<td>52 (94.5)</td>
<td>148 (72.9)</td>
<td>0.248 (&lt;LOQ, 1.612)</td>
</tr>
</tbody>
</table>
### Table 4. Mean pharmacokinetic parameters for serum asparaginase activity

<table>
<thead>
<tr>
<th>Parameter a (units)</th>
<th>Group A (n = 26)</th>
<th>Group B (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/F (liters/m²)</td>
<td>12.8 (55) b</td>
<td>8.3 (50)</td>
</tr>
<tr>
<td>k_a (h⁻¹)</td>
<td>0.149 (62)</td>
<td>c</td>
</tr>
<tr>
<td>k_e (h⁻¹)</td>
<td>0.031 (35)</td>
<td>0.055 (17)</td>
</tr>
<tr>
<td>CL/F (liters/h/m²)</td>
<td>0.40 (53)</td>
<td>0.46 (49)</td>
</tr>
<tr>
<td>t_{1/2,z} (h)</td>
<td>22.1 (35)</td>
<td>12.6 (17)</td>
</tr>
<tr>
<td>t_max (h) d</td>
<td>14.0 (38)</td>
<td>18.4 (17)</td>
</tr>
<tr>
<td>A_max (IU/mL) d</td>
<td>1.25 (57)</td>
<td>1.10 (49)</td>
</tr>
</tbody>
</table>

a Abbreviations: V/F, apparent extravascular volume of distribution; k_a, apparent rate constant for absorption of enzyme activity; k_e, apparent rate constant for elimination of enzyme activity; CL/F, apparent extravascular clearance of enzyme activity; t_{1/2,z}, apparent biological half-life of enzyme activity; t_max, time of maximum serum enzyme activity; A_max, maximum serum enzyme activity.

b Percent coefficient of variation in parentheses.

c For group B, k_a = k_e = k.

d Predicted values for the initial dose of the drug.
Figure Legends

Fig 1  Analysis of Patients Enrolled on AALL07P2; pharmacokinetic (PK); pharmacodynamic (PD)

Fig. 2. (A) Correlation between the observed and model predicted asparaginase activity in 510 serum samples obtained from 54 patients during the initial course of therapy. The overall goodness of fit of the one-compartment open model with first-order absorption to the experimental data for individual patients is indicated by the closeness of the correlation coefficient (0.94) and slope of the best-fit line (0.86) to unity. (B) Mean serum asparaginase activity-time profile for the six 25,000-IU/m² doses of IM Erwinia asparaginase given during the first course of therapy for 24 patients receiving the initial dose on a Wednesday. Data points are the geometric mean values of the observed asparaginase activity at each sample time shown together with 1-SD unit error bars. The continuous line is the best-fit curve determined by nonlinear regression analysis of the mean profile.
Figure 1. Analysis of Patients Enrolled on AALL07P2

- 59 Enrolled
  - 58 Received *Erwinia* asparaginase
    - Safety Population
  - 55 Evaluable AALL07P2
  - 53 Patients with PK data
    - 1 ineligible - withdrew from frontline therapy;
    - 2 inevaluable - received *E. coli* asparaginase by error;
    - received wrong study drug
    - 1 ineligible - received study drug before enrollment
Figure 2

A

Predicted asparaginase activity (IU/mL)

Observed asparaginase activity (IU/mL)

B

Acetate activity (IU/mL)

Time (days)
Asparaginase has been used in the treatment of childhood acute lymphoblastic leukemia for over 30 years. However, the utilization of pegylated asparaginase (Peg-Asp) has been hindered by asparaginase allergy. In previous studies, the development of allergy to Asp, which is produced by Erwinia species, after exposure to Asp has been observed. In this study, we report a case of a patient with ALL who developed a severe allergic reaction to Asp after exposure to Peg-Asp. The patient was successfully treated with Erwinia asparaginase (EAsp) after a single dose of EAsp (250 U/m2). This case highlights the importance of considering EAsp as an alternative to Peg-Asp in patients with asparaginase allergy.