

EXPERT OPINION

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Erwinia asparaginase in pediatric acute lymphoblastic leukemia

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Introduction: Asparaginase is a major component of therapy for acute lymphoblastic leukemia (ALL) and has been used for over 40 years. Hypersensitivity reactions limit the use and efficacy of asparaginase products. However, *Erwinia* asparaginase gained the Food and Drug Administration (FDA) approval in November 2011, for use in patients with allergic reactions to *Escherichia coli*-derived asparaginase.

Areas covered: *Erwinia* asparaginase is an enzyme that hydrolyzes the amino acid asparagine. This review examines the properties of *Erwinia* asparaginase compared to the two other preparations of asparaginase available for use in the United States. Results of selected clinical trials involving *Erwinia* asparaginase, including the pivotal study resulting in FDA approval, are presented.

Expert opinion: *Erwinia* asparaginase is well tolerated, and it is effective in achieving asparaginase levels associated with efficacy in the treatment of ALL. With FDA approval of *Erwinia* asparaginase, oncologists now have an alternative for ALL patients who become hypersensitive to *E. coli*-derived asparaginase. Future studies will be needed to establish optimal dosing of *Erwinia* asparaginase (e.g., intravenous vs. intramuscular) and to better define the most appropriate indications for its use in patients previously treated with *E. coli*-derived asparaginase.

Keywords: acute lymphoblastic leukemia, allergy, antibody, *Erwinia* asparaginase, treatment

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1. Introduction

Since its initial use in childhood acute lymphoblastic leukemia (ALL) in the 1960s [1], asparaginase has become an essential component of multi-agent chemotherapy. As single agent therapy, asparaginase demonstrated response rates of 20 – 68% [2-7], firmly establishing it as an active agent for ALL. In combination therapy, asparaginase demonstrated efficacy in both induction and post-induction phases of therapy [8-13], confirming its role in multi-agent chemotherapy.

There are three preparations of asparaginase available for use in the United States, native *Escherichia coli* (*E. coli*) asparaginase (Elspar[®], Merck & Co., Inc., Whitehouse Station, NJ), PEG-asparaginase (Oncaspar[®], Sigma-Tau Pharmaceuticals, Inc., Gaithersburg, MD), and *Erwinia* asparaginase (Erwinaze[®]; EUSA Pharma [US] Inc., Doylestown, PA), see Drug Summary Box 1. Native *E. coli* asparaginase and PEG-asparaginase are derived from *E. coli*, whereas *Erwinia* asparaginase is derived from *Erwinia chrysanthemi*. Due to its longer half-life and the resultant fewer doses required per treatment period, the most commonly utilized preparation in North America is PEG-asparaginase. PEG-asparaginase has also been preferred due to its lower immunogenicity [14-19].

Children's Oncology Group (COG) ALL clinical trials utilize PEG-asparaginase beginning with induction therapy, with the exception of infant ALL protocols that uses *E. coli* asparaginase. Because patients with allergy to PEG-asparaginase

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Box 1. Drug summary.

Drug name	<i>Erwinia</i> asparaginase
Phase	Launched
Indication	Acute lymphoblastic leukemia when hypersensitivity to <i>E. coli</i> -derived asparaginase has developed
Pharmacology description	Catalyzes the hydrolysis of asparagine to aspartic acid and ammonia
Route of administration	Intramuscular
Pivotal trial	AALL07P2, pilot study, to determine if <i>Erwinia</i> asparaginase administered to patients with hypersensitivity to PEG-asparaginase provides a 48-h trough asparaginase activity ≥ 0.1 IU/mL

have antibodies that are cross-reactive with native *E. coli* asparaginase, *Erwinia* asparaginase is the only available asparaginase preparation for these patients. This article discusses results of *Erwinia* asparaginase in clinical studies for childhood ALL, including the pivotal AALL07P2 study that resulted in Food and Drug Administration (FDA) approval, and discusses the overall role of *Erwinia* asparaginase in treatment for patients with ALL.

2. Background

2.1 Mechanism of action

Erwinia asparaginase is a selective enzyme that hydrolyzes the amino acid asparagine to aspartic acid and ammonia (Box 1). Leukemic blasts often lack the capability to produce their own asparagine. Thus, asparagine can be considered an essential amino acid for leukemic blasts, and depletion of asparagine by asparaginase selectively kills these cells. While some leukemic blasts lack or have very low levels of asparagine synthetase [20,21], drug resistance to asparaginase resulting in an increased production of asparagine can be demonstrated in exposed cells. Exposure to asparaginase has been shown, both *in vitro* and *in vivo*, to upregulate asparagine synthetase mRNA expression in hyperdiploid leukemic cells as well as leukemic cells with/without ETV6-RUNX1 fusion [22,23]. Although increased asparagine synthetase mRNA expression has been correlated with poorer outcome in patients lacking ETV6-RUNX1 fusion [22], it has not been correlated with asparaginase resistance in patients with hyperdiploid or ETV6-RUNX1 fusion positive leukemia [22,23]. Bone marrow mesenchymal cells, whose expression of asparagine synthetase mRNA has been shown to be 20 times that of the leukemic cells, may provide an alternate mechanism of protection for the leukemic cells by providing increased concentrations of asparagine within the microenvironment [24]. Thus, drug resistance may occur through multiple mechanisms, including upregulation of asparagine synthetase and increased concentration of asparagine in the leukemic microenvironment, leading to an increase in cellular asparagine concentration [24-27].

2.2 Pharmacokinetics

There are significant differences in the pharmacokinetic properties of the three asparaginase preparations, with PEG-asparaginase having the longest and *Erwinia* asparaginase having the shortest half-life (Table 1) [15-18,28]. However, peak concentration following intramuscular administration occurs latest with PEG-asparaginase and earliest with *Erwinia* asparaginase [14].

The pharmacokinetic parameter that is felt to be most closely associated with efficacy is trough concentration. Both early studies in nonhuman primates [29] as well as reports of clinical studies from Europe [30,31] and from COG [28], have concluded that 0.1 IU/mL is a target level of asparaginase activity based on an association with asparagine depletion [32,33], although levels as low as 0.05 IU/mL have also been associated with asparagine depletion [34]. Assays that measure asparagine in the presence of asparaginase have had significant technical limitations, and the more reliable measure for clinical application is asparaginase activity. Because of the different half-lives for the three asparaginase products, they cannot be readily interchanged without careful consideration of the appropriate dose and schedule for each agent.

2.3 Allergic reactions

Allergic reactions to asparaginase preparations include localized reactions, urticaria, and anaphylaxis. Because the asparaginase enzyme is not expressed in normal human cells [35], immunological reactions may develop and have been reported in up to two-thirds of patients receiving *E. coli*-derived products [11,16,19,28,36-43]. In the DFCI 91-01 clinical trial in which patients were randomized to native *E. coli* asparaginase versus PEG-asparaginase (both administered intramuscularly), patients who received PEG-asparaginase had a reduced risk of hypersensitivity reactions compared to those randomized to native *E. coli* asparaginase [19,44]. The rate of *Erwinia* asparaginase allergic reactions have been reported to range from 5 to 33% [41,43,45-48] and increases with increasing exposures [37]. The AALL07P2 study evaluating *Erwinia* asparaginase reported grade 3/4 Allergic Reaction/Hypersensitivity in 5 of 55 patients (9%) [49], whereas the *Erwinia* asparaginase expanded access protocol reported such reactions in 27 of 572 patients (5%) [50].

Variables such as drug formulation, mode of administration, and age have been examined for their influence on the occurrence of allergic reactions to asparaginase products. The impact of steroid treatment at the time of asparaginase administration on the development of antibodies has not been studied, although is thought to reduce the incidence of allergic reactions. Although previous reports suggested that the intravenous route of administration resulted in lower rates of allergic reactions compared to intramuscular route of administration [36,51], recent studies demonstrate comparable rates of allergic reactions with either route of administration [52-55]. Likewise, age does not appear to influence rates of allergic reactions, with children < 10 years of age, > 10 to < 18 years of age, and adults

Table 1. Asparaginase pharmacokinetics.

Preparation	Peak concentration	Elimination half-life
Native <i>E. coli</i> asparaginase (25,000 IU/m ² IM)	< 1 d	1.44 + 0.35 d
PEG-asparaginase (2,500 IU/m ² IM)	3 – 4 d	5.73 + 3.24 d
<i>Erwinia</i> asparaginase (25,000 IU/m ² IM)	1 – 2 d	0.65 + 0.13 d

d: Day; IM: Intramuscular.

having similar rates of allergic reactions [45,56]. In the St. Jude Total Protocol XV, allergic reactions were higher among patients treated on the low-risk arm than among those on the standard/high-risk arm, supporting the role of chemotherapy-induced immunosuppression in modulating the immune response to asparaginase [57].

Once an allergic reaction occurs, it is recommended to switch to a different asparaginase preparation, although the development of allergic reactions to asparaginase was not associated with a lower event free survival (EFS) on several studies [19,41,42,45]. It should be noted that on these studies substitution with a different asparaginase preparation following the development of clinical allergy routinely occurred and may have affected outcome. For example, 42 of 215 children (20%) with newly diagnosed ALL enrolled on Dana-Farber Cancer Institute (DFCI) ALL Consortium Protocol 00-01 [43] (planned administration of 30 weekly doses of intramuscular *E. coli* asparaginase) developed allergic reactions and were switched to twice-weekly intramuscular *Erwinia* asparaginase (25,000 IU/m²). At a median follow-up of approximately 5 years, EFS of the 42 patients who were switched to *Erwinia* asparaginase was 86% (standard error +/- 5%) compared with 81% (standard error +/- 3%) for the 170 patients without *E. coli* asparaginase allergy. However, for the DFCI 91-01 clinical trial [19], patients unable to receive more than 25 of 30 planned weeks of asparaginase because of experiencing an asparaginase-related dose-limiting toxicity (e.g., pancreatitis, thrombosis/hemorrhage) had a lower EFS compared to patients who received the prescribed asparaginase treatment, 73% (+/-7%) versus 90% (+/-2%), respectively [19], supporting the importance of maintaining asparaginase treatment.

2.4 Anti-asparaginase antibody formation

Allergic reactions are associated with the development of anti-asparaginase IgG antibodies. These antibodies decrease the effectiveness of asparaginase by either increasing asparaginase clearance and/or neutralizing asparaginase activity [16,18,28,58-61]. Antibody detection is most commonly accomplished through an enzyme-linked immunosorbent assay (ELISA), although a more sensitive assay, surface plasmon resonance (SPR-Biacore), has been developed [60]. Anti-asparaginase antibodies that develop after exposure to native *E. coli* asparaginase often

demonstrate cross-reactivity to the PEG-asparaginase preparation, but not to the *Erwinia* asparaginase preparation [62,63]. Thus, substitution of *Erwinia* asparaginase in patients with allergic reactions to *E. coli* asparaginase preparations is often recommended. However, for patients on the ALL-Berlin-Frankfurt-Munster (BFM) 2000 or the ALL-Relapse (REZ) BFM 2002 clinical trials, an effect of anti-asparaginase antibody levels on PEG-asparaginase enzymatic activity during second-line treatment following initial native *E. coli* asparaginase therapy was only observed when anti-*E. coli* asparaginase antibody levels were high. For patients with moderate or intermediate antibody levels, treatment with PEG-asparaginase produced adequate plasma asparaginase levels [63].

Anti-asparaginase antibodies have been detected in the majority of children and adults treated with native *E. coli* asparaginase [57,64,65], although the criterion for positivity varies among laboratories. However, the rate of antibody detection has been reported to be lower after exposure to the PEG-asparaginase preparation compared to either the native *E. coli* asparaginase or *Erwinia* asparaginase preparations [16,28,66]. Additionally, anti-asparaginase antibodies have been demonstrated in patients without clinical allergic reactions (silent hypersensitivity) and allergic reactions have occurred in patients without detectable anti-asparaginase antibodies [35,57,59,65,67-69].

Similar to clinical allergy, the detection of anti-asparaginase antibodies was not associated with lower EFS in the St. Jude Children's Research Hospital ALL Total XIII-HR study [41]. Because clinical allergy was present in the majority of patients with detectable antibodies, and substitution with *Erwinia* asparaginase occurred for most patients with clinical allergy, this substitution may have affected the outcome measure. Conversely, in the CCG-1961 study, the detection of anti-asparaginase antibodies in patients without clinical allergic reactions was associated with an increased rate of early events [37]. In the St. Jude Total XV protocol in which native *E. coli* asparaginase was used, anti-asparaginase antibody levels inversely associated with serum asparaginase activity, and high antibody levels were associated with a lower risk of osteonecrosis and a higher risk of CNS relapse [57,70]. Both these effects may be explained by the inverse relationship between asparaginase drug levels and dexamethasone clearance [57,70]. In the Total XV study, the frequency of anti-asparaginase antibodies was higher for patients treated on the low-risk arm versus the standard/high-risk arm, suggesting that the intensive chemotherapy reduces the rate of antibody formation [57].

2.5 Nonallergic adverse events

Although hypersensitivity is the most common toxicity associated with *Erwinia* asparaginase therapy, other clinically significant toxicities include thrombosis/bleeding (2%/1%), abnormal transaminases (3%), hyperbilirubinemia (1%), and pancreatitis (4%) [50]. When *Erwinia* asparaginase was used in newly diagnosed patients with no previous asparaginase exposure, rates of thrombosis/bleeding, liver

function abnormalities, and pancreatitis were 1%, 4%, and 1 – 2%, respectively [56,71]. These reports suggest that rates of nonallergic toxicities associated with *Erwinia* asparaginase may be similar to the rates commonly seen with other asparaginase preparations.

3. Clinical efficacy

Two clinical studies directly compared the effectiveness of *Erwinia* asparaginase to native *E. coli* asparaginase. In retrospect, both trials were flawed in using doses/schedules of *Erwinia* asparaginase that were insufficient to maintain adequate asparaginase levels throughout the treatment period. The European Organisation for Research and Treatment of Cancer—Children's Leukemia Group (EORTC-CLG) 58881 compared *Erwinia* asparaginase 10,000 IU/m² twice weekly × 12 doses intravenously (IV) to native *E. coli* asparaginase 10,000 IU/m² twice weekly IV × 12 doses in 700 newly diagnosed children with ALL or lymphoblastic lymphoma. Patients randomized to *Erwinia* asparaginase versus *E. coli* asparaginase achieved a lower rate of complete remission following induction (91 vs. 94.5%, $p = 0.078$) and had an inferior EFS (59.8 vs. 73.4%, $p = 0.0004$). Toxicity was similar in the two groups, except that coagulation abnormalities were more common in patients randomized to *E. coli* asparaginase [71]. The DFCI 95-01 compared *Erwinia* asparaginase 25,000 IU/m² weekly intramuscularly (IM) × 20 doses to native *E. coli* asparaginase 25,000 IU/m² weekly IM × 20 doses in 491 newly diagnosed children with ALL or lymphoblastic lymphoma. Patients randomized to *Erwinia* asparaginase had an inferior EFS (78 vs. 89%, $p = 0.01$), but lower rates of any clinically significant toxicity (10 vs. 24%, $p < 0.01$) [56]. The dosage of *Erwinia* asparaginase was not biologically equal to that of *E. coli* asparaginase, as an *Erwinia* asparaginase dose of 25,000 IU/m² administered every 2 – 3 days is required to maintain adequate asparaginase levels. Therefore, it is likely that the poorer outcomes reported in these two studies resulted from suboptimal asparaginase dosing in patients treated on the *Erwinia* asparaginase arms. Of note, the estimated trough levels for *E. coli* asparaginase during weekly treatment of 25,000 IU/m² are in the presumed therapeutic range (0.1 IU/mL), whereas trough levels for *Erwinia* asparaginase using this same dose are much less than 0.1 IU/mL [14].

The Children's Oncology Group study AALL07P2 tested the hypothesis that *Erwinia* asparaginase 25,000 IU/m² IM × 6 doses administered on a Monday, Wednesday, Friday schedule in patients with clinical allergy to PEG-asparaginase would achieve a trough asparaginase activity at 48 h > 0.1 IU/mL. For 48 evaluable patients, all patients in course 1 achieved serum trough asparaginase concentrations ≥ 0.1 IU/mL at either 48-h ($n = 35$) or 72-h ($n = 13$) post dose 3. Eighty percent (28/35) of those evaluated at 48 h and 38% (5/13) evaluated at 72 h had serum asparaginase activity levels > 0.4 IU/mL. Based on the results of this pivotal study, the FDA granted approval for

the use of *Erwinia* asparaginase in November 2011 for patients allergic to *E. coli*-derived asparaginase. The AALL07P2 results are similar to those reported for a subset of patients enrolled on the DFCI ALL Consortium Protocol 00-01, which used twice-weekly dosing of *Erwinia* asparaginase at 25,000 IU/m² [72]. While the 3-day asparaginase trough levels were generally ≥ 0.1 IU/mL, fewer than 50% of the 4-day asparaginase trough levels were < 0.1 IU/mL, supporting the thrice-weekly schedule used in AALL07P2.

4. Expert opinion

Erwinia asparaginase has been utilized with a range of doses and schedules as part of multi-agent chemotherapy for patients with ALL for decades. The enzyme was first registered in the United Kingdom in 1985 and has since been registered in Portugal, Netherlands, Austria, Ireland, Canada, and Lebanon. In November 2011, it was approved by FDA with the recommended dose of 25,000 IU/m² administered IM three times a week (Monday/Wednesday/Friday) for six doses for each planned dose of pegaspargase [50,52]. In contrast to both *E. coli* preparations that are approved for IM and IV administration, *Erwinia* asparaginase is FDA-approved only for IM administration.

Erwinia asparaginase has been reported to have similar or lower levels of toxicities compared to *E. coli* asparaginase preparations [56,71,73]. A nonrandomized historical comparison using identical doses of *Erwinia* and *E. coli* asparaginase (6000 IU/m² thrice weekly) showed that patients receiving *E. coli* asparaginase had significantly higher incidence of neurotoxicity, pancreatitis, and life-threatening sepsis compared to patients receiving *Erwinia* asparaginase [73]. Only two studies have directly compared *Erwinia* asparaginase to *E. coli* asparaginase preparations in asparaginase naïve patients [56,71]. These studies, like the nonrandomized comparison, failed to adjust the dose of *Erwinia* asparaginase based on its shorter half-life, and thus the dosing of asparaginase preparations were not biologically equivalent. It is likely that toxicities of *Erwinia* and *E. coli* asparaginase products are reasonably comparable when doses/schedules are adjusted to maintain comparable durations of exposure above the target 0.1 IU/mL level, as the rate of toxicities with *E. coli* preparations appears to be related to the intensity of asparaginase use [74,75].

There are several potential areas for future research regarding use of *Erwinia* asparaginase. One unresolved issue is the relative safety and pharmacokinetic efficacy of administering *Erwinia* asparaginase by the intravenous versus the intramuscular route. There is clinical experience with intravenous administration of *Erwinia* asparaginase, primarily from Europe. One report noted that peak asparaginase levels were higher for intravenous administration while there was a depot effect for intramuscular administration (10,000 IU/m²), such that trough levels tend to be higher for the intramuscular route [76]. A second report using twice-weekly administration

(30,000 IU/m²) during reinduction therapy showed similar trough drug levels for the intramuscular and intravenous routes [77]. In the United States, comparable pharmacokinetic and safety data will be required to document that adequate trough levels are maintained with the intravenous route using the Monday–Wednesday–Friday schedule and to confirm that the safety profile with this dose/schedule using the intravenous route is acceptable.

A second area for future research is evaluation of a pegylated formulation of *Erwinia* asparaginase. A pegylated form of recombinant *Erwinia* asparaginase is being developed for ALL patients with hypersensitivity to *E. coli* asparaginase products [78]. Preclinical results indicate that the pegylated form is longer acting than the native *Erwinia* asparaginase product [79]. A Phase 1 trial for the intravenous administration of this agent has been developed (NCT01551524). Like the pegylated form of *E. coli* asparaginase, a pegylated *Erwinia* asparaginase product should allow less frequent administration and may also be less immunogenic.

A third area for future research is to assess *Erwinia* asparaginase role in those patients without hypersensitivity to *E. coli*-derived asparaginase. For example, can therapeutic drug monitoring for asparaginase be used to identify patients without hypersensitivity reactions with inadequate asparaginase levels who might benefit from switching to *Erwinia* asparaginase? Alternatively, can monitoring for the development of anti-asparaginase antibodies be used to identify patients who can benefit from *Erwinia* asparaginase? In a relapsed ALL population receiving PEG-asparaginase, no asparaginase activity was

observed immediately after drug administration for 7 of 53 samples (13%), an observation most likely attributable to the presence of anti-asparaginase antibodies [80]. While it seems logical that patients such as this with “silent hypersensitivity” to *E. coli*-derived asparaginase may benefit from switching to *Erwinia* asparaginase, clinical data are needed to confirm this hypothesis. A study evaluating anti-*E. coli* asparaginase antibody levels in children receiving frontline ALL therapy identified relationships between the level of anti-*E. coli* asparaginase antibodies and serum asparaginase activity in patients receiving *E. coli* asparaginase and PEG-asparaginase [63]. Similar results were reported for patients receiving *E. coli* asparaginase on the St. Jude Total XV clinical trial [57]. Thus, an important area for future research is determining whether either therapeutic drug monitoring or monitoring for levels of anti-asparaginase antibodies can be used to guide the use of *Erwinia* asparaginase and lead to improved anti-leukemia activity.

In summary, oncologists now have an alternative asparaginase preparation available for patients who develop hypersensitivity reactions to *E. coli* asparaginase preparations and have dosing guidelines based on the targeted level of plasma asparaginase activity. The availability of *Erwinia* asparaginase opens avenues for future research to improve outcome for patients with ALL.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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