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L-asparaginase treatment in acute lymphoblastic leukemia: a focus on *Erwinia* asparaginase

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Abstract

Asparaginases are a cornerstone of treatment protocols for acute lymphoblastic leukemia (ALL) and are used for remission induction and intensification treatment in all pediatric regimens and in the majority of adult protocols. Extensive clinical data have shown that intensive asparaginase treatment improves clinical outcomes in childhood ALL. Three asparaginase preparations are available; the native asparaginase derived from *Escherichia coli* (*E. coli*-asparaginase), a pegylated form of this enzyme (PEG-asparaginase) and a product isolated from *Erwinia chrysanthemi*, i.e. *Erwinia* asparaginase. Clinical hypersensitivity reactions and silent inactivation due to antibodies against *E.coli*-asparaginase, lead to inactivation of E-Coli asparaginase in up to 60% of cases. Current treatment protocols include *E. coli*-asparaginase or PEG-asparaginase for first-line treatment of ALL. Typically, patients exhibiting sensitivity to one formulation of

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Conflict of interest:

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asparaginase are switched to another product to ensure they receive the most efficacious treatment regimen possible. Erwinia asparaginase is used as a second- or third-line treatment in European and US protocols. Despite the universal inclusion of asparaginase in such treatment protocols, there is much debate regarding the optimal formulation and dosage of these agents. This manuscript provides an overview of available evidence to make recommendations for optimal use of Erwinia asparaginase in the treatment of ALL.

Keywords

acute lymphoblastic leukemia; asparagine depletion; asparaginase; Erwinia asparaginase; Erwinase; ALL

Introduction

The long-term outcome of acute lymphoblastic leukemia (ALL) has improved dramatically over the last few decades through the development of effective treatments and well-designed treatment protocols. Long-term event-free survival rates in children are currently around 80%¹⁻⁶ and overall survival rates are close to or exceeding 90%.⁴ Although overall survival rates in adults have improved in recent years, only 38–50% achieve long-term survival.^{7;8} The superior outcome achieved in childhood ALL has been attributed to a higher proportion of favorable genetic subtypes, more effective treatment options and better compliance with treatment by patients, parents and doctors when compared with adult ALL patients, alongside a poorer tolerance of adults to some chemotherapy regimens.⁹⁻¹¹ While the majority of recent regimens for adult ALL are based on pediatric schedules, there is room for further treatment refinement in these patients.^{9;10;12;12-17}

Among the drugs used in the treatment of ALL are bacterial-derived enzymes, referred to as asparaginases.^{6;18} Three main types of asparaginase have been used to date: native asparaginase derived from *Escherichia coli* (*E. coli*-asparaginase: Kidrolase[®], EUSA Pharma; Elspar[®], Ovation Pharmaceuticals; Crasnitin[™], Bayer; Leunase[®], sanofi-aventis; Asparaginase medac[™], Kyowa Hakko); a pegylated form of the native *E. coli*-asparaginase (polyethylene glycol [PEG]-asparaginase: Oncaspar[™], Enzon Pharmaceuticals Inc);¹⁹ and an enzyme isolated from *Erwinia chrysanthemi*, referred to as Erwinia asparaginase (Erwinase[®], EUSA Pharma).¹⁸ It is important to note that some of these preparations are no longer available in all countries. A fourth, new, recombinant *E. coli*-asparaginase preparation is currently undergoing clinical evaluation; it is engineered to have an amino acid sequence identical to that of Asparaginase medac[™], with initial data showing a comparable efficacy and toxicity profile to those of the other *E. coli*-asparaginases.²⁰ An asparaginase encapsulated into homologous red blood cells has recently been proposed as a new approach to maintain enzyme activity, while reducing formation of anti-asparaginase antibodies.²¹ In addition, a pegylated form of recombinant Erwinia asparaginase is under preclinical study.²²

The parenteral administration of asparaginase results in rapid and complete deamination of the amino acid asparagine and, to a lesser extent glutamine,²³⁻²⁶ leading to depletion of asparagine, especially in the plasma^{23;27-31} and, in part, the cerebrospinal fluid (CSF).^{32;33} Differences in the biological activity among available *E. coli*-asparaginase preparations have been suggested.³² The tolerated dose has varied among trials,^{4,25,34} which is also suggestive of differences in the relative potency of the available asparaginase products.

Despite being an essential drug used in all treatment protocols for ALL, there is much debate regarding the optimal formulation and dosage of asparaginase. We provide an

overview of available data on the use of asparaginases, and focus on *Erwinia* asparaginase which has been less well studied compared with other forms.

Asparaginase therapy in ALL

Efficacy data for asparaginases

Extensive clinical data support the use of asparaginase therapy in pediatric ALL^{2;4;6;35-38} and the benefit of intensive asparaginase treatment compared with less intensive regimens has been demonstrated (Figure 1).^{2;38-41} In a study conducted by the Dana-Faber Cancer Institute (DFCI) ALL Consortium and designed to improve outcomes and minimize toxicities in pediatric patients with standard-risk or high-risk ALL, 377 children were enrolled to receive a high-dose native *E.coli* asparaginase (25 000 IU/m² weekly) or PEG-asparaginase (2 500 IU/m² every other week) for 30 weeks during intensification therapy. The estimated 5-year event-free survival rate was significantly higher than that of a previously conducted DFCI ALL Consortium study (83%±2% vs 74% ±3%; p<0.01), a finding which was attributed to the prolonged asparaginase intensification.² In addition, in this study children who tolerated more than 25 weekly doses of asparaginase had a better event-free survival than those who received 25 or fewer doses.² Furthermore, a randomized study carried out by the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) determined the efficacy of a BFM-type modified chemotherapy regimen with or without prolonged use of high-dose native *E.coli* asparaginase (25 000 IU/m² weekly for 20 weeks) during continuation therapy in 355 children with standard-risk ALL.⁴¹ Children given asparaginase had a significantly increased 10-year disease-free survival (87.5% vs 78.7%) and overall survival (93.7% vs 88.6%), with a 40% reduction in the relative risk of failure compared with patients who were not treated with asparaginase.⁴¹ This finding supports previous data of Amylon et al³⁸ showing that high-dose native *E.coli* asparaginase (25 000 IU/m² weekly for 20 weeks) during consolidation significantly improved complete continuous remission in pediatric patients with T-cell ALL and lymphoblastic lymphoma compared with patients treated with lower-dose asparaginase regimen (71.3% vs 57.8%, respectively). The randomized studies conducted by Moghrabi et al⁴⁰ and Duval et al³⁹ made clear that asparaginase preparations with a shorter half-life result in a poorer event-free survival, albeit less toxicity, compared to the use of asparaginase preparation with a longer half-life given at the same dose and frequency (Figure 1). It is also noteworthy that in a study carried out by Rizzari et al,⁴² no significant difference in disease-free survival was observed between patients who received standard treatment (10 000 IU/m² asparaginase for 4 doses during reinduction) and those who received high-dose treatment (25 000 IU/m² asparaginase weekly for 20 weeks during reinduction and early continuation)

As a result of these trials, asparaginases are now a universal component of ALL therapy and are used for remission induction and intensification treatment in every pediatric regimen for ALL. However, much debate remains regarding the optimal formulation and dosage of asparaginase in the treatment of ALL. Therapy aims to achieve serum asparagine depletion but no critical minimum value for efficacy has yet been established.^{24;25;43} A serum level of asparaginase >100 IU/L corresponds to depletion of asparagine (ie below the level of quantification)²⁷ and therefore is often considered the target trough asparaginase level; complete asparagine depletion is observed less frequently with enzyme concentrations below this level.^{43;44} However, there is some evidence to suggest that trough asparaginase levels of below 50 IU/L can also result in serum and CSF asparagine depletion.⁴⁴

Toxicity of asparaginases: hypersensitivity

Asparaginases are associated with a unique set of side effects. Hypersensitivity reactions, due to anti-asparaginase antibody production, have been observed in up to 60% of patients at

some time during *E. coli* asparaginase therapy.⁴⁵ The development of these antibodies appears to be more commonly observed with native *E. coli*-asparaginase^{28;46;47} compared with the pegylated enzyme.^{28;48} (Table 1) Symptoms of clinical hypersensitivity include anaphylaxis, pain, edema, Quincke's edema, urticaria, erythema, rash and pruritis.⁴⁶ The route of administration determines the clinical symptoms with a greater incidence of major skin reactions observed with intramuscular (IM) administration compared with intravenous (IV) administration.⁵² Clinical hypersensitivity occurs almost exclusively in post-induction regimens (ie intensification, re-induction)^{50;53} when asparaginase has not been given for weeks or months. There are several possible explanations for the rarity of allergic reactions during remission induction. For example, there is a delay in an effective immune response due to the time taken for complement activation and the subsequent production of antibodies¹⁸ the symptoms associated with allergy might be masked by intensive corticosteroids treatment that occurs during induction,¹⁸ and the frequency of dosing during induction may have a desensitizing effect, since allergic reactions are rarely observed in this phase despite measurable antibody production. Some studies have shown that the incidence of hypersensitivity to asparaginase is similar between age groups,^{2;54} although others have suggested that infants and younger patients develop antibody and hypersensitivity reactions less frequently than teenagers and adult patients.¹⁸

Antibodies produced in response to asparaginases do not always lead to clinical hypersensitivity, but may instead cause rapid inactivation of the asparaginase, resulting in suboptimal asparagine depletion. This is commonly referred to as 'silent hypersensitivity' or 'silent inactivation'^{45;55;56} and may occur in approximately 30% of the patients.⁴⁵ Development of anti-asparaginase antibodies can thus confer resistance to asparaginase therapy and is associated with higher plasma levels of asparagine⁵⁷ and reduced therapeutic efficacy in some,^{55;58} but not all, studies.^{46;47} This inconsistency of anti-asparaginase antibodies as a prognostic indicator may be explained by the efficacy of the overall treatment regimens and the use of alternative asparaginase preparations following allergic reactions, which may mitigate the adverse effects of silent hypersensitivity.

Typically, patients exhibiting clinical allergy symptoms to one formulation of asparaginase are switched to another product to ensure they receive the most efficacious treatment regimen possible.^{45;56} However, since patients with silent hypersensitivity lack clinical symptoms and routine antibody monitoring is often not implemented, asparaginase switching does not usually occur in this setting.⁴⁵ PEG-asparaginase has a relatively lower immunogenicity due to the covalent conjugation to monomethoxy polyethylene glycol⁵⁹ and often replaces *E. coli*-asparaginase in patients who develop allergic reaction. This switch may not be optimal because antibodies against *E. coli*-asparaginase can cross-react with PEG-asparaginase.^{49;60} Moreover, PEG-asparaginase may also induce silent inactivation⁵⁶ with antibodies resulting in a fast decline in asparaginase activity.⁶¹ Switching from PEG-asparaginase after an allergic reaction to *E. coli*-asparaginase is not considered a viable treatment option.⁶²

Other toxicity associated with asparaginases

Pancreatitis occurs in 4–18% of pediatric patients, depending upon the definition used in the study, and can cause significant morbidity.^{54;63;64} Adolescents appear to be at higher risk for developing this condition than younger children.⁵⁴ Pancreatitis tends to occur after the first few weeks of asparaginase, suggesting a predisposition to this complication rather than a cumulative drug effect.⁶⁴ Re-treatment with asparaginase after an episode of pancreatitis is associated with a high risk of recurrence⁶⁴ and so further doses of asparaginase are often omitted, which may negatively impact event-free survival.² Other asparaginase-related toxicities include abnormalities of hemostasis (including central nervous system [CNS] thromboses and hemorrhage, and peripheral deep venous thromboses in 2–4% of patients),

hyperglycemia and abnormalities of lipid metabolism.^{59;65} As with pancreatitis, thrombotic complications are more common in adolescents and adults than in younger children.⁵⁴ In adult patients, liver toxicity with elevated liver enzymes or increased bilirubin is a frequent clinical problem.⁸

Erwinia asparaginase in ALL

Currently, there are no widely accepted guidelines for the use of asparaginases, especially Erwinia asparaginase. A number of comparative studies have been conducted with Erwinia asparaginase and native *E. coli* preparations; the dose and schedules of asparaginase in these studies have been inconsistent and outcomes have been variable.^{39;40;66;67} However, the efficacy of Erwinia asparaginase following hypersensitivity to *E. coli*-asparaginase preparations has been demonstrated.^{45;68} The differences in these results highlight the need for recommendations to provide guidance for the optimal use of Erwinia asparaginase in the treatment of ALL.

Efficacy data for Erwinia asparaginase

Eden et al⁶⁶ carried out a non-randomized study (UKALL VIII) comparing the toxicity of IM administration of Erwinia asparaginase with *E. coli*-asparaginase (6000 IU/m² three-times weekly for 3 weeks) in 758 unselected children with ALL. No apparent difference in event-free survival was observed after 4.5 years of follow-up, but the incidence of neurotoxicity, pancreatitis and life-threatening sepsis was significantly lower in children treated with Erwinia asparaginase compared with those who received *E. coli*-asparaginase (neurotoxicity: 2% vs 4%; pancreatitis: 0% vs 2%; sepsis: 18% vs 20%).⁶⁶ Results from this early trial led to the first randomized study (European Organisation for Research and Treatment of Cancer-Children's Leukemia Group [EORTC-CLG] 58881) comparing Erwinia asparaginase with *E. coli*-asparaginase³⁹ and included 700 children (aged <18 years) with ALL (93%) or lymphoblastic non-Hodgkin's lymphoma (7%). Patients were randomized to receive the same dosage of either asparaginase (10 000 IU/m² IV twice-weekly for a total of eight doses in the induction phase and four doses in the re-induction phase). Significantly more patients administered Erwinia asparaginase failed to achieve complete remission compared with those who received *E. coli*-asparaginase (4.9% vs 2%) and the relapse rate was higher, resulting in reduced event-free survival. Overall 6-year survival was also significantly superior but coagulopathy was more common in patients administered *E. coli*-asparaginase compared with the Erwinia asparaginase group (83.9% vs 75.1%).³⁹

A subsequent randomized study, DFCI-ALL-95-01, compared administration of Erwinia asparaginase (25 000 IU/m² once in induction followed by once-weekly doses for 20 weeks during intensification) with the same doses of *E. coli*-asparaginase in 286 ALL patients (aged 0–18 years).⁴⁰ The 139 children given Erwinia asparaginase had significantly reduced toxicity (10% vs 24%, $p < 0.01$) and fewer allergic reactions (6% vs 14%, $p = 0.03$) compared with 147 *E. coli*-asparaginase-treated patients, but had significantly lower 5-year event-free survival (78% \pm 4% vs 89% \pm 3%).⁴⁰ There were also significantly more relapses involving the CNS in children receiving Erwinia asparaginase compared with *E. coli*-asparaginase-treated patients (6% vs 1%).⁴⁰

Another study by Kwok et al⁶⁷ compared the efficacy of Erwinia asparaginase and *E. coli*-asparaginase in 116 children with ALL. Erwinia asparaginase was administered at a dose of 10 000 IU/m² IM and *E. coli*-asparaginase at 7500 to 10 000 IU/m² IM twice-weekly for eight doses during remission induction. Patients treated with Erwinia asparaginase were 6.7 times more likely to have residual leukemia levels $\geq 10^{-2}$ in bone marrow compared with *E. coli*-asparaginase-treated patients.⁶⁷

Due to the shorter half-life of Erwinia asparaginase compared with the *E. coli*-derived preparations,⁵⁵ a higher dose and increased frequency of treatment is required to ensure adequate serum enzyme activity and complete serum asparagine depletion. It is therefore possible that the inferior outcome of patients treated with Erwinia asparaginase in these trials (with parallel decreases in adverse reactions) is a result of insufficient dose and frequency of this preparation.^{11;40;62;69} Indeed, Boos et al²⁵ reported that only 26% of samples from ALL patients had complete depletion of asparagine (ie ≤ 0.1 $\mu\text{mol/L}$) 3 days after administration of Erwinia asparaginase (10 000 IU/m² at 3-day intervals). In addition, physiological asparagine levels recovered faster after Erwinia asparaginase than *E. coli* preparations.²⁵

In one study, Erwinia asparaginase (30 000 IU/m² IV or IM) was given daily during induction therapy and twice a week for 2 weeks during re-induction phase. The trough levels (measured immediately before the next administration) were below 100 IU/L in approximately two-thirds of samples during reinduction.⁴³ Consequently, the majority of patients failed to achieve complete depletion of asparagine during re-induction. Similarly, in a DFCI ALL Consortium trial, in which patients switched to Erwinia asparaginase (25 000 IU/m² twice-weekly) after allergy to *E. coli*-asparaginase, 83% of patients had serum enzyme activity levels at or above 100 IU/L 3 days after administration, but only 42% of patients maintained that level 4 days post-dosing.⁶⁸ These data highlight that even with relatively high Erwinia asparaginase doses (25 000–30 000 IU/m²), a twice-weekly regimen was still associated with inadequate enzyme levels in most patients.^{43;68} Despite these findings, treatment with twice-weekly Erwinia asparaginase after *E. coli*-asparaginase allergy did not adversely impact rates of event-free survival in the DFCI ALL Consortium trial.⁶⁸

Evidence from Viera Pinheiro et al²⁶ suggests increased dosing frequency enhances Erwinia asparaginase activity. In this study, patients with ALL and non-Hodgkin's lymphoma were administered Erwinia asparaginase (20 000 IU/m² three-times weekly) and trough asparagine levels and asparaginase activity were assessed 2 and 3 days following therapy.²⁶ Mean serum asparaginase trough levels were above the target level of 100 IU/L 2 days after administration of Erwinia asparaginase (mean asparaginase level: 156 IU/L), although the activity fell after 3 days (mean asparaginase activity: 50 IU/L). Finally, Erwinia asparaginase administered at 10 000 U/m² IV every second day resulted in a median trough activity of 115 U/L 2 days after administration but asparaginase activities were below 100 U/L in 45% of samples.⁷⁰ Taken together, these data show that even a regimen of three-times weekly dosing (with a 2-day interval at weekends) yields inadequate asparaginase trough activity for at least part of the treatment schedule (typically at the weekend). In this regard, all the comparative studies in which Erwinia asparaginase yielded 'inferior' outcomes included less frequent and/or lower absolute doses than those used by Viera Pinheiro et al²⁶ and therefore serum asparagine levels may not have been sufficiently depleted.

Second-line treatment with Erwinia asparaginase

Despite the apparently inferior outcomes of comparative studies of Erwinia asparaginase with *E. coli*-derived preparations, a study of 1001 high-risk pediatric ALL patients treated with nine doses of native *E. coli*-asparaginase during induction (6000 IU/m² three-times weekly for 3 weeks) demonstrated the efficacy of switching products after clinical hypersensitivity.⁴⁵ Results from an interim analysis of 280 patients, who were evaluated for at least 30 months after induction, showed that 41% developed clinical allergic reactions with positive antibody formation and were switched to Erwinia asparaginase. The antibody-positive patients with allergic symptoms were switched to Erwinia asparaginase, resulting in a reduction in their hazard ratio for treatment failure from 3.2 to 0.6. In contrast, 29% of

patients had silent hypersensitivity and continued to receive *E. coli*-asparaginase; these children had poorer outcomes.⁴⁵ This demonstrates that awareness of the presence of asparaginase antibodies (in the absence of allergy) and subsequent switching to Erwinia asparaginase might mitigate the adverse effects of silent hypersensitivity.

Studies have shown cross-reactivity between patients' antibodies against *E. coli*-asparaginase and PEG-asparaginase, but not between those against *E. coli*-asparaginase and Erwinia asparaginase.^{49;60} Moreover, asparagine concentrations were less depleted by PEG-asparaginase than by Erwinia asparaginase in a small study of patients with antibodies against *E. coli*-asparaginase.⁵⁷ Interestingly, one study showed that patients may also develop antibodies to the non-protein PEG moiety of PEG-asparaginase.⁷¹ This was associated with rapid clearance of PEG-asparaginase in a subgroup of pediatric patients who otherwise did not present a clinical manifestation of hypersensitivity or allergy. Furthermore, a population pharmacokinetic model demonstrated a fast decline in asparaginase activity in a group of patients, most likely related to the development of antibodies against PEG-asparaginase.⁶¹ It has therefore been suggested that anti-PEG level monitoring/screening or asparaginase activity measurements could allow for modification in PEG-asparaginase dosing or the use of an alternative asparaginase.^{61;71} So far, the presence of anti-PEG antibodies has not been confirmed by others. Routine antibody assessment or measurement of asparaginase levels has been proposed to predict future allergic reaction or to alert physicians to the possibility of silent hypersensitivity.^{18;26;46}

As yet, there are no data from large well-designed studies to demonstrate a preference for Erwinia asparaginase over PEG-asparaginase in patients developing hypersensitivity to *E. coli*-asparaginase and there is no consensus opinion on this. After allergic reactions to *E. coli* preparations, substitution with an alternative asparaginase should be based on drug monitoring.²⁵ Erwinia asparaginase appears to be well tolerated in children with previous allergy to *E. coli*-asparaginase.⁶⁸ Allergic reactions to Erwinia asparaginase have also been reported in up to 33% of patients switching to Erwinia asparaginase after clinical hypersensitivity to native *E. coli*-asparaginase.^{68;72}

Current status of and recommendations for the use of Erwinia asparaginase

Both *E. coli*-asparaginase and PEG-asparaginase can be used as first-line treatment in pediatric ALL protocols, depending upon country. Before a temporary interruption in 2002 that resulted from manufacturing issues related to vial stoppers, Erwinia asparaginase was considered the best alternative in cases of clinical hypersensitivity to these enzymes.⁶² Erwinia asparaginase production was reinstated in 2006 and previous European licenses are planned for reinstatement, together with a process of mutual recognition in other European countries and full approval in the USA.

Which patients should receive Erwinia asparaginase?

Patients developing allergic reactions to one asparaginase should be switched to an alternative product, to ensure maximum clinical benefit in terms of survival. Second-line asparaginase therapy should be dictated by protocols or regulatory/availability factors and the type of asparaginase used in front-line therapy; some protocols advise Erwinia asparaginase as a preferable preparation after allergic reaction to native *E. coli*-asparaginase, whilst others prescribe PEG-asparaginase as replacement for native *E. coli*-asparaginase and Erwinia asparaginase as third-line drug.

Several clinical trial groups in Europe and the United States allow the use of Erwinia asparaginase as a second-line agent (eg Nordic Society of Pediatric Hematology and

Oncology [NOPHO], German Multicenter Acute Lymphoblastic Leukemia Study Group [GMALL], EORTC-58951, French Acute Lymphoblastic Leukemia group [FRALLE], Children's Oncology Group [COG]), DFCI ALL Consortium and, for others, as a third-line treatment (AIEOP, ALL-BFM-2000, Dutch Childhood Oncology Group [DCOG-ALL-10], Czech Republic protocols). Selection of the individual asparaginase is determined by availability, treatment protocol and treatment status of the patients (ie asparaginase-naïve or relapsed) (Table 2) and various *Erwinia* asparaginase dosing regimens are in use (Table 3).

Recommendations

- *Erwinia* asparaginase should be used for the second- or third-line treatment of ALL, depending upon regulatory requirements, in patients developing hypersensitivity to *E. coli* asparaginase preparations
- *Erwinia* asparaginase should be prescribed if switching from PEG-asparaginase is required (ie second-line use of native *E. coli*-asparaginase is not justified)

Erwinia asparaginase dosing and schedule

Due to the short half-life of *Erwinia* asparaginase,⁵⁵ a higher dose and increased dosing frequency is required to ensure optimal asparagine depletion. Current evidence suggests that *Erwinia* asparaginase should be administered at dosages of at least 20 000 IU/m² three-times weekly, ie every second day with a 3-day interval at the weekend.²⁶ Twice-weekly dosing at higher doses (25 000–30 000 IU/m²) has been associated with suboptimal trough serum enzyme activity, but not consistently with inferior event-free survival^{43;68} and, as a result, this dosing regimen is still utilized by some groups.

As *Erwinia* asparaginase requires frequent dosing to maintain asparagine depletion, therapeutic drug monitoring data (specifically for serum asparaginase levels) could assist in determining whether increasing the interval between doses is possible and could therefore help to minimize inconvenience to both patients and physicians. Furthermore, the development of pegylated *Erwinia* asparaginase with a longer half-life would make the dosing schedule more convenient for patients.

Recommendations

- *Erwinia* asparaginase should be administered at dosages of at least 20 000 IU/m² multiple times per week (eg three-times weekly)

Duration of treatment

The optimal duration of *Erwinia* asparaginase treatment has yet to be established, although it has been suggested that prolonged intensification results in improved survival. This was demonstrated in a study by Silverman et al², where the 5-year event-free survival rate of patients who received at least 26 weeks of asparaginase therapy was significantly better than those who tolerated 25 weeks or fewer of therapy (90% vs 73%). This study, together with the studies presented above and summarized in Figure 1, suggest that prolonged and intensified therapy with asparaginase improves outcome of children with ALL.

If *Erwinia* asparaginase is used as second-line treatment to replace native *E. coli*-asparaginase or PEG-asparaginase the duration of treatment depends on the protocol and the yielded duration of asparagine depletion. Also, the duration of asparaginase treatment will depend on the backbone of combination chemotherapy that is given.

Recommendations

- Prolonged intensification with asparaginase to optimize survival benefits

Route of administration of Erwinia asparaginase

Intravenous administration results in higher peak plasma concentrations, while IM administration results in a concurrent slower increase of asparaginase activity due to the depot effect. Accordingly, administration of 10 000 U/m² Erwinia asparaginase applied every second day results in median trough activities of 115 U/L (determined from 58 samples of 15 patients) when applied intravenously, and of 151 U/L (determined from 39 samples of 14 patients) when applied intramuscularly. After IM administration, only 15% of analyzed samples showed asparaginase activities below the desired activity of 100 U/L, while 45% of samples were below 100 U/L, when Erwinia asparaginase was administered intravenously.⁷⁰

However, Rizzari et al⁴⁴ found no significant differences in mean enzyme activity or frequency of samples showing complete asparagine depletion after IV or IM administration of Erwinia asparaginase 10 000 IU/m² every 3 days (eight doses) administered in the induction phase.⁴⁴ Similarly, Albertsen et al⁴³ found comparable complete asparagine depletion in patients given a more intense regimen of IV or IM administration at 30 000 IU/m² daily for 10 days in the induction phase.⁴³ In this study, however, Erwinia asparaginase administered via the IM route produced trough asparaginase plasma levels significantly lower (by approximately 28%) than IV administration. During the subsequent re-induction phase (30 000 IU/m² twice-weekly for 2 weeks), no differences were observed between the two routes in terms of trough asparaginase activities or in the proportion of patients who failed to achieve complete asparagine depletion.⁴³ Finally, no significant differences have been observed between two routes of administration of Erwinia asparaginase 30 000 IU/m² twice-weekly for 2 weeks as a re-induction regimen in terms of neutralizing asparaginase antibody formation.⁵⁰

The results of studies investigating the optimal route for the administration of asparaginase are inconsistent and therefore further studies are required to determine whether IV or IM administration of Erwinia asparaginase is associated with any meaningful clinical differences.²⁴

Recommendations

- No recommendations are made for the route of administration as more data are required to define the optimal route. However, most groups in Europe currently use IV asparaginases, while North American groups more often administer this agent intramuscularly

Monitoring of asparaginase trough levels and/or depletion of asparagine

Initially, the US Food and Drug Administration required that asparagine levels were used as the primary outcome measure in clinical trials. Asparaginase therapy aims to achieve serum asparagine depletion, but no critical minimum value for efficacy has yet been established^{24;25;43} and asparagine levels are difficult to measure accurately when asparaginase is present in blood because the enzyme can continue to breakdown asparagine *ex vivo* if the sample is not immediately processed and stored on ice. Therefore, monitoring of asparaginase levels is more reliable than measurement of asparagine itself. A serum level of asparaginase >100 IU/L and possibly >50 IU/L corresponds to depletion of asparagine (ie below the level of quantification)^{44;73}; complete asparagine depletion is observed less frequently with enzyme concentrations below this level.^{43;44} However, clinical testing to measure asparaginase levels or asparagine depletion is not routinely carried out, although therapeutic drug monitoring is offered in Europe to guide therapeutic decisions (Boos et al. personal communication).

Recommendations

- Due to technical difficulties in measuring serum asparagine levels, monitoring asparaginase levels is more reliable and therefore recommended for adaptation of asparaginase dosing in individual cases and for trials in which regulatory authorities ask for pharmacokinetic and pharmacodynamic endpoints

Monitoring of *Erwinia* asparaginase antibody levels

Although it has been advocated previously to determine anti-asparaginase antibody levels to determine whether alterations in dosing regimen should be employed to overcome the risk of silent hypersensitivity, monitoring of asparaginase levels should be sufficient to identify silent hypersensitivity, since not all antibodies lead to asparaginase inactivation.

Recommendations

- No recommendations are made for monitoring antibody status

Conclusions

Advances in therapies for ALL have led to improved long-term survival rates for pediatric and adult patients. Asparaginases form a cornerstone of ALL treatment protocols with three main preparations for use in treatment protocols: the native *E. coli*-asparaginase, a pegylated form (PEG-asparaginase) and an alternative enzyme isolated from *Erwinia chrysanthemi*, referred to as *Erwinia* asparaginase. Despite the availability of these agents, much debate remains regarding the optimal formulation and dose for the treatment of pediatric and adult ALL patients. This manuscript aims to provide recommendations, based on data available in the literature, to ensure optimal use of *Erwinia* asparaginase. Patients who receive an asparaginase as first-line treatment for ALL and develop anti-asparaginase antibodies should be switched to another asparaginase preparation to ensure maximum survival benefit. Monitoring of asparaginase levels is preferable to assess the extent of serum asparagine depletion and to identify cases of silent inactivation. *Erwinia* asparaginase is a valid second- or third-line therapy, depending upon protocols, regulatory factors and availability. Evidence from published studies suggests that *Erwinia* asparaginase should be administered at a dose of at least 20 000 IU/m² three-times weekly, via either the IV or IM route. Further clinical and pharmacokinetic studies of *Erwinia* asparaginase will help to optimize the use of this agent.

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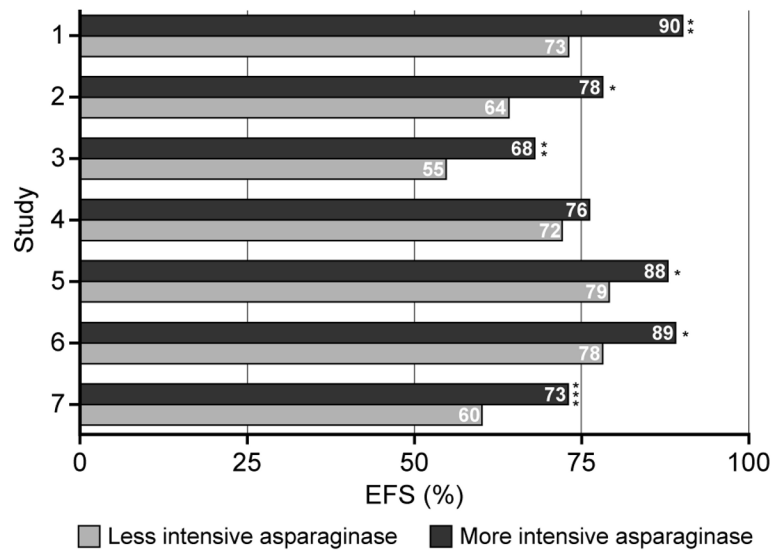


Figure 1.
Effect of intensification with asparaginases on event-free survival

1. Silverman et al. ⁽²⁾
2. Amylon et al. ⁽³⁸⁾
3. Amylon et al. ⁽³⁸⁾
4. Rizzari et al. ⁽⁴²⁾
5. Pession et al. ⁽⁴¹⁾
6. Moghrabi et al. ⁽⁴⁰⁾
7. Duval et al. ⁽³⁹⁾

EFS, event-free survival

Intensification studies

1 Less than or greater than 25 weeks of treatment

2–5 Additional 20 weeks of treatment

6–7 *Erwina* vs *E.Coli*-ASP

*p<0.05; **p<0.01; ***p<0.001

Table 1

Incidence of specific antibodies induced by the three main asparaginase types

Asparaginase type	Dose	Concomitant steroid medications	Antibody-positive patients	Citation
<i>E. Coli</i>	10 000 IU/m ² IM three-times weekly for nine doses during induction and nine during re-induction	Prednisolone	35.5%	Woo et al. 2000 (46)
	6000 IU/m ² IM three-times weekly for nine (induction) and six (intensification) doses	Prednisolone/dexamethasone	26–42%	Avramis et al. 2002 (28)
PEG	6000 IU/m ² SC two-times weekly for 14 doses (induction/intensification)	Prednisolone	20%	Larson et al. 1998 (47)
	2500 IU/m ² IM for a total of four doses (induction) and one dose (intensification)	Dexamethasone	11%	Hawkins et al. 2004 (48)
Erwinia	2500 IU/m ² IM for one dose (induction) and one dose (delayed intensification)	Prednisolone/dexamethasone	2–11%	Avramis et al. 2002 (28)
	10 000 IU/m ² IM three-times weekly for a total of nine doses (induction/re-induction)		33%	Wang et al. 2003 (49)
	30 000 IU/m ² IV or IM daily for a total of 10 doses (induction), twice-weekly for a total of four doses (re-induction)	Prednisolone	21%	Albertsen et al. 2002 (50)
	30 000 IU/m ² IV or IM daily for a total of 10 doses (induction), twice-weekly for a total of four doses (re-induction)	Prednisolone/dexamethasone	8–10%	Albertsen et al. 2002 (53)

E. Coli, *Escherichia coli*; IM, intramuscular; SC, subcutaneous; PEG, polyethylene glycol; IV, intravenous

Table 2

Current regional use of asparaginases (source: EUSA Pharma)

	North America, UK, Australia and New Zealand	Europe (BFM zone)	Rest of World
Children	Naïve patients	First-line: <i>E. coli</i> -asparaginase Second-line: Erwinia asparaginase	First-line: <i>E. coli</i> -asparaginase Second-line: Erwinia asparaginase or PEG-asparaginase
	Relapsed	First-line: PEG-asparaginase Second-line: Erwinia asparaginase	First-line: <i>E. coli</i> -asparaginase Second-line: Erwinia asparaginase or PEG-asparaginase
Adults	Naïve patients	First-line: <i>E. coli</i> -asparaginase or PEG-asparaginase Second-line: Erwinia asparaginase or PEG-asparaginase	First-line: <i>E. coli</i> -asparaginase Second-line: Erwinia asparaginase or PEG-asparaginase

BFM, Berlin-Frankfurt-Münster; PEG, polyethylene glycol; *E. Coli*, *Escherichia coli*

Table 3

ALL protocols currently used for Erwinia asparaginase (second- or third-line treatment)

Protocol	Second-line treatment
NOPHO	Erwinia asparaginase 20 000 IU/m ² two–three-times/week (×6)
AIEOP	NB: Erwinia asparaginase as third-line Erwinia asparaginase 20 000 IU/m ² every other day
GMALL-07/2003 and 01/2003	Erwinia asparaginase 20 000 IU/m ² three-times weekly (×5); IV (10 000 IU/m ² in patients older than 55 years)
COG	Erwinia asparaginase 25 000 IU/m ² three-times weekly (×6); IM
COALL-07-03	Erwinia asparaginase 45 000 IU/m ² (×2)
Czech Republic	NB: Erwinia asparaginase as third-line Induction and late intensification • Erwinia asparaginase 10 000 IU/m ² twice-weekly HR blocks • Erwinia asparaginase 10 000 IU/m ² twice-weekly First relapse • Erwinia asparaginase 10 000 IU/m ² twice-weekly NB: Erwinia asparaginase as third-line Induction • Erwinia asparaginase 10 000 IU/m ² two–three times/week Intensification (standard or medium risk) • Erwinia asparaginase 10 000 IU/m ² two–three times/week HR blocks Erwinia asparaginase 10 000 IU/m ² , two–three times/week Protocol I • Erwinia asparaginase 10 000 IU/m ² every 2 days IM/IV Protocol II • Erwinia asparaginase 10 000 IU/m ² every 2 days IM/IV Block HR-I Erwinia asparaginase 10 000 IU/m ² every 2 days IM/IV Erwinia asparaginase 20 000 IU/m ² two–three times/week; IM Induction, delayed intensification(s): Erwinia asparaginase 12 000 IU/m ² three times/week; IM, ie double dose compared to <i>E.coli</i> asparaginase
DCOG ALL-10	
BFM-2000	
EORTC-58951	
FRALLE- 2000	

Protocol	Second-line treatment
St Jude	<p>Induction:</p> <p>Erwinia asparaginase 20 000 IU/m² three times/week (×6) IM;</p> <p>Post-remission: 30 000 or 42 000 IU/m² twice-weekly IM for 30 weeks (standard-/high-risk patients), twice-weekly for 4 weeks during first and second reinduction (low-risk patients)</p> <p>Post-induction consolidation:</p> <p>Erwinia Asparaginase 25 000 IU/m² twice-weekly IM for 30 weeks</p>
DFCI ALL Consortium	

NOPHO, Nordic Society of Pediatric Hematology and Oncology; AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; GMALL, German Multicenter Acute Lymphoblastic Leukemia Study Group; COG, Children's Oncology Group; COALL, Cooperative Study Group for Childhood Acute Lymphoblastic Leukemia; DCOG, Dutch Childhood Oncology Group; BFM, Berlin-Frankfurt-Münster; EORTC, European Organization for Research and Treatment of Cancer; FRALLE, French Acute Lymphoblastic Leukemia group; DFCl, Dana-Faber Cancer Institute; IV, intravenous; IM, intramuscular; HR, high risk