Review Article

L-Asparaginase Treatment in Acute Lymphoblastic Leukemia

A Focus on *Erwinia* Asparaginase

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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 treatment 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*, ie, *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 asparaginase are switched to another 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, debate on the optimal formulation and dosage of these agents continues. This article provides an overview of available evidence for optimal use of *Erwinia* asparaginase in the treatment of ALL. ***Cancer* 2011;117:238–49.** © *2010 American Cancer Society.*

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The long-term outcome of acute lymphoblastic leukemia (ALL) has improved dramatically during the last few decades because of 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% to 50% achieve long-term survival.[7,8] Compared with adult ALL patients, who have a poorer tolerance to some chemotherapy regimens,[9-11] childhood ALL patients achieve a superior outcome, attributed to a higher proportion of favorable genetic subtypes, more effective treatment options, and better compliance with treatment by patients, parents, and physicians. Although the majority of recent regimens for adult ALL patients are based on pediatric treatment schedules, there is room for further treatment refinement in these patients.[9,10,12-17]

Among the drugs used in the treatment of ALL are bacteria-derived enzymes, referred to as asparaginases.[6,18] Three main types of asparaginase have been used so far: 1) native asparaginase derived from *Escherichia coli* (*E. coli* asparaginase: Kidrolase, EUSA Pharma, Oxford, UK; Elspar, Ovation Pharmaceuticals, Deerfield, Illinois; Crasnitin, Bayer AG, Leverkusen, Germany; Leunase, Sanofi-Aventis, Paris, France; Asparaginase Medac, Kyowa Hakko, Tokyo, Japan), 2) a pegylated form of the native *E. coli* asparaginase (polyethylene glycol [PEG]-asparaginase: Oncaspar, Sigma-Tau Pharmaceuticals, Inc.,

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Gaithersburg, MD),[19] and 3) an enzyme isolated from *Erwinia chrysanthemi*, referred to as *Erwinia* asparaginase (Erwinase, EUSA Pharma, Oxford, UK).[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 efficacy and toxicity profile comparable 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 antiasparaginase 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 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 its use as an essential drug used in all treatment protocols for ALL, asparaginase's optimal formulation and dosage are still being debated. We provide an overview of available data on the use of asparaginases with a 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 (Fig. 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/m^2 weekly) or PEG-asparaginase (2 500 IU/m^2 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%; $P < .01$), a finding that 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/m^2 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 an 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 from Amylon et al[38] showing that high-dose native *E. coli* asparaginase (25 000 IU/m^2 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 a lower-dose asparaginase regimen (71.3% vs 57.8%, respectively). The randomized studies conducted by Moghrabi et al[40] and Duval et al[39] made clear that asparaginase preparations with a shorter half-life result in a poorer

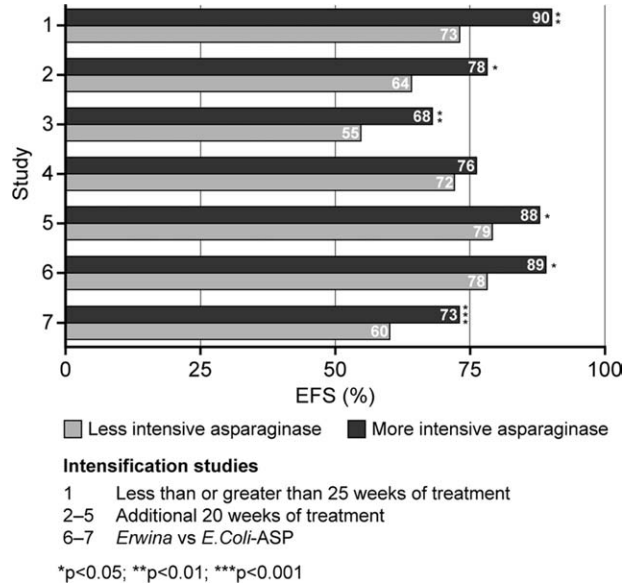

Figure 1. The effect of intensification with asparaginases on event-free survival is shown. EFS indicates event-free survival; Study 1, Silverman[2]; Studies 2 and 3, Amylon[38]; Study 4, Rizzari[42]; Study 5, Pession[41]; Study 6, Moghrabi[40]; Study 7, Duval.[39]

Table 1. Incidence of Specific Antibodies Induced by the Three Main Asparaginase Types

Asparaginase Type	Dose	Concomitant Steroid Medications	Antibody-Positive Patients	Citation
E. coli	10 000 IU/m^2 IM 3x/wk for 9 doses during induction and 9 during reinduction	Prednisolone	35.5%	Woo 2000[46]
	6000 IU/m^2 IM 3x/wk for 9 (induction) and 6 (intensification) doses	Prednisolone/dexamethasone	26-42%	Avramis 2002[28]
	6000 IU/m^2 SC 2x/wk for 14 doses (induction/intensification)	Prednisolone	20%	Larson 1998[47]
PEG	2500 IU/m^2 IM for a total of 4 doses (induction) and 1 dose (intensification)	Dexamethasone	11%	Hawkins 2004[48]
	2500 IU/m^2 IM for 1 dose (induction) and 1 dose (delayed intensification)	Prednisolone/dexamethasone	2-11%	Avramis 2002[28]
Erwinia	10 000 IU/m^2 IM 3x/wk for a total of 9 doses (induction/reinduction)	—	33%	Wang 2003[49]
	30 000 IU/m^2 IV or IM daily for a total of 10 doses (induction), 2x/wk for a total of 4 doses (reinduction)	Prednisolone	21%	Albertsen 2002[50]
	30 000 IU/m^2 IV or IM daily for a total of 10 doses (induction),2x/wk for a total of 4 doses (reinduction)	Prednisolone/dexamethasone	8-10%	Albertsen 2002[53]

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

event-free survival, albeit less toxicity, compared with the use of asparaginase preparation with a longer half-life given at the same dose and frequency (Fig. 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/m^2 asparaginase for 4 doses during reinduction) and those who received high-dose treatment (25 000 IU/m^2 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 crucial 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 antiasparaginase 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 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 postinduction regimens (ie, intensification, reinduction)[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 antibodies,[18] 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, as 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 antiasparaginase antibodies can, thus, confer resistance to asparaginase therapy and is associated with higher plasma levels of asparagine[57] and reduced therapeutic efficacy in some,[55,58] but not all, studies.[46,47] This inconsistency of antiasparaginase antibodies as a prognostic indicator may be explained by the efficacy of the overall treatment regimens and the use of alternative asparaginase preparations after 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, because 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 glycol[59] 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 inactivation[56] 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 Toxicities Associated With Asparaginases

Pancreatitis occurs in 4% to 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] Retreatment with asparaginase after an episode of pancreatitis is associated with a high risk of recurrence,[64] 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% to 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. Several 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 after 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 al[66] carried out a nonrandomized study (UKALL VIII) comparing the toxicity of IM administration of *Erwinia* asparaginase with *E. coli* asparaginase (6000 IU/m^2 3 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* asparaginase[39] and included 700 children (aged <18 years) with ALL (93%) or lymphoblastic non-Hodgkin lymphoma (7%). Patients were randomized to receive the same dosage of either asparaginase (10 000 IU/m^2 IV twice-weekly for a total of 8 doses in the induction phase and 4 doses in the reinduction 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/m^2 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%; $P < .01$) and fewer allergic reactions (6% vs 14%; $P = .03$) compared with 147 patients treated with *E. coli* asparaginase 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 those receiving *E. coli* asparaginase (6% vs 1%).[40]

Another study by Kwok et al[67] 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/m^2 IM and *E. coli* asparaginase at 7500 to 10,000 IU/m^2 IM twice-weekly for 8 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 patients treated with *E. coli* asparaginase.[67]

Due to the shorter half-life of *Erwinia* asparaginase compared with *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 al[25] reported that only 26% of samples from ALL patients had complete depletion of asparagine (ie, $\leq$0.1 μmol/L) 3 days after administration of *Erwinia* asparaginase (10,000 IU/m^2 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/m^2 IV or IM) was given daily during induction therapy and twice a week for 2 weeks during reinduction 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 reinduction. Similarly, in a DFCI ALL Consortium trial, in which patients switched to *Erwinia* asparaginase (25,000 IU/m^2 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 postdosing.[68] These data highlight that even with relatively high *Erwinia* asparaginase doses (25,000-30,000 IU/m^2), 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 al[26] suggests increased dosing frequency enhances *Erwinia* asparaginase activity. In this study, patients with ALL and non-Hodgkin lymphoma were administered *Erwinia* asparaginase (20 000 IU/m^2 3-times weekly) and trough asparagine levels and asparaginase activity were assessed 2 and 3 days after 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/m^2 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 3-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 al,[26] 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 9 doses of native *E. coli* asparaginase during induction (6000 IU/m^2 3-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 nonprotein 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 United States.

Which Patients Should Receive Erwinia *Asparaginase?*

Patients developing allergic reactions to a particular 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 and 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, whereas others prescribe PEG-asparaginase as replacement for native *E. coli* asparaginase and *Erwinia* asparaginase as third-line drugs.

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-naive or relapsed), and various *Erwinia* asparaginase dosing regimens are in use (Table 2; 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.

Table 2. Current Regional Use of Asparaginases (Source: EUSA Pharma)

		North America, UK, Australia, New Zealand	Europe (BFM Zone)	Rest of World
Children	Naive patients	First-line: PEG-asparaginase Second-line: Erwinia asparaginase	First-line: *E. coli*-asparaginase Second-line: Erwinia asparaginase or PEG-asparaginase	First-line: *E. coli*-asparaginase Second-line: Erwinia asparaginase or PEG-asparaginase
	Relapsed patients	First-line: PEG-asparaginase Second-line: Erwinia asparaginase	First-line: PEG-asparaginase Second-line: Erwinia asparaginase	First-line: *E. coli*-asparaginase Second-line: Erwinia asparaginase or PEG-asparaginase
Adults	Naive patients	First-line: *E. coli*-asparaginase or PEG-asparaginase Second-line: Erwinia asparaginase or PEG-asparaginase	First-line: *E. coli*-asparaginase or PEG-asparaginase Second-line: Erwinia asparaginase	First-line: *E. coli*-asparaginase Second-line: Erwinia asparaginase or PEG-asparaginase

BFM indicates Berlin-Frankfurt-Munster; PEG, polyethylene glycol; *E. coli*, *Escherichia coli*.

Table 3. ALL Protocols Currently Used for Erwinia asparaginase (Second-Line or Third-Line Treatment)

Protocol	Treatment
NOPHO	Erwinia asparaginase 20 000 IU/m^2 2-3x/wk (x6)
Second-line	
AIEOP	Erwinia asparaginase 20 000 IU/m^2 every other day
Third-line	
GMALL 07/2003 and 01/2003	Erwinia asparaginase 20 000 IU/m^2 3x/wk (x5); IV(10 000 IU/m^2
Second-line	in patients aged >55 y)
COG	Erwinia asparaginase 25 000 IU/m^2 3x/wk (x6); IM
Second-line	
COALL-07-03	Erwinia asparaginase 45 000 IU/m^2 (x2)
Czech Republic	Induction and late intensification:
Third-line	Erwinia asparaginase 10 000 IU/m^2 2x/wk
	HR blocks:
	Erwinia asparaginase 10 000 IU/m^2 2x/wk
	First relapse:
	Erwinia asparaginase 10 000 IU/m^2 2x/wk
DCOG ALL-10	Induction:
Third-line	Erwinia asparaginase 10 000 IU/m^2 2-3x/wk
	Intensification (standard or medium risk):
	Erwinia asparaginase 10 000 IU/m^2 2-3x/wk
	HR blocks:
	Erwinia asparaginase 10 000 IU/m^2, 2-3x/wk
BFM-2000	Protocol 1:
Second-line	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-1:
	Erwinia asparaginase 10 000 IU/m^2 every 2 days IM/IV
EORTC-58951	Erwinia asparaginase 20 000 IU/m^2 2-3x/wk; IM
Second-line	
FRALLE- 2000	Induction, delayed intensification(s): Erwinia asparaginase 12 000 IU/m^2
Second-Line	3x/wk; IM, ie double dose compared with *E .coli* asparaginase
St Jude	Induction:
Second-line	Erwinia asparaginase 20 000 IU/m^2 3x/wk (x6) IM;
	Post-remission: 30 000 or 42 000 IU/m^2 2x/wk IM for 30 wks (standard/high-risk patients), 2x/wk for 4 wks during first and second reinduction (low-risk patients)
DFCI ALL Consortium	Postinduction consolidation:
Second-line	Erwinia asparaginase 25 000 IU/m^2 2x/wk IM for 30 wks

NOPHO indicates Nordic Society of Pediatric Hematology and Oncology; AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; GMALL, German Multileft 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; DFCI, Dana-Faber Cancer Institute; IV, intravenous; IM, intramuscular; HR, high risk.

- *Erwinia* asparaginase should be prescribed when 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 are required to ensure optimal asparagine depletion. Current evidence suggests that *Erwinia* asparaginase should be administered at dosages of at least 20,000 IU/m^2 3-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/m^2) 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 used 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/m^2 multiple times per week (eg, 3 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 al,[2] 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, suggests that prolonged and intensified therapy with asparaginase improves outcome of children with ALL.

When *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

- Use prolonged intensification with asparaginase to optimize survival benefits.

Route of Administration of Erwinia *Asparaginase*

Intravenous (IV) administration results in higher peak plasma concentrations, whereas IM administration results in a concurrent slower increase of asparaginase activity due to the depot effect. Accordingly, administration of 10,000 U/m^2 *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, whereas 45% of samples were below 100 U/L when *Erwinia* asparaginase was administered intravenously.[70]

However, Rizzari et al[44] 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/m^2 every 3 days (8 doses) administered in the induction phase.[44] Similarly, Albertsen et al[43] found comparable complete asparagine depletion in patients given a more intense regimen of IV or IM administration at 30,000 IU/m^2 daily for 10 days in the induction phase.[43] In this study, however, *Erwinia* asparaginase administered by the IM route produced trough asparaginase plasma levels significantly lower (by approximately 28%) than IV administration. During the subsequent reinduction phase (30,000 IU/m^2 twice-weekly for 2 weeks), no differences were observed between the 2 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 2 routes of administration of *Erwinia* asparaginase 30,000 IU/m^2 twice-weekly for 2 weeks as a reinduction 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, whereas North American groups more often administer this agent by intramuscularly.

Monitoring of Asparaginase Trough Levels and/or Depletion of Asparagine

Initially, the US Food and Drug Administration required that asparagine levels be used as the primary outcome measure in clinical trials. Asparaginase therapy aims to achieve serum asparagine depletion, but no critical minimal 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

- Because of 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 antiasparaginase antibody levels to discover whether alterations in dosing regimen should be used to overcome the risk of silent hypersensitivity, monitoring of asparaginase levels should be sufficient to identify silent hypersensitivity because 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 3 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 on the optimal formulation and dose for the treatment of pediatric and adult ALL patients. This article 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 antiasparaginase antibodies should be switched to another asparaginase preparation to ensure maximal 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/m^2 3-times weekly, by either the IV or IM route. Further clinical and pharmacokinetic studies of *Erwinia* asparaginase will help optimize the use of this agent.

CONFLICT OF INTEREST DISCLOSURES

This work was supported in part by grant CA-21,765 from the US National Institutes of Health, by the American Lebanese Syrian Associated Charities, and by EUSA Pharma. Pieters is involved in scientific collaborations with different companies producing and developing asparaginases. Hunger is the Ergen Family Chair in Pediatric Cancer. Boos served personally as consultant and participated in advisory boards for different asparaginase-selling companies, including EUSA Pharma and former license holders. In addition, Boos is also involved in scientific collaborations with different companies producing and developing asparaginase. Rizzari is involved in scientific researches supported by different companies producing and/or marketing asparaginase products. Silverman served on an advisory board for EUSA Pharma and as a consultant for Enzon Pharmaceuticals. Baruchel received an honorarium from OPI for a lecture.

Goekbuget is involved in scientific collaborations with different companies producing and developing asparaginases. Schrappe is involved in scientific collaborations with different companies producing and developing asparaginases. Pui received an honorarium from EUSA Pharma for a lecture.

REFERENCES

1. Silverman LB, Declerck L, Gelber RD, et al. Results of Dana-Farber Cancer Institute Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia 1981-1995. *Leukemia.* 2000;14:2247-2256.
2. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood.* 2001; 97:1211-1218.
3. Pui CH, Sandlund JT, Pei D, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St Jude Children's Research Hospital. *Blood.* 2004;104:2690-2696.
4. Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N Engl J Med.* 2009;360:2730-2741.
5. Möricke A, Reiter A, Zimmermann M, et al. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. *Blood.* 2008;111:4477-4489.
6. Pieters R, Carroll WL. Biology and treatment of acute lymphoblastic leukemia. *Pediatr Clin North Am.* 2008;55:1-20.
7. Kantarjian H, Thomas D, O'Brien S, et al. Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone Hyper-CVAD., a dose-intensive regimen, in adult acute lymphocytic leukemia. *Cancer.* 2004;101:2788-2801.
8. Goekbuget N, Baumann A, Beck J, et al. PEG-asparaginase in adult acute lymphoblastic leukemia ALL: efficacy and feasibility analysis with increasing dose levels. *Blood.* 2008;112. Abstract 302.
9. de Bont JM, Holt B, Dekker AW, van der Does-van den Berg, Sonneveld P, Pieters R. Significant difference in outcome for adolescents with acute lymphoblastic leukemia treated on pediatric vs adult protocols in the Netherlands. *Leukemia.* 2004;18:2032-2035.
10. Schiffer CA. Differences in outcome in adolescents with acute lymphoblastic leukemia: a consequence of better regimens? Better doctors? Both? *J Clin Oncol.* 2003;21:760-761.
11. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. *Lancet.* 2008;371:1030-1043.
12. Boissel N, Auclerc MF, Lheritier V, et al. Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials. *J Clin Oncol.* 2003;21:774-780.
13. Huguet F, Girard N, Guerche CS, Hennequin C, Mornex F, Azria D. Chemoradiotherapy in the management of locally advanced pancreatic carcinoma: a qualitative systematic review. *J Clin Oncol.* 2009;27:2269-2277.
14. Huguet F, Raffoux E, Thomas X, et al. Towards a pediatric approach in adults with acute lymphoblastic leukemia ALL.: the GRAALL-2003 Study. *Blood.* 2006;108. Abstract 147.
15. Storring JM, Brandwein J, Gupta V, et al. Treatment of adult acute lymphoblastic leukemia ALL. with a modified DFCI pediatric regimen-The Princess Margaret Experience. *Blood.* 2006;108. Abstract 1875.
16. Ramanujachar R, Richards S, Hann I, et al. Adolescents with acute lymphoblastic leukaemia: Outcome on UK national paediatric ALL97. and adult UKALLXII/E2993. trials. *Pediatr Blood Cancer.* 2006;48:254-261.
17. Linker C, Damon L, Ries C, Navarro W. Intensified and shortened cyclical chemotherapy for adult acute lymphoblastic leukemia. *J Clin Oncol.* 2002;20:2464-2471.
18. Avramis VI, Tiwari PN. Asparaginase native ASNase or pegylated ASNase. in the treatment of acute lymphoblastic leukemia. *Int J Nanomedicine.* 2006;1:241-254.
19. Oncospar. Summary of Product Characteristics. *Sigma-Tau Pharmaceuticals, Inc*. 2006.
20. Pieters R, Appel I, Kuehnel HJ, et al. Pharmacokinetics, pharmacodynamics, efficacy, and safety of a new recombinant asparaginase preparation in children with previously untreated acute lymphoblastic leukemia: a randomized phase 2 clinical trial. *Blood.* 2008;112:4832-4838.
21. Bertrand Y, Thomas X, Baruchel A, et al. GRASPALL 2005.01 Clinical Study L-asparaginase loaded into red blood cells is effective at depleting serum asparagine in children and adults with relapsed acute lymphoblastic leukaemia ALL. *Blood.* 2008;112. Abstract 306.
22. Allas S, Sahakian P, Fichtner I, Abribat T. Pharmacokinetics and pharmacodynamics in mice of a pegylated recombinant *Erwinia Chrysanthemi*-derived l-asparaginase. *Blood.* 2009;114. Abstract 2033.
23. Miller HK, Salser JS, Balis ME. Amino acid levels following L-asparagine amidohydrolase EC. 3.5.1.1. therapy. *Cancer Res.* 1969;29:183-187.
24. Avramis VI, Martin-Aragon S, Avramis EV, Asselin BL. Pharmacoanalytical assays of Erwinia asparaginase Erwinase. and pharmacokinetic results in high-risk acute lymphoblastic leukemia HR ALL. patients: simulations of Erwinase population PK-PD models. *Anticancer Res.* 2007;27:2561-2572.
25. Boos J, Werber G, Ahlke E, et al. Monitoring of asparaginase activity and asparagine levels in children on different asparaginase preparations. *Eur J Cancer.* 1996;32A:1544-1550.
26. Vieira Pinheiro JP, Ahlke E, Nowak-Gottl U, et al. Pharmacokinetic dose adjustment of Erwinia asparaginase in protocol II of the paediatric ALL/NHL-BFM treatment protocols. *Br J Haematol.* 1999;104:313-320.
27. Appel IM, Kazemier KM, Boos J, et al. Pharmacokinetic, pharmacodynamic and intracellular effects of PEG-asparaginase in newly diagnosed childhood acute lymphoblastic leukemia: results from a single agent window study. *Leukemia.* 2008;22:1665-1679.
28. Avramis VI, Sencer S, Periclou AP, et al. A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. *Blood.* 2002;99:1986-1994.
29. Ollenschlager G, Roth E, Linkesch W, Jansen S, Simmel A, Modder B. Asparaginase-induced derangements of glutamine metabolism: the pathogenetic basis for some drug-related side-effects. *Eur J Clin Invest.* 1988;18:512-516.
30. Broome JD. L-Asparaginase: discovery and development as a tumor-inhibitory agent. *Cancer Treat Rep.* 1981;65(suppl 4): 111-114.

31. Dinndorf PA, Gootenberg J, Cohen MH, Keegan P, Pazdur R. FDA drug approval summary: pegaspargase Oncaspar. for the first-line treatment of children with acute lymphoblastic leukemia ALL. *Oncologist.* 2007;12:991-998.
32. Ahlke E, Nowak-Gottl U, Schulze-Westhoff P, et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in children with acute lymphoblastic leukaemia. *Br J Haematol.* 1997; 96:675-681.
33. Vieira Pinheiro JP, Wenner K, Escherich G, et al. Serum asparaginase activities and asparagine concentrations in the cerebrospinal fluid after a single infusion of 2,500 IU/m2. PEG asparaginase in children with ALL treated according to protocol COALL-06-97. *Pediatr Blood Cancer.* 2006;46:18-25.
34. Liang DC, Hung IJ, Yang CP, et al. Unexpected mortality from the use of E. coli L-asparaginase during remission induction therapy for childhood acute lymphoblastic leukemia: a report from the Taiwan Pediatric Oncology Group. *Leukemia.* 1999;13:155-160.
35. Ertel IJ, Nesbit ME, Hammond D, Weiner J, Sather H. Effective dose of L-asparaginase for induction of remission in previously treated children with acute lymphocytic leukemia: a report from Children's Cancer Study Group. *Cancer Res.* 1979;39:3893-3896.
36. Clavell LA, Gelber RD, Cohen HJ, et al. Four-agent induction and intensive asparaginase therapy for treatment of childhood acute lymphoblastic leukemia. *N Engl J Med.* 1986; 315:657-663.
37. Sallan SE, Hitchcock-Bryan S, Gelber R, Cassady JR, Frei E, 3rd, Nathan DG. Influence of intensive asparaginase in the treatment of childhood non-T-cell acute lymphoblastic leukemia. *Cancer Res.* 1983;43:5601-5607.
38. Amylon MD, Shuster J, Pullen J, et al. Intensive high-dose asparaginase consolidation improves survival for pediatric patients with T cell acute lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric Oncology Group study. *Leukemia.* 1999;13:335-342.
39. Duval M, Suciu S, Ferster A, et al. Comparison of Escherichia coli-asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer-Children's Leukemia Group phase 3 trial. *Blood.* 2002;99:2734-2739.
40. Moghrabi A, Levy DE, Asselin B, et al. Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia. *Blood.* 2007;109:896-904.
41. Pession A, Valsecchi MG, Masera G, et al. Long-term results of a randomized trial on extended use of high dose L-asparaginase for standard risk childhood acute lymphoblastic leukemia. *J Clin Oncol.* 2005;23:7161-7167.
42. Rizzari C, Valsecchi MG, Arico M, et al. Effect of protracted high-dose L-asparaginase given as a second exposure in a Berlin-Frankfurt-Munster-based treatment: results of the randomized 9102 intermediate-risk childhood acute lymphoblastic leukemia study—a report from the Associazione Italiana Ematologia Oncologia Pediatrica. *J Clin Oncol.* 2001; 19:1297-1303.
43. Albertsen BK, Schroder H, Jakobsen P, Muller HJ, Carlsen NT, Schmiegelow K. Monitoring of Erwinia asparaginase therapy in childhood ALL in the Nordic countries. *Br J Clin Pharmacol.* 2001;52:433-437.
44. Rizzari C, Zucchetti M, Conter V, et al. L-asparagine depletion and L-asparaginase activity in children with acute lymphoblastic leukemia receiving i.m. or i.v. Erwinia C. or E. coli L-asparaginase as first exposure. *Ann Oncol.* 2000;11:189-193.
45. Panosyan EH, Seibel NL, Martin-Aragon S, et al. Asparaginase antibody and asparaginase activity in children with higher-risk acute lymphoblastic leukemia: Children's Cancer Group Study CCG-1961. *J Pediatr Hematol Oncol.* 2004; 26:217-226.
46. Woo MH, Hak LJ, Storm MC, et al. Hypersensitivity or development of antibodies to asparaginase does not impact treatment outcome of childhood acute lymphoblastic leukemia. *J Clin Oncol.* 2000;18:1525-1532.
47. Larson RA, Fretzin MH, Dodge RK, Schiffer CA. Hypersensitivity reactions to L-asparaginase do not impact on the remission duration of adults with acute lymphoblastic leukemia. *Leukemia.* 1998;12:660-665.
48. Hawkins DS, Park JR, Thomson BG, et al. Asparaginase pharmacokinetics after intensive polyethylene glycol-conjugated L-asparaginase therapy for children with relapsed acute lymphoblastic leukemia. *Clin Cancer Res.* 2004;10:5335-5341.
49. Wang B, Relling MV, Storm MC, et al. Evaluation of immunologic cross-reaction of antiasparaginase antibodies in acute lymphoblastic leukemia ALL. and lymphoma patients. *Leukemia.* 2003;17:1583-1588.
50. Albertsen BK, Schroder H, Jakobsen P, et al. Antibody formation during intravenous and intramuscular therapy with Erwinia asparaginase. *Med Pediatr Oncol.* 2002;38:310-316.
51. Albertsen BK, Schmiegelow K, Schroder H, et al. Anti-Erwinia asparaginase antibodies during treatment of childhood acute lymphoblastic leukemia and their relationship to outcome: a case-control study. *Cancer Chemother Pharmacol.* 2002;50:117-120.
52. Nesbit M, Chard R, Evans A, Karon M, Hammond GD. Evaluation of intramuscular versus intravenous administration of L-asparaginase in childhood leukemia. *Am J Pediatr Hematol Oncol.* 1979;1:9-13.
53. Albertsen BK, Schroder H, Ingerslev J, et al. Comparison of intramuscular therapy with Erwinia asparaginase and asparaginase Medac: pharmacokinetics, pharmacodynamics, formation of antibodies and influence on the coagulation system. *Br J Haematol.* 2001;115:983-990.
54. Barry E, DeAngelo DJ, Neuberg D, et al. Favorable outcome for adolescents with acute lymphoblastic leukemia treated on Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium Protocols. *J Clin Oncol.* 2007;25: 813-819.
55. Asselin BL. The three asparaginases. Comparative pharmacology and optimal use in childhood leukemia. *Adv Exp Med Biol.* 1999;457:621-629.
56. Wenner KA, Vieira Pinheiro JP, Escherich G, et al. Asparagine concentration in plasma after 2,500 IU/m2. PEG-asparaginase i.v. in children with acute lymphoblastic leukemia. *Klin Padiatr.* 2005;217:321-326.
57. Hak LJ, Relling MV, Cheng C, et al. Asparaginase pharmacodynamics differ by formulation among children with newly diagnosed acute lymphoblastic leukemia. *Leukemia.* 2004;18: 1072-1077.
58. Zalewska-Szewczyk B, Andrzejewski W, Mlynarski W, Jedrychowska-Danska K, Witas H, Bodalski J. The anti-asparagines antibodies correlate with L-asparagines activity and may affect clinical outcome of childhood acute lymphoblastic leukemia. *Leuk Lymphoma.* 2007;48:931-936.

59. Fu CH, Sakamoto KM. PEG-asparaginase. *Expert Opin Pharmacother.* 2007;8:1977-1984.
60. Zalewska-Szewczyk B, Gach A, Wyka K, Bodalski J, Mlynarski W. The cross-reactivity of anti-asparaginase antibodies against different L-asparaginase preparations. *Clin Exp Med.* 2009;9:113-116.
61. Hempel G, Muller HJ, Lanvers-Kaminsky C, Wurthwein G, Hoppe A, Boos J. A population pharmacokinetic model for pegylated-asparaginase in children. *Br J Haematol.* 2010;148: 119-125.
62. Gadner H, Masera G, Schrappe M, et al. The Eighth International Childhood Acute Lymphoblastic Leukemia Workshop 'Ponte di legno meeting'. report: Vienna, Austria, April 27-28, 2005. *Leukemia.* 2006;20:9-17.
63. Alvarez OA, Zimmerman G. Pegaspargase-induced pancreatitis. *Med Pediatr Oncol.* 2000;34:200-205.
64. Kearney SL, Dahlberg SE, Levy DE, Voss SD, Sallan SE, Silverman LB. Clinical course and outcome in children with acute lymphoblastic leukemia and asparaginase-associated pancreatitis. *Pediatr Blood Cancer.* 2009;53:162-167.
65. Parsons SK, Skapek SX, Neufeld EJ, et al. Asparaginase-associated lipid abnormalities in children with acute lymphoblastic leukemia. *Blood.* 1997;89:1886-1895.
66. Eden OB, Shaw MP, Lilleyman JS, Richards S. Non-randomised study comparing toxicity of Escherichia coli and Erwinia asparaginase in children with leukaemia. *Med Pediatr Oncol.* 1990;18:497-502.
67. Kwok CS, Kham SK, Ariffin H, Lin HP, Quah TC, Yeoh AE. Minimal residual disease MRD. measurement as a tool to compare the efficacy of chemotherapeutic drug regimens using *Escherichia Coli*-asparaginase or *Erwinia*-asparaginase in childhood acute lymphoblastic leukemia ALL. *Pediatr Blood Cancer.* 2006;47:299-304.
68. Vrooman LM, Supko JG, Neuberg DS, et al. Erwinia asparaginase after allergy to E. coli asparaginase in children with acute lymphoblastic leukemia. *Pediatr Blood Cancer.* 2010;54: 199-205.
69. Ogawa C, Ohara A, Manabe A, et al. Treatment outcome of discontinued l-asparaginase in children with standard-risk acute lymphoblastic leukemia: Tokyo Children's Cancer Study Group TCCSG. Study L99-15. *Blood* 2005;106. Abstract 878.
70. Schrey D, Borghorst S, Lanvers-Kaminsky C, et al. Therapeutic drug monitoring of asparaginase in the ALL-BFM 2000 Protocol between 2000 and 2007. *Pediatr Blood Cancer.* 2010;54:952-958.
71. Armstrong JK, Hempel G, Koling S, et al. Antibody against polyethylene glycol. adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients. *Cancer.* 2007; 110:103-111.
72. Billett AL, Carls A, Gelber RD, Sallan SE. Allergic reactions to Erwinia asparaginase in children with acute lymphoblastic leukemia who had previous allergic reactions to Escherichia coli asparaginase. *Cancer.* 1992;70:201-206.
73. Rizzari C, Citterio M, Zucchetti M, et al. A pharmacological study on pegylated asparaginase used in front-line treatment of children with acute lymphoblastic leukemia. *Haematologica.* 2006;91:24-31.