



Optimizing asparaginase therapy for acute lymphoblastic leukemia

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Asparaginases are important agents used in the treatment of children with acute lymphoblastic leukemia (ALL). Three types of asparaginase are currently available: two are derived from *Escherichia coli* [native asparaginase and pegylated asparaginase (PEG-asparaginase)] and one from *Erwinia chrysanthemi* (crisantaspase). All three products share the same mechanism of action but have different pharmacokinetic properties, which do not make them easily interchangeable. Among the known toxicities and side-effects, allergic reactions and silent inactivation represent the most important limitations to the prolonged use of any asparaginase product, with associated reduced therapeutic effects and poorer outcomes. Routine real time monitoring can help to identify patients with silent inactivation and facilitate a switch to a different product to ensure continued depletion of asparagine, completion of the treatment schedule and maintenance of outcomes. However, the most appropriate second-line treatment is still a matter of debate. PEG-asparaginase has lower immunogenicity and a longer half-life than native *Escherichia coli* (*E. coli*) asparaginase, which makes it useful for both first-line and second-line use with a reduced number of doses. However, PEG-asparaginase displays cross-reactivity with native *E. coli* asparaginase that may harm its therapeutic effects. Crisantaspase does not display cross-reactivity to either of the *E. coli*-derived products, which has made crisantaspase the second-line treatment option in a number of recent protocols. As crisantaspase has a much shorter biological half-life than the *E. coli*-derived products, the appropriate dosage and administration schedule are of paramount importance in delivering treatment with this product. In the ongoing trial AIEOP-BFM ALL 2009 (Associazione Italiana Ematologia Oncologia Pediatrica - Berlin-Franklin-Munster), in which PEG-asparaginase is used first-line, one dose of PEG-asparaginase is substituted by seven doses of crisantaspase given intravenously at 20 000 IU/m² on alternate days when clinical allergy or silent inactivation is present. Based on the indications of different protocols, lack of cross-reactivity to the *E. coli*-derived products and taking into consideration regulatory factors and availability, crisantaspase may be considered a viable second-line therapy.

Keywords

acute lymphoblastic leukemia, asparaginase, childhood, hypersensitivity, silent inactivation

INTRODUCTION

Asparaginase is an established treatment for acute lymphoblastic leukemia (ALL); it exploits a key weakness of ALL cells, their inability to synthesize asparagine. In this regard, asparaginase has to be considered as a 'targeted therapy'.

This article outlines current strategies to fully exploit the targeted treatment applied by using asparaginase and to optimize treatment over an extended period of time. The use of asparaginase is associated with the onset of hypersensitivity reactions that have been reported to impact on treatment outcomes. Therefore, strategies to identify and manage hypersensitivity will be discussed, together with approaches to ensure continued treatment with asparaginase to maintain benefit.

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KEY POINTS

- Asparaginases are used in the treatment of ALL; three types are currently available: two are derived from *E. coli* [native asparaginase and pegylated asparaginase (PEG-asparaginase)] and one from *Erwinia chrysanthemi* (crisantaspase).
- To gain the full benefit from treatment with any asparaginase, it is essential to complete the full dose and schedule.
- Patients may develop resistance to asparaginase, which can take the form of an allergic reaction or silent inactivation, both of which may potentially reduce the therapeutic benefit of asparaginase.
- Asparaginases derived from *E. coli* show cross-reactivity to each other; crisantaspase does not exhibit cross-reactivity to *E. coli* asparaginase and if used second-line in patients with allergy to *E. coli* asparaginase allows the achievement of adequate asparagine depletion.
- Routine real time monitoring can help to identify patients with silent inactivation and facilitate a switch to a different asparaginase product to ensure continued depletion of asparagine and completion of the treatment schedule.

THE ASPARAGINASES

It is well established that asparaginase has a key role in the treatment of ALL. Since the 1960s, it has been recognized that a single dose of asparaginase can achieve remission for 60 days in 65% of children with ALL. The rationale behind the use of asparaginase in ALL is that lymphoblastic leukemia cells are unable to synthesize adequate amounts of asparagine and depend on extracellular sources [1]. Asparaginase is able to deplete serum asparagine and starves lymphoblastic leukemia cells of asparagine. The resultant asparagine depletion leads to reduced DNA, RNA and protein synthesis and ultimately to the death of the leukemic cell [1].

Three types of asparaginase are currently available, derived from two different bacterial sources: *Escherichia coli* and *Erwinia chrysanthemi* [2]. Native asparaginase and pegylated asparaginase (PEG-asparaginase) are both derived from *E. coli* and crisantaspase is derived from *Erwinia chrysanthemi* [2]. Crisantaspase is known as asparaginase *Erwinia chrysanthemi* in the United States; however, we will use crisantaspase in this article. All three types share the same mode of action; however, differences in the pharmacokinetic properties of the asparaginases mean that the three agents are not interchangeable at the same dose and frequency [2,3].

Asparaginase activity levels should be at least 100 IU/l in order to achieve depletion of plasma asparagine to less than 0.1 $\mu\text{mol/l}$ and an optimal therapeutic effect [2,4–6]. Normal levels of asparagine are approximately 65 $\mu\text{mol/l}$ [7]; suboptimal doses of asparaginase will reduce asparagine levels to 25–40 $\mu\text{mol/l}$; however, this is defined as inadequate depletion of asparagine and will not result in a clinical effect [8].

OPTIMAL DOSING SCHEDULES ACHIEVE MAXIMAL CLINICAL BENEFIT

The maximum clinical benefit is seen with asparaginase when patients receive intensive asparaginase treatment through optimal dosing and treatment schedules, which result in sustained depletion of asparagine in the leukemic cells. An early Children's Cancer Group study carried out in the 1970s revealed that patients receiving a dose of 300 IU/m² of native *E. coli* asparaginase over 4 weeks had a remission rate of 9.5%, compared with a rate of 62.5% in patients receiving 12 000 IU/m² [9]. More recent studies using asparaginase have revealed a significant benefit in terms of treatment outcomes [event-free survival (EFS) [10,11], disease-free survival (DFS) [12] and continuous complete remission rate (CCR) [13]] in patients receiving intensive asparaginase treatment schedules (Fig. 1).

In a study carried out by the Dana-Farber Clinical Institute, standard and high-risk children were treated with an extended 30 weeks of high-dose asparaginase during intensification ($n=352$). At 5-year follow-up, EFS in children who received less than 25 weeks of the planned asparaginase therapy was significantly poorer than in those who received 26 weeks or more of therapy (73 versus 90%, $P<0.01$) [10]. A significant improvement in EFS with continued therapy was also seen in a retrospective analysis of children by the Tokyo Children's Cancer Study Group in children with newly diagnosed standard risk ALL [11]. The study demonstrated that children who received more than 50% of the scheduled dose had a significantly improved 5-year EFS versus those who received less than 50% of the scheduled dose (92.9 versus 74.1%, respectively, $P<0.025$).

Significant improvement in DFS with continued asparaginase was also demonstrated in a study carried out in Italy, The Netherlands and Hungary using the framework of the International Berlin-Frankfurt-Muenster (BFM) Study Group. The study randomized standard risk children aged less than 15 years ($n=355$) treated with a modified BFM backbone to receive or not to receive 20 additional weeks of asparaginase during continuation therapy.

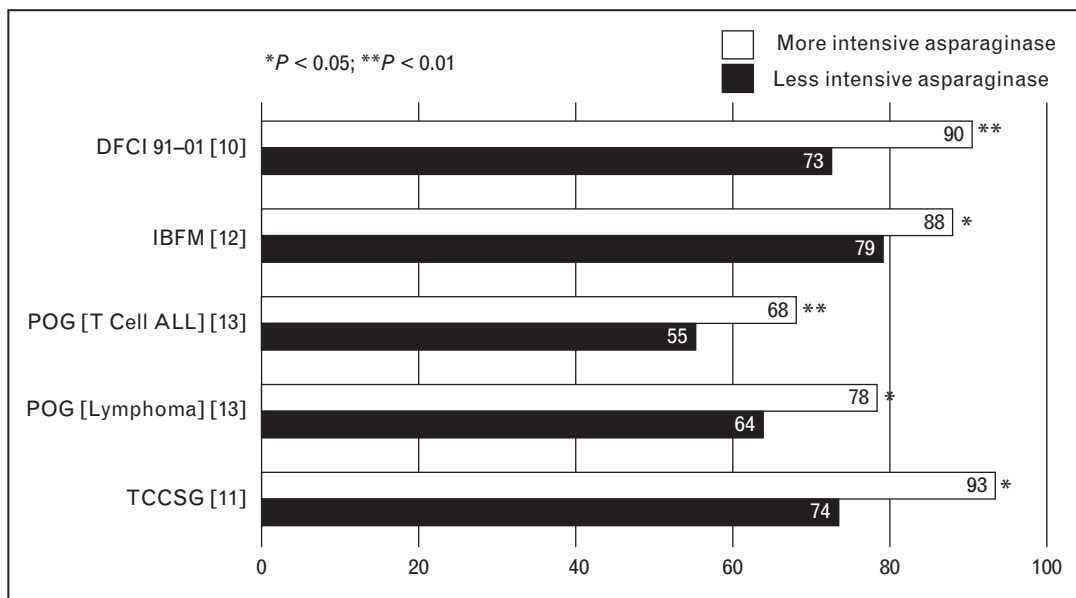


FIGURE 1. Effects of more intensive versus less intensive asparaginase treatment on outcomes [10–13]. DFCI: less than or greater than 25 weeks of treatment; IBFM, POG: additional 20 weeks of treatment; TCCSG: less than or greater than 50% of scheduled treatment. The numbers on each bar refer to the clinical outcomes measured in each study. EFS for DFCI 91-01 and TCCSG, DFS for IBFM and CCR for POG. DFCI, Dana-Farber Clinical Institute; IBFM, International Berlin-Frankfurt-Munster Group; POG, Pediatric Oncology Group; TCCSG, Tokyo Children's Cancer Study Group. Reproduced with permission from John Wiley & Sons, Inc. [2].

At 9-year follow-up, DFS in children who received an extra 20 weeks of treatment with asparaginase was significantly improved (87.5 versus 78.7%, $P=0.03$) [12].

Work by the Pediatric Oncology Group in children with T-cell ALL ($n=357$) or advanced stage lymphoma ($n=195$) demonstrated a significant improvement in CCR with continued asparaginase [13]. Patients were randomized to no asparaginase or an additional 20 weeks of asparaginase consolidation. At 4-year follow-up, CCR in children with ALL who received an extra 20 weeks of treatment with asparaginase was significantly improved compared with those who did not receive the additional 20 weeks of treatment (68 versus 55%, $P<0.001$). A similar picture was seen in lymphoma patients (78 versus 64%, $P<0.001$).

Two studies carried out in the 1990s comparing native *E. coli* asparaginase and crisantaspase using identical dosing schedules revealed inferior overall survival (OS) and EFS with crisantaspase and demonstrate the importance of using the appropriate dose and schedule of asparaginase [14,15]. The pharmacokinetics of the asparaginases differ, which has a clear impact on dosing requirements. The DFCI ALL Consortium Protocol 95-01 randomized 491 children aged less than 18 years to crisantaspase or native *E. coli* asparaginase at a dose of 25 000 IU/m² intramuscularly (i.m.) once during induction (day 4)

and weekly for 20 weeks during intensification [14]. Crisantaspase was associated with an inferior 5-year EFS compared with native *E. coli* asparaginase (78 ± 4 versus $89 \pm 3\%$, $P<0.01$). The European Organisation for Research and Treatment of Cancer–Children's Leukemia Group (EORTC-CLG) 58881 trial randomized 700 children with ALL or lymphoblastic lymphoma to either native *E. coli* asparaginase or crisantaspase at a dose of 10 000 IU/m² intravenously (i.v.) twice weekly. A higher proportion of patients in the crisantaspase group failed to achieve complete remission compared with the native *E. coli* asparaginase group (4.9 versus 2.0%, $P=0.038$). Furthermore, both EFS and OS were lower at 6 years in the crisantaspase group compared with the native *E. coli* asparaginase group (EFS: 59.8 versus 73.4%, $P=0.0004$ and OS: 75.1 versus 83.9%, $P=0.002$) [15]. It is well established that sustained depletion of asparagine is key to effective treatment with the asparaginases and these studies demonstrate the importance of giving the appropriate dose to ensure adequate asparaginase activity and sustained depletion of asparagine.

RESISTANCE TO ASPARAGINASES

In common with all large proteins, asparaginase has the potential to elicit an immune response resulting in the production of anti-asparaginase antibodies.

Antiasparaginase antibodies are the main cause of resistance to asparaginase and result in reduced activity of asparaginase and adverse clinical outcomes [16–19]. Patients with antiasparaginase antibodies have insufficient asparaginase for effective depletion of asparagine [16,17] and in some cases, asparaginase activity may be undetectable [18]. It is clear that there will be a loss of effect if levels of asparaginase are inadequate; such patients have significantly poorer outcomes in terms of median DFS and OS compared to patients with adequate asparaginase activity and complete asparagine depletion [19].

Asparaginase resistance can be symptomatic, with signs of clinical hypersensitivity, or asymptomatic without any signs [2]. Asymptomatic resistance is termed silent inactivation and is potentially more dangerous than clinical hypersensitivity, as there are no clinical signs of allergy to alert the physician to the presence of asparaginase resistance [2]. A study of 154 children with ALL treated with *E. coli* asparaginase measured antibodies in plasma on day 29 and revealed that 35% of children had antibodies of whom 56% had clinical hypersensitivity and 44% had silent inactivation [20].

The signs and symptoms of clinical hypersensitivity include urticaria, rash, laryngeal edema, bronchospasm, hypotension and occasionally anaphylactic shock [21]. Clinical hypersensitivity is a common reason for discontinuation of treatment [22]. Allergic clinical reactions are graded according to the Common Terminology Toxicity Criteria [23] and a reaction of Grade 2–4 is an indication to discontinue treatment [24,25].

Published rates of clinical hypersensitivity vary greatly between studies, due to differences in dosing, route of administration, duration of treatment, concomitant medication and the asparaginase preparation used in treatment. In addition, not all patients who have antibodies to asparaginase will develop an overt clinical hypersensitivity reaction. However, comparatively high hypersensitivity rates (up to 36% with 5000 IU/m² i.v. every 3 days for eight doses in induction followed by 10 000 IU/m² i.v. for four doses in re-induction) have been reported with native *E. coli* asparaginase [17]. Hypersensitivity rates to native *E. coli* asparaginase are higher than those reported with either PEG-asparaginase (4–8%) [16,26] or with crisantaspase [27–29]. Rates with crisantaspase depend on whether it is used as first-line treatment or second-line after hypersensitivity to *E. coli* asparaginase. Hypersensitivity rates have been reported at 0% for first-line treatment and up to 33% in patients receiving 25 000 IU/m² i.m. twice a week for repeated doses as second-line treatment [27–29].

Up to 60% of patients receiving asparaginase may develop antibodies [18]. It appears that crisantaspase and PEG-asparaginase may have a lower rate of antibody development than native *E. coli* asparaginase [16–18,26,28,29]. Antibody rates with native *E. coli* asparaginase (5000 IU/m² i.v. every 3 days for eight doses in induction followed by 10 000 IU/m² i.v. for four doses for re-induction) are about 40% [17], rates with PEG-asparaginase range from 11 to 18% [16,26] and rates with crisantaspase are 8 to 21% [27,28] depending on the schedule and the dose.

IMPACT OF ANTIBODIES ON ASPARAGINASE ACTIVITY

The presence of antibodies (whether the symptoms of allergy are present or not) has a significant impact on outcomes. A study of 47 children with newly diagnosed ALL followed up for a median of 30 months demonstrated that those children who developed antibodies (IgG or IgM) to asparaginase had significantly poorer outcomes in terms of EFS and OS compared to children without antibodies (EFS: 57 versus 79%, $P < 0.02$ and OS: 57 versus 89%, $P < 0.01$) [17]. A similar picture is seen in patients with silent inactivation. An interim analysis of 280 children with high-risk ALL after 30 months revealed that 29% of children had silent inactivation. Those children with silent inactivation had significantly higher leukemic event rates compared with children without antibodies (16 versus 5.2%, $P = 0.01$) [18].

A further study confirms the negative impact of antibodies on asparaginase activity [16]. The Children's Cancer Group 1962 protocol determined the correlation between antibody levels and the proportion of samples with asparaginase activity sufficient for asparagine depletion (>100 IU/l) at each stage of therapy (induction, delayed intensification 1 and 2) in patients receiving either native *E. coli* or PEG-asparaginase. The study revealed that most children (89–93% depending on the stage of therapy) with low antibody levels had asparaginase activity more than 100 IU/l. In contrast, only 50–64% of children with high antibody levels had asparaginase activity of more than 100 IU/l.

Antibodies to native *E. coli* asparaginase have an impact on the activity of subsequent lines of therapy [30]. A retrospective analysis of 329 patients (1221 samples) receiving asparaginase as part of ALL-BFM 2000 for front line ALL and ALL-REZ for relapsed ALL revealed that patients with high levels of antibodies (>200 AU/ml) to native *E. coli* asparaginase had levels of asparaginase below the threshold of 100 IU/l when switched to PEG-asparaginase as

second-line treatment. However, crisantaspase treatment in patients with high levels of antibodies to *E. coli* asparaginase resulted in asparaginase activity levels over the 100 IU/l threshold.

CROSS-REACTIVITY OF THE ASPARAGINASES

Crisantaspase is immunologically distinct from asparaginase derived from *E. coli* (native *E. coli* asparaginase or PEG-asparaginase) [31]. Studies demonstrate that there is considerable cross-reactivity between the native and pegylated forms of *E. coli* asparaginase, but not between *E. coli*-derived asparaginase and crisantaspase [32]. There is a substantial reduction in half-life in *E. coli* asparaginase (whether native or pegylated) after exposure to native *E. coli* asparaginase. Indeed, the half-life of native *E. coli* asparaginase in patients with a hypersensitivity reaction is undetectable and the half-life of PEG-asparaginase is reduced from around 6 days to less than 2 days [3,33]. This reduction in half-life results in subtherapeutic levels of asparaginase and inadequate depletion of asparagine.

MANAGEMENT OPTIONS IN PATIENTS WITH RESISTANCE TO ASPARAGINASE

There are three management options for patients with hypersensitivity to a native *E. coli* asparaginase product:

- (1) Cease asparaginase treatment.
- (2) Continue with the same product by using prophylaxis against the symptoms of hypersensitivity.
- (3) Switch to a different product as a substitute.

Ceasing treatment when additional products are available is not considered a viable option. The use of prophylaxis (steroids or antihistamines) is not appropriate, as prophylaxis will mask the symptoms of hypersensitivity; however, patients will still have reduced asparaginase activity.

Switching to another product can be a difficult choice, as data from large, well designed studies to demonstrate a preference for one preparation over another are still lacking and there is no definitive consensus opinion on the appropriate choice. PEG-asparaginase has a relatively lower immunogenicity compared with native *E. coli* preparations and is currently still used as second-line treatment in some protocols [16,26,30]; however, the evidence that antibodies against *E. coli* asparaginase can cross-react with PEG-asparaginase makes this switch sub-optimal in some cases [2,31]. An option deemed

more rational is the substitution of native *E. coli* asparaginase with crisantaspase, as there is no cross-reactivity between the two agents [2,32].

In patients with hypersensitivity to *E. coli* asparaginase, a switch to crisantaspase allows the achievement of adequate asparagine depletion and maintains clinical outcomes [29]. A study in 215 children with newly diagnosed ALL who received 30 weekly doses of native *E. coli* asparaginase i.m. was carried out as part of the DFCI ALL Consortium Protocol 00-01. Crisantaspase was substituted at a dose of 25 000 IU/m² administered i.m. twice weekly at 3-day and 4-day intervals in patients experiencing allergy to native *E. coli* asparaginase. Allergy to native *E. coli* asparaginase developed in 21% of patients ($n=45$), 42 of whom were switched to crisantaspase. Nadir serum asparaginase activity (NSAA) was measured in 38 of the switched patients, and 89% had adequate asparaginase activity (>100 IU/l). At median follow-up of 5.4 years, EFS was not significantly different between patients switched to crisantaspase because of hypersensitivity to native *E. coli* asparaginase and patients remaining on native *E. coli* asparaginase, [86 ± 5 versus $81 \pm 3\%$, respectively ($P=0.55$), see Fig. 2]. This study illustrates that switching patients with hypersensitivity to crisantaspase enables clinicians to ensure that their patients receive continued exposure to asparaginase and maintain an outcome similar to that obtained in patients without hypersensitivity.

Further evidence to support a switch to crisantaspase in patients with antibodies or hypersensitivity to *E. coli* asparaginase comes from an interim analysis of the Children's Cancer Group Study 1961 study of 1001 children with high-risk ALL [18]. Patients received native *E. coli* asparaginase

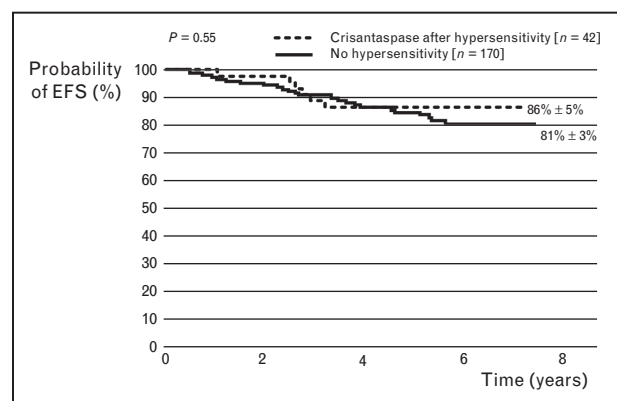


FIGURE 2. EFS in patients without hypersensitivity remaining on native *Escherichia coli* asparaginase and in patients receiving crisantaspase after experiencing a hypersensitivity reaction to native *E. coli* asparaginase. Reproduced with permission from John Wiley & Sons, Inc. [29].

(6000 IU/m² i.m.) for nine doses during induction. One-half of early responders continued to receive native *E. coli* asparaginase and the rest of the early responders, together with slow early responders, received PEG-asparaginase. Patients with hypersensitivity reactions were switched to crisantaspase, regardless of antibody status. An interim analysis of 280 patients followed for at least 30 months after induction revealed that patients with antibodies but no clinical hypersensitivity reaction and not switched to crisantaspase had a significantly higher incidence of events compared with those with antibodies and clinical reactions who were switched to crisantaspase (16 versus 2.6%, hazard ratio = 3.2, $P = 0.01$). The investigators also evaluated asparaginase activity in 88 of the patients who were positive for antibodies 7–14 days following asparaginase administration. No detectable asparaginase activity was found in 81 (94%) of the antibody-positive patients tested.

In general, the type of second-line asparaginase therapy is dictated by protocols, regulatory and availability factors and by the type of asparaginase used in front-line therapy; currently, there is general agreement that crisantaspase should be used after an allergic reaction or when silent inactivation to PEG-asparaginase product is detected. Whichever alternate asparaginase is used, pharmacological monitoring should be used.

THERAPEUTIC MONITORING

Asparaginase can be monitored directly in plasma; in addition, asparagine itself can also be measured in either plasma or cerebrospinal fluid as a surrogate measure of asparaginase activity. Asparaginase activity shows considerable variation depending on the individual patient and also the line of treatment used.

Therapeutic monitoring can also be helpful to identify patients with inadequate asparaginase activity. A recent paper reported the results of therapeutic monitoring of asparaginase activity in a single center enrolled in the ALL-BFM 2000 trial [34]. Over a 5-year period (1999–2005), 127 patients (1355 samples) were monitored for asparaginase activity. First-line therapy included native *E. coli* asparaginase (5000 IU/m² in induction and 10 000 IU/m² in re-induction), which resulted in asparaginase activity below the threshold of 100 IU/l in 7% of patients in induction and 29% (including those with allergic reaction) in re-induction. Second-line treatment for patients with clinical allergy or silent inactivation was with PEG-asparaginase (1000 IU/m²), which resulted in asparaginase activity below the threshold of 100 IU/l

in 17% of patients receiving PEG-asparaginase. Overall, only 10% of children went on to require treatment with third-line crisantaspase due to lack of asparaginase activity with PEG-asparaginase.

A retrospective study of 763 patients (2074 samples) enrolled in the ALL-BFM 2000 trial from 114 hospitals (2000–2007) revealed a wide range of asparaginase activity regardless of the asparaginase used [35]. In ALL-BFM 2000, first-line therapy with native *E. coli* asparaginase (5000 IU/m²) during induction phase resulted in asparaginase activity below the threshold of 100 IU/l in 11% of samples collected 3 days after drug administration. Second-line treatment with PEG-asparaginase (1000 IU/m²) was performed in children with clinically overt allergic reactions and resulted in asparaginase activity below the threshold of 100 IU/l in 30% of patients evaluated 7 days after drug administration. Third-line treatment with crisantaspase (10 000 IU/m²) was given after an allergic reaction to previous asparaginase preparations and resulted in asparaginase activity below the threshold of 100 IU/l in 33% of samples drawn 2 days after drug administration. There were fewer samples with activity below 100 IU/l in patients receiving crisantaspase i.m. compared with crisantaspase i.v. (15 versus 45%) and median levels were higher in the i.m. group (151.5 versus 115.0 IU/l) [35]. Figure 3 illustrates the interpatient variability with PEG-asparaginase (1000 IU/m²) given during re-intensification.

In a review published by Pieters *et al.* [2], monitoring of asparaginase levels is recommended for adaptation of asparaginase dosing in individual cases and to ensure that such patients receive alternative treatment to enable continued depletion of asparagine and a therapeutic effect. Monitoring asparaginase activity is the most reliable option for therapeutic monitoring, is relatively simple and inexpensive and commercial test kits are available. There is currently some debate around measuring plasma asparagine or antibody levels as both are more complex to measure than plasma asparaginase levels.

EVOLUTION OF ASPARAGINASE TREATMENT AND MONITORING IN THE AIEOP-BFM ALL 2000 AND 2009 TRIALS

The AIEOP-BFM ALL 2000 (Associazione Italiana Ematologia Oncologia Pediatrica - Berlin-Franklin-Munster) protocol was a BFM-based chemotherapy treatment wherein patients received native *E. coli* asparaginase given i.v. (BFM) or i.m. (AIEOP) as first-line therapy, PEG-asparaginase as second-line therapy and crisantaspase, if available, as third-line

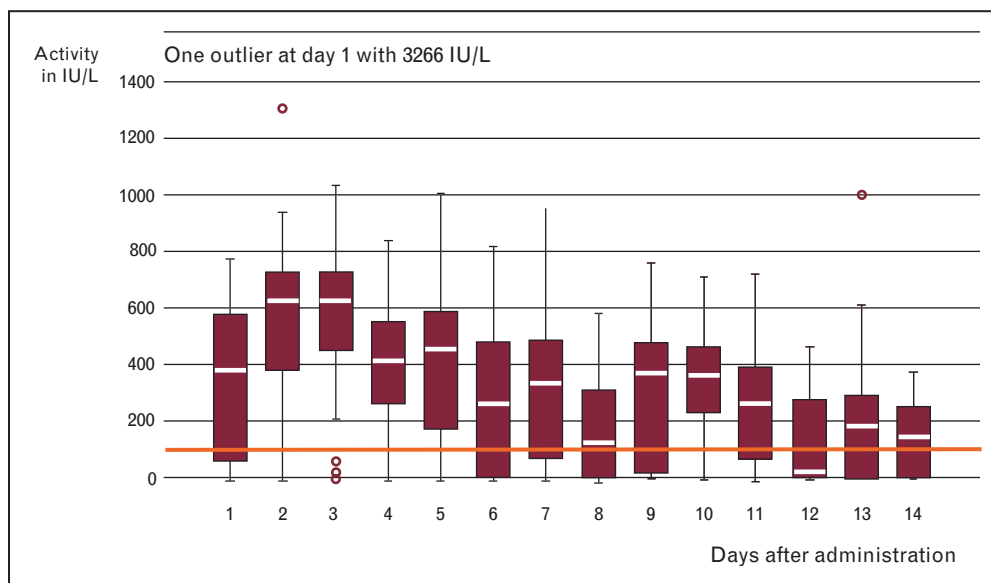


FIGURE 3. Time course of asparaginase activity in the population of patients receiving PEG-asparaginase (1000 IU/m²) as second-line treatment after an allergic reaction to native *Escherichia coli* asparaginase during re-induction treatment. Reproduced with permission from John Wiley & Sons, Inc. [35].

therapy [36]. Data on clinical allergy rates derived from patients treated in Germany showed that clinical allergy was seen in 38.1% (359/942) of standard and intermediate-risk patients exposed to at least two asparaginase-containing treatment elements (protocol I or protocol II/first protocol III) with native *E. coli* asparaginase. Patients with clinical allergy were switched to PEG-asparaginase. Of those patients with clinical allergy, an additional 6.4% (23/359) experienced a second allergic reaction to PEG-asparaginase. Rates of clinical allergy were higher in high-risk patients; 56.3% (112/199) for the first allergic reaction to native *E. coli* asparaginase and 42.9% (48/112) for the second reaction to PEG-asparaginase (unpublished data). Pharmacological monitoring was not routinely carried out in the countries participating in this study.

The ongoing AIEOP-BFM ALL 2009 trial uses i.v. PEG-asparaginase as first-line treatment and includes two randomized studies (in intermediate-risk and high-risk patients, respectively) which consider intensified use of PEG-asparaginase. Intensive treatment with asparaginase has been demonstrated to improve outcomes; however, as the number of doses of asparaginase increases as does the risk of hypersensitivity. In addition, a pharmacological study to monitor asparaginase activity in plasma (and asparagine levels in the cerebrospinal fluid) throughout the whole treatment has been implemented as a standard protocol request. Samples are periodically sent (and analyzed) to central laboratories in Italy and Germany to ensure uniform testing procedures. Samples of patients receiving

PEG-asparaginase during any exposure beyond the first exposure (i.e. the exposure planned in induction, namely phase IA) are monitored in real time to ensure that asparaginase is at therapeutic levels and that patients will eventually receive the appropriate asparaginase treatment. This particular monitoring enables a timely switch to crisantaspase as second-line therapy to maintain asparagine depletion in cases of silent inactivation (for clinical hypersensitivity, the indication to switch to crisantaspase is already indicated by the protocol guidelines based on clinical judgment). For each substituted dose of PEG-asparaginase (2500 IU/m²), crisantaspase is given i.v. at 20 000 IU/m² for seven doses on alternate days. The preliminary cumulative rate of clinical allergic reactions observed in this trial is 4.4% (15/337) in non high-risk patients and 19.3% (18/93) in high-risk patients (unpublished data) and is considerably lower than the rates in the previous AIEOP-BFM ALL 2000 trial.

CONCLUSION

The asparaginases are valuable agents in the treatment of children with ALL and the ability to tolerate asparaginase treatment is considered a valuable predictor of outcome. PEG-asparaginase is increasingly used first-line in clinical practice. Although rates of clinical allergy and silent inactivation are lower with PEG-asparaginase than with native *E. coli* asparaginase, they remain important side-effects and result in a number of patients not receiving the full treatment course and, thus, the full benefit of treatment.

Routine real time monitoring can help to identify patients with silent inactivation and facilitate a switch to a different product to ensure continued depletion of asparagine, completion of the treatment schedule and maintenance of outcomes. Although there is cross-reactivity between asparaginases derived from *E. coli*, crisantaspase does not display this to either form of *E. coli*-derived asparaginase and allows patients to maintain treatment duration if given at the appropriate dose and schedule. As a consequence, several protocols, such as the AIEOP-BFM ALL 2009, specify crisantaspase as the second-line asparaginase product to be used for the treatment of patients with an allergic reaction or silent inactivation during first-line treatment with PEG-asparaginase. There is a need for drug monitoring programs to be established to ensure real time monitoring of asparaginase activity to guarantee that patients with hypersensitivity or silent inactivation are switched to a different product. Based on the indications of different protocols and taking into consideration regulatory factors and availability, crisantaspase may be used as a viable second-line therapy. Data derived from several publications indicate that crisantaspase should be used at a dose of at least 20 000 IU/m² on alternate days (or three times weekly) by either the i.v. or i.m. route.

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Conflicts of interest

There are no conflicts of interest.

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