

ORIGINAL PAPER

Asparaginase encapsulated in erythrocytes as second-line treatment in hypersensitive patients with acute lymphoblastic leukaemia

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Summary

Asparaginase is essential in treating acute lymphoblastic leukaemia (ALL). Asparaginase-related hypersensitivity causes treatment discontinuation, which is associated with decreased event-free survival. To continue asparaginase treatment after hypersensitivity, a formulation of asparaginase encapsulated in erythrocytes (eryaspase) was developed. In NOR-GRASPALL 2016 (NCT03267030) the safety and efficacy of eryaspase was evaluated in 55 patients (aged 1–45 years; median: 6.1 years) with non-high-risk ALL and hypersensitivity to asparaginase conjugated with polyethylene glycol (PEG-asparaginase). Eryaspase (150 u/kg) was scheduled to complete the intended course of asparaginase (1–7 doses) in two Nordic/Baltic treatment protocols. Forty-nine (96.1%) patients had asparaginase enzyme activity (AEA) ≥ 100 iu/l 14 $\pm$ 2 days after the first eryaspase infusion [median AEA 511 iu/l; interquartile range (IQR), 291–780], whereas six of nine (66.7%) patients had AEA ≥ 100 iu/l 14 $\pm$ 2 days after the fourth infusion (median AEA 932 iu/l; IQR, 496–163). The mean terminal half-life of eryaspase following the first infusion was 15.3 $\pm$ 15.5 days. Few asparaginase-related adverse events were reported; five patients (9.1%) developed clinical allergy associated with enzyme inactivation. Replacement therapy was successfully completed in 50 patients (90.9%). Eryaspase was well tolerated, and most patients had AEA levels above the therapeutic target after the first infusion. The half-life of eryaspase confirmed that a 2-week schedule is appropriate.

KEYWORDS

acute lymphoblastic leukaemia, asparaginase, asparaginase encapsulated in erythrocytes, hypersensitivity

Asparaginase is indispensable when treating acute lymphoblastic leukaemia (ALL) and has contributed to improving survival^{1–3}; however, asparaginase has a significant toxicity burden.⁴ Recombinant asparaginase from *Escherichia coli* conjugated with polyethylene glycol (PEG-asparaginase) is often used as

first-line treatment, but many patients develop hypersensitivity,^{4,5} expressed as a clinical allergy (13%–15%)^{2,6,7} or silent inactivation (3%–8%).^{5,8,9} Hypersensitivity leads to discontinuation of scheduled asparaginase therapy and is linked with immunological inactivation of asparaginase enzyme activity

(AEA), both of which have been associated with a decrease in event-free survival.^{5,10,11} Replacing planned asparaginase doses with other asparaginase preparations is crucial.^{5,11,12}

Eryaspase is L-asparaginase encapsulated in allogenic erythrocytes developed to prolong the half-life of asparaginase and reduce hypersensitivity and other asparaginase-related toxicities.^{13,14} Eryaspase utilises erythrocytes to actively transport asparagine and glutamine from the plasma into the cell. Encapsulated asparaginase facilitates the hydrolysis and reduces the levels of circulating asparagine and glutamine, which in turn deprives lymphoblasts of these amino acids, triggering apoptosis.^{15,16} Crucially, encapsulated asparaginase prevents the recognition of asparaginase epitopes by the host's immune system. Patients with asparaginase-related hypersensitivity can be re-exposed with eryaspase, even if their prior hypersensitivity reaction was severe.^{13,14}

In this study, we combined eryaspase with multi-agent chemotherapy in patients with ALL and hypersensitivity to PEG-asparaginase to evaluate the pharmacological profile and effect of asparaginase-related toxicities.

PATIENTS AND METHODS

Study design and patients

NOR-GRASPALL 2016 was a multinational, multi-centre, investigator-initiated, single-arm phase II trial conducted in Denmark, Estonia, Finland, Lithuania, Norway and Sweden (Data Supplement). Eligible patients were aged 1–45 years and diagnosed with first-line, non-high-risk, Philadelphia chromosome-negative, B-cell precursor or T-cell ALL. Adult patients were included only in Denmark and Norway (Data Supplement). Patients with documented hypersensitivity to PEG-asparaginase (first-line treatment) and treated according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL2008 protocol (NCT00819351) or the ALLTogether pilot protocol (NCT04307576) were eligible for inclusion. Hypersensitivity was validated either in the form of clinical allergy and/or plasma AEA below the lower level of quantification in two samples drawn 14 days after the last PEG-asparaginase administration. Clinical allergy was confirmed with low plasma AEA if samples were available. All patients had Karnofsky/Lansky score above 50.

Patients were assessed for inadequate organ functions that prohibited further asparaginase administration including history of pancreatitis; history of serious haemorrhage or thrombosis associated with prior asparaginase therapy or pre-existing coagulopathy (e.g. haemophilia); and a history of transfusion reactions or any contraindication to receive blood transfusions.

Data presented herein are for the 55 patients recruited into the study that received at least one dose of eryaspase. The Evaluable Patient Population for pharmacokinetic analyses was defined as patients who provided data on whole-blood AEA at 14 ± 2 days following the first eryaspase administration, which encompassed the 53 patients with evaluable pharmacokinetic parameters (Figure 1).

The study was approved by the National Medicines Agencies in all participating countries (EudraCT no. 2016–004451-70), relevant ethical committees and registered at www.clinicaltrials.gov (NCT03267030). The study complied with the Declaration of Helsinki. Informed consent was obtained from parents/guardians, or patients (as age-appropriate) before enrolment.

Asparaginase treatment and risk-group stratification

In the NOPHO ALL2008 and ALLTogether pilot protocols, PEG-asparaginase was used as the standard preparation. In the NOPHO ALL2008 protocol, non-high-risk patients were scheduled for five doses of PEG-asparaginase (1000 iu/m^2 /dose, administered intramuscularly), starting at treatment Day 30 at 2-week intervals during the consolidation phase and then three additional doses at 6-week intervals during the delayed intensification and first maintenance phase. In the ALLTogether pilot protocol, PEG-asparaginase treatment ($1500 \text{ iu/m}^2 < 16$ years and $1000 \text{ iu/m}^2 \geq 16$ year, administered intravenously) was scheduled from treatment Day 4 in induction. Non-high-risk patients were stratified at treatment Day 29 into either standard-risk, intermediate low-risk, or intermediate high-risk risk groups in which they received a total of four, five, or eight doses of PEG-asparaginase at 2-week intervals, respectively. Risk-grouping and treatment details are given in the data supplement (Tables S2 and S3).

Patients were included in the present study after developing hypersensitivity to PEG-asparaginase treatment and eryaspase (150 u/kg) substituted the remaining doses of PEG-asparaginase. Patients initiated treatment at either 2- or 6-week intervals according to the treatment protocol and time of inclusion (Figure 2).

Pharmacological and immunogenicity assessments

Rigorous sampling for whole-blood pharmacokinetics and immunological analyses was performed following each infusion of eryaspase. Samples of whole blood were collected at regular intervals for all administrations: 10 min before the infusion, immediately after the infusion, 24 h post infusion, as well as 3, 6, 10, 14, 21 and 42 days post infusion. AEA was analysed using L-aspartic β -hydroxamate after the erythrocytes were lysed. Asparaginase antibodies were analysed using a modified version of semi-quantitative, indirect enzyme-linked immunosorbent assay (ELISA).¹⁷ Cerebrospinal fluid (CSF) was collected when lumbar punctures were performed in accordance with the NOPHO ALL2008/ALLTogether pilot protocols during the inclusion period. Asparagine levels in CSF samples were evaluated via ion-pair liquid chromatography coupled to tandem mass spectrometry as previously described.¹⁸ Further details regarding sampling and analyses are described in the data supplement.

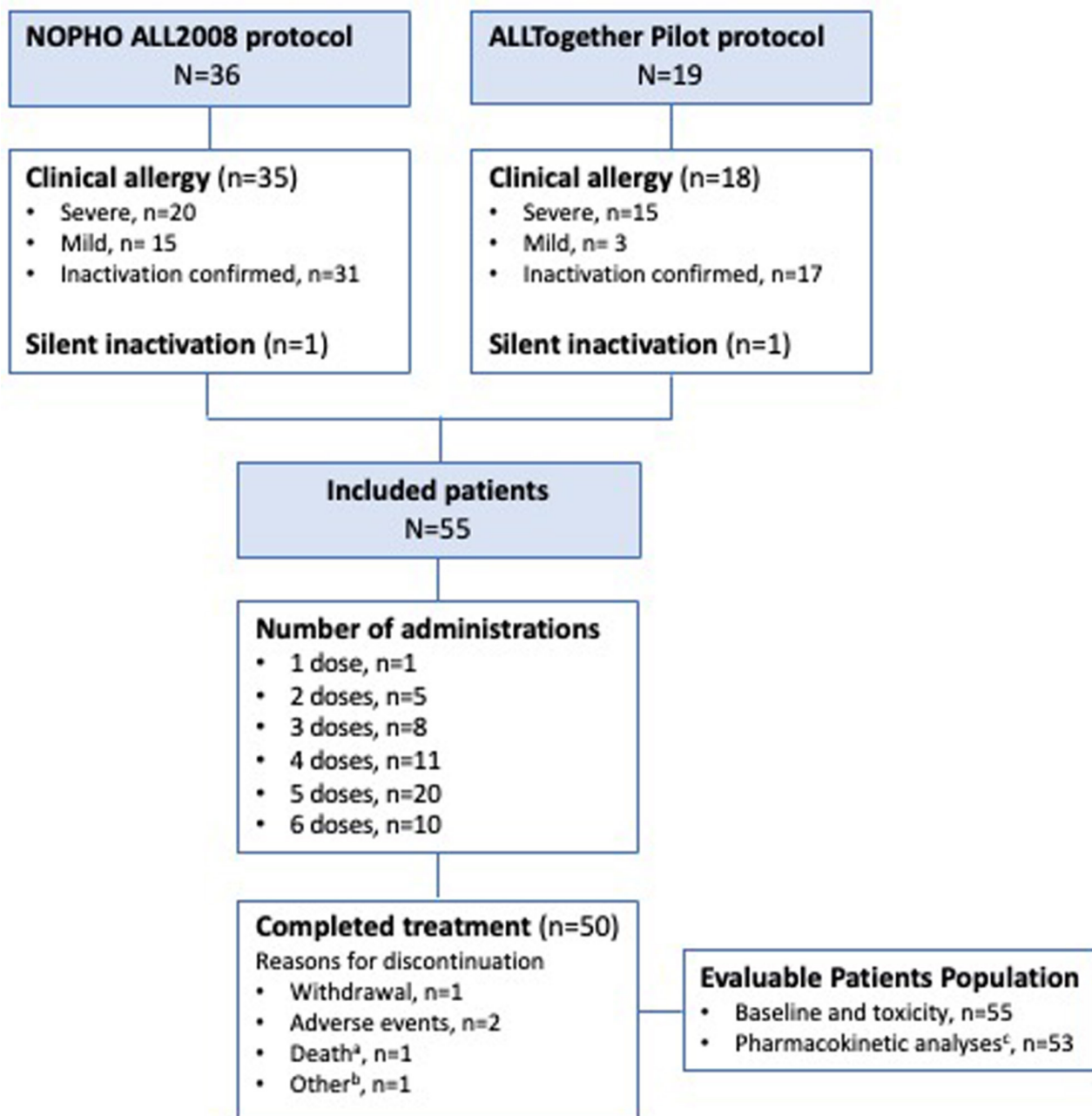


FIGURE 1 NOR-GRASPALL 0032016 CONSORT diagram stratified by treatment protocol and hypersensitivity reaction prior to inclusion. One patient died during the inclusion period, but it occurred beyond the 30-day period after the last dose of the study drug. One patient developed irregular antibodies. The Evaluable Patient Population for pharmacokinetic analyses was defined as patients who provided data on asparaginase enzyme activity levels on Day 14 (± 2 days) following the first administration of eryaspase. NOPHO, Nordic Society of Paediatric Haematology and Oncology [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/bjh.18152)]

Assessment of asparaginase-related adverse events

Asparaginase-related adverse events (AEs) were reported and graded according to the Ponte di Legno (PdL) toxicity consensus definitions.¹⁹ All other AEs were graded according to the Common Terminology Criteria for AEs (CTCAE) version 4.0.¹⁹ All events were recorded from start of treatment until 30 days after the last administration. Events of leukopenia, thrombocytopenia, anaemia, infection, or fever

that led to hospitalisation or prolonged hospitalisations were not reported as AEs.

Outcome measure

The primary end-points evaluated the pharmacological profile including AEA levels, including the percentage of patients with AEA above 100 u/l at 14 ± 2 days following the first infusion [i.e. the observed concentration at

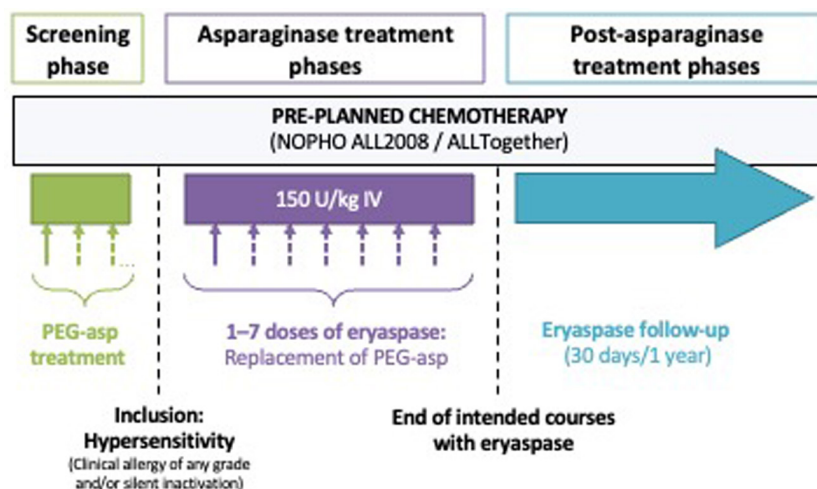


FIGURE 2 NOR-GRASPALL 2016 study diagram. Schematics of the NOPHO ALL2008 protocol and ALLTogether pilot protocol are presented. In the NOPHO ALL2008^a protocol, eight doses of PEG-asparaginase were planned (five doses every 2 weeks, three doses every 6 weeks). In the ALLTogether pilot protocol, the number of PEG-asparaginase doses was planned according to the patients' assigned risk group (standard risk: four doses; intermediate risk [IR]-eight doses). All doses planned with 2-week intervals. NOPHO, Nordic Society of Paediatric Haematology and Oncology; PEG, polyethylene glycol [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/bjh.18152)]

the end of each dosing interval (C_{trough}) for all patients. The area under the curve (AUC) was characterised over the dose interval of 14 days and extrapolated to infinity [i.e. $\text{AUC}(0-\infty)$] to estimate clearance (CL) and volume of distribution (V_{ss}). Secondary end-points included the percentage of patients on a 2-week regimen with asparaginase activity above 100 u/l at 14 ± 2 days following the fourth infusion. According to the previously determined half-life ($t_{1/2}$) for whole-blood asparaginase at 14 ± 2 days, patients would be near the steady state; hence, the pharmacokinetics over a dose interval can be used to estimate CL and V_{ss} . Pharmacodynamic evaluation included concentrations of asparagine in the CSF.

Safety and tolerability were evaluated as incidence of hypersensitivity (clinical allergy or silent inactivation), or AEs that occurred within 30 days after the last administration. Event-free survival was assessed 1 year after the last administration.

Statistical analyses

The data was analysed with the software package SAS® Version 9.4 (SAS Institute, Cary, NC, USA) and GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, CA, USA). Continuous data were presented as the mean with standard deviation (SD), median with interquartile range (IQR) and range. Pharmacokinetic parameters were determined using Phoenix® WinNonLin® 6.4 (Certara USA, Inc., Princeton, NJ, USA). Pharmacokinetic statistical summaries included the mean, SD, median, minimum, maximum, geometric mean and coefficient of variation (CV). The relationship of AEA and CSF asparagine was displayed graphically with a polynomial non-linear regression (locally estimated scatterplot smoothing).

RESULTS

Between August 2017 and July 2020, 55 patients were included in the study: 53 children (96.4%) and two adults (3.6%); median age was 6.1 years (IQR, 3.5–10.6). Patients were included after a median of three doses (range, 2–6) of PEG-asparaginase. Of the included patients, 53 patients (96.4%) had clinical allergy to PEG-asparaginase, of those 46 (87%) had confirmed inactivation of PEG-asparaginase in two or more samples. Remaining patients had inadequate sampling. Clinical allergy was classified as severe in 31 patients (58.5%) and mild in 22 patients (41.5%, [Figure 1](#)). Two patients (3.6%) with silent inactivation were included in the study. All patient characteristics are listed in [Table 1](#).

All 55 patients were included when evaluating baseline and toxicity, and the Evaluable Patient Population for pharmacokinetic analysis included 53 patients with adequate sampling 14 ± 2 days following the first eryaspase infusion ([Figure 1](#)).

Replacement therapy with eryaspase was completed in 50 patients (90.9%) who received the remaining intended asparaginase treatment after hypersensitivity. All patients received at least one dose of eryaspase, with a median of five infusions (range, 1–6) per patient. Thirty patients (54.5%) received five doses or less ([Figure 1](#)).

Pharmacokinetics

A total of 1665 samples from 53 patients were included in the pharmacokinetic analysis. Two patients were excluded due to missing sampling or qualification. Of the 53 patients, 49 (92.5%) patients had an AEA of 100 iu/l or more 14 ± 2 days after the first infusion (median AEA 511 iu/l; IQR, 291–780 iu/l; [Figure 3](#)). Nine patients were included in the analyses

TABLE 1 Patient characteristics

Characteristics	No. (range or %) N = 55
Age [median (IQR)]	6.1 (3.5–10.6)
<18 years	53
>18 years	2
Sex	
Male	38 (69.1%)
Female	17 (30.9%)
Immunophenotype	
Pre B-ALL	52 (94.5%)
T-ALL	3 (5.5%)
Treatment protocol and risk group	
NOPHO ALL2008	36 (65.5%)
Standard risk	17 (30.9%)
Intermediate risk	18 (32.7%)
ALLTogether pilot protocol	19 (34.5%)
Standard risk (very low risk)	4 (7.3%)
Intermediate risk, low	3 (5.5%)
Intermediate risk, high	13 (23.6%)
CNS disease at diagnosis ^a	
No blasts	46 (83.6%)
>5 WBC/ μ l blasts	8 (24.5%)
Hypersensitivity characteristics	
Clinical allergy	53 (96.4%)
Mild	22 (42.3%) ^b
Severe	30 (58.8%)
Inactivation of PEG-asparaginase confirmed	48 (90.6%) ^c
Silent inactivation	2 (3.6%)
PEG-asparaginase doses before inclusion, median (range)	3 (2–4)

Abbreviations: ALL, acute lymphoblastic leukaemia; CNS, central nervous system; IQR, interquartile range; N, number of patients; NOPHO, Nordic Society of Paediatric Haematology and Oncology; PEG, polyethylene glycol; WBC, white blood cell.

^aThe datum is missing for one patient.

^bInactivation confirmed in 21 of 22 cases.

^cIn five patients less than two samples were available for enzyme activity analysis.

of AEA at 14 ± 2 days following the fourth infusion in which six patients (66.7%) displayed AEA of 100 iu/l or more (median AEA 932 iu/l; IQR, 496–163; Figure 3). Pharmacokinetic parameters for the fourth infusion only included patients on a 2-week regimen as these patients would be at or near the steady state. The number of patients with AEA of 100 iu/l or more and medians of AEA levels for doses 1–4 are included in the data supplement (Figure S1). According to the 6-week schedule, 109 of 151 samples that were drawn from 37 patients at 14 ± 2 days after an eryaspase infusion had an AEA of 100 iu/l or more (median: 422 iu/l; IQR, 252–732), and 41 of 99 samples (41.4%) that were drawn from 37 patients at 42 ± 5 days after an eryaspase administration displayed an AEA of 100 iu/l or more (median: 261 iu/l; IQR, 146–482).

The mean terminal $t_{1/2}$ and SD following the first infusion was 15.3 ± 15.5 days. The mean CL was 0.01 l/day/kg and exhibited high variability (CV = 80.5%). The mean terminal $t_{1/2}$ and SD following the fourth infusion was 13.2 ± 11.2 days, with high variability following the fourth infusion (CV = 84.8%; Table 2).

Thirty-two patients (60.4%) developed asparaginase-directed antibodies during eryaspase treatment. Furthermore, four of five patients with an asparaginase-related allergy were antibody-positive at the time of the allergic reaction and when inactivation occurred.

Pharmacodynamic measurements

The concentration of asparagine in CSF samples was assessed in 164 samples from 49 patients, of these 110 samples had a corresponding AEA measurement (± 48 h). Notably, CSF asparagine concentrations tended to decline with elevated AEA (Figure 4). Asparagine levels stratified per patient are included in the data supplement (Figure S2).

Adverse events

A clinical allergy was reported in 10 patients after eryaspase and two of these patients experienced severe hypersensitivity events according to PdL toxicity consensus definitions,¹⁹ leading to the discontinuation of their pre-planned eryaspase treatment (Table 3). One of the patients had mild allergic symptoms after the first infusion, but continued the eryaspase treatment and developed a severe allergy after the fourth dose. Notably, retrospective analysis revealed low AEA in the patient. The other patient developed a severe allergy after the fourth eryaspase administration; however, this patient had AEA levels above the threshold until the allergic reaction occurred. Nonetheless, both patients developed an allergy after a dose administered within the 6-week interval. Of the eight patients with mild allergic symptoms, only three patients had an enzyme activity of less than 100 iu/l 14 days post infusion. One patient had AEA levels below the lower level of quantification 14 days post administrations (three doses, 6-week interval), but did not experience any clinical symptoms. Osteonecrosis was reported in one patient (grade 2), and no patients experienced any events of thromboembolism or pancreatitis. Most reported events (15/23) were grade 2 or less. One event was fatal, but judged to be unrelated to eryaspase.

Overall survival

All patients were followed for one year after their last eryaspase dose. Fifty-three patients (96.4%) sustained complete remission at the end of follow-up. One patient died during the enrolment period owing to haemophagocytic lymphohistiocytosis, which was judged to be unrelated to eryaspase.

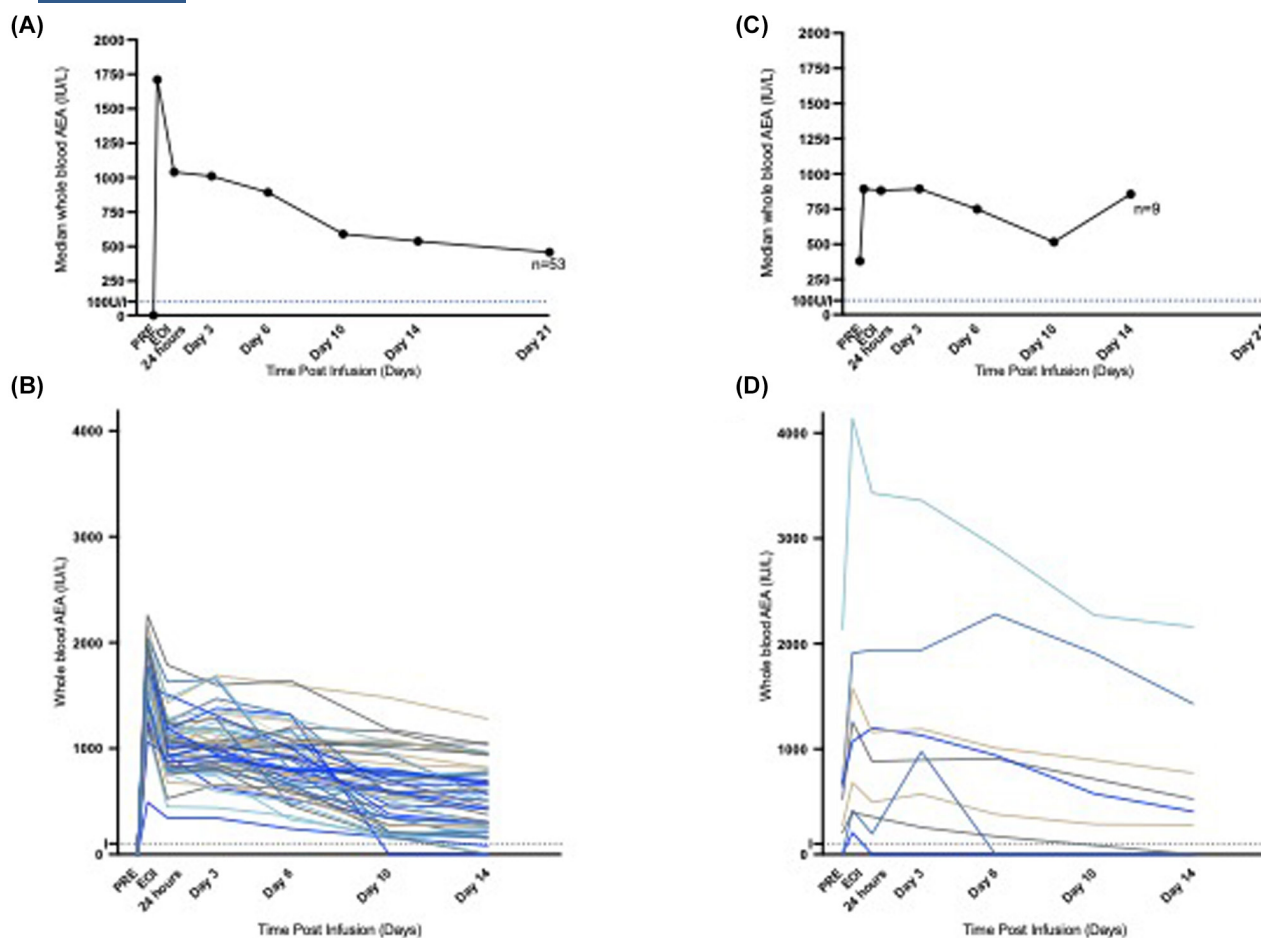


FIGURE 3 Plots of AEA over time following the first and fourth dose of eryaspase. (A) Plot of median AEA after the first infusion. (B) Spaghetti plot of individual AEA measurements combined for each patient after the first infusion for all patients in the Pharmacokinetic Patient Population ($n = 53$). (C) Plot of median AEA after the fourth infusion. (D) Spaghetti plot of individual AEA measurements combined for each patient after the fourth infusion for all evaluable patients in the Pharmacokinetic Patient Population ($n = 9$). AEA, asparaginase enzyme activity; EOI, end-of-infusion sample; PRE, pre-infusion sample [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/bjh.18152)]

One patient had a central-nervous-system relapse in the follow-up period.

DISCUSSION

This phase II trial evaluated the feasibility of eryaspase as a substitution therapy in non-high-risk ALL patients who developed hypersensitivity to prior PEG-asparaginase treatment in two large, contemporary ALL treatment protocols. This is the first study to evaluate eryaspase in children and young adults with first-line ALL.

Overall, eryaspase treatment was well tolerated. We found that a small proportion of patients ($n = 5$, 9.1%) developed allergic reactions accompanied by enzyme inactivation when eryaspase was administered after hypersensitivity to PEG-asparaginase; moreover, these data were similar to those in previous studies that showed low allergic reactions rates after eryaspase treatment.¹⁴ In this study, the mean terminal $t_{1/2}$ following the first infusion was 15.3 days, which was consistent with the mean terminal $t_{1/2}$ of 16–20 days reported in

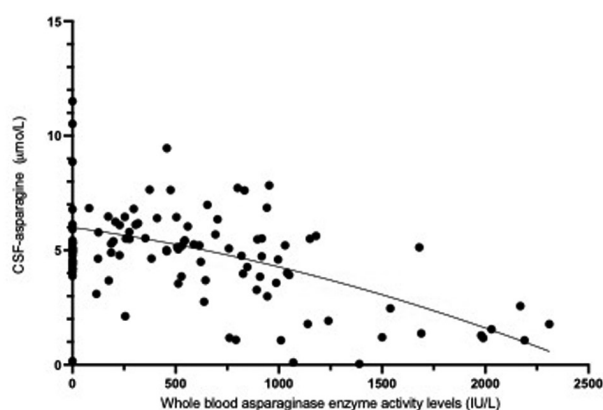
another eryaspase pharmacokinetic study.^{13,14,20} Thus, data from this trial supports the 2-week interval regimen. This finding provides an alternative to the usual second-line asparaginase treatment after PEG-asparaginase hypersensitivity, *Erwinia chrysanthemi*-derived L-asparaginase (*Erwinia*), which has a short half-life (16 h); consequently, a new infusion is required every second/third day, and 6–7 doses are recommended to replace each planned PEG-asparaginase dose. Administrations at 2-week intervals reduce the number of hospital visits, which improves the patient's quality of life and reduces associated medical costs.^{21–23} Importantly, there has been an intermittent, worldwide shortage of *Erwinia* caused by drug supply issues, thereby compromising the outcome for patients.¹¹ Discontinuing pre-planned asparaginase treatments owing to hypersensitivity has corresponded with substantially inferior outcomes of ALL.^{2,5,10,11} Therefore, a replacement therapy is crucial to minimise the risk of ALL relapse.¹¹

A specific threshold of response has not been defined owing to a lack of comparable studies on eryaspase including additional information on eryaspase pharmacokinetics.

TABLE 2 Whole-blood asparaginase pharmacokinetic parameters after the first and fourth infusions of eryaspase (150 u/kg)

Statistic	$t_{1/2}$ (days)	$C_{\max}$ (u/l)	AUC_{inf} (day $\times$ u/l)	CL(l/day/kg)	V_{ss} (l/kg)
First infusion					
N	52	53	52	52	52
Mean (SD)	15.3 (15.5)	1644 (333)	28 261 (27369)	0.0098 (0.0079)	0.12 (0.04)
Median	10.1	1700	20 071	0.0075	0.12
Min, Max	2.8, 75.1	492, 2260	3380, 149 568	0.001, 0.044	0.1, 0.3
Geometric mean	10.7	1602	20 420	0.0073	0.12
% CV	101	20.3	96.9	80.5	32.4
Fourth infusion					
N	11	12	11	11	11
Mean (SD)	13.2 (11.2)	1268 (1082)	30 104 (32260)	0.0435 (0.0636)	0.34 (0.18)
Median	9.04	1046	17 913	0.0135	0.29
Min, Max	1.4, 37	208, 4140	734, 96 639	0.004, 0.217	0.1, 0.6
Geometric mean	9.37	940	13 128	0.0206	0.29
% CV	84.8	85.3	107	146	54.5

Abbreviations: AUC_{inf} , area under the curve from time zero extrapolated to infinity following the first infusion; $C_{\max}$, maximum observed concentration after each administration; CL, clearance; CV, coefficient of variation; Max, maximum; Min, minimum; N, number of patients; SD, standard deviation; V_{ss} , distribution volume at steady state.

**FIGURE 4** Observed CSF asparagine measurements relative to whole-blood asparaginase enzyme activity with a polynomial non-linear regression (LOESS). CSF, cerebrospinal fluid

The threshold of AEA above 100 u/l has been considered to be appropriate and safe for other asparaginase formulations and is widely used as threshold for the adequate therapeutic concentration.²³ The threshold for an acceptable frequency of hypersensitivity reactions depends on availability of other second-line options, severity of the allergic reactions, and number of doses administered before development of allergic reactions. Notably, the rates of hypersensitivity rates in this study are comparable with PEG-asparaginase (13%–22%),^{2,7,8} and with *Erwinia* asparaginase (3%–9%).^{21,24,25} However, a well-powered randomised controlled trial comparing *Erwinia* asparaginase and eryaspase is essential to confirm the efficacy. The therapeutic target of 100 iu/l after the first infusion was reached in ~90% of patients, except for four instances: one patient had a mild allergy associated with the first infusion

and developed anaphylaxis after the fourth dose, whereas one patient had mild allergic symptoms and two patients had no allergic symptoms. Given the half-life of eryaspase, drug accumulation was expected such that C_{trough} of whole-blood AEA levels would increase towards a steady state. Contrastingly, accumulation was not observed in some of the patients and whole-blood AEA concentrations declined during repeated infusions. This observation would account for the lower C_{trough} whole-blood AEA level that occurred following the fourth infusion. The reason for the declining whole-blood AEA during repeated infusions is not known but might be associated with an emerging inactivation or allergy. Additionally, very few patients were included in the analyses after the fourth dose as all patients following the NOPHO ALL2008 protocol entered the 6-week regimen for the last three doses. In accordance with the treatment protocol, the samples were stored and analysed at the end of the study, which may have inadvertently introduced variation in the storage; moreover, a reduction in AEA levels over time could not be excluded despite storage conditions. Importantly, the method of measuring AEA after eryaspase administrations and after lysis of the erythrocytes requires validation, with further investigation of the AEA level needed to ensure asparagine depletion is necessary. Ideally, plasma asparagine levels should be measured during future studies; however, this has been proven difficult in a clinical setting.^{26–28} Asparaginase-directed antibodies were found in 60.4% (32/53), but most (28/32) patients did not further develop clinical allergy or inactivation of asparaginase, and our findings are consistent with those of previous studies.¹³ Since antibodies cannot penetrate the erythrocyte membrane, the produced asparaginase antibodies may not be targeting the encapsulated asparaginase, but

TABLE 3 Incidence of adverse events during treatment with eryaspase

Adverse events	All treated patients (N = 55)	
	Any grade	Grade ≥3
Patients with any AE	17 (30.9%)	6 (10.9%)
Any AE	23	8
Hypersensitivity	11 ^a	2
Rash/urticaria ^b	4	0
Other ^c	3	0
Anaphylaxis	2	2
Pyrexia	2	0
Hyperlipidaemia	2	0
Increased liver enzymes	2	0
Bone pain	1	0
Constipation	1	0
Hemophagocytic lymphohistiocytosis	1	1
Leukoencephalopathy	1	NA ^d
Nausea	1	0
Osteonecrosis	1	0
Stomatitis	1	1
Weight decrease	1	0
Fatal AEs	1	1 (1.8%)
AEs leading to discontinuation of treatment	2	3 (3.6%)

Specific events of infections, neutropenia, thrombocytopenia and anaemia were not reported according to the Study Protocol.

Abbreviations: AE, adverse event; N, number of patients; NA, not available.

^aIn 10 patients, one patient had mild allergy after the first infusion and anaphylaxis after the fourth dose.

^bOne patient reported a pruritic rash.

^cPatients reported a cough, a coarse feeling in throat, malaise, and/or flushing.

^dGrade unknown.

rather a small fraction of free asparaginase. Future work will examine the neutralising effect of the asparaginase antibodies. Despite the presence of asparaginase antibodies, re-exposure with eryaspase after hypersensitivity to PEG-asparaginase was possible without triggering allergic reactions in most patients (89.1%). These results confirm that encapsulating asparaginase in erythrocytes prevented an immune response.

As eryaspase utilises erythrocytes, it is difficult to distinguish drug-related hypersensitivity reactions from transfusion-like reactions. Interestingly, while pyrexia and flushing were reported in some patients and these reactions might be associated with the eryaspase infusions, adequate AEA levels were sustained. Thus, therapeutic drug monitoring of asparaginase activity is crucial when treating patients with eryaspase.

We found a tendency for CSF asparagine concentrations to decline after eryaspase administration. The total number of samples was too scarce to make a statistically meaningful interpretation of the results. Previous studies have found

that asparagine depletion was significantly reduced after eryaspase treatment.^{13,29} As CSF sampling is invasive, we collected CSF samples when a pre-planned lumbar puncture was performed in accordance with the NOPHO ALL2008 and ALLTogether pilot protocols. Under most circumstances, the CSF sampling time did not correspond with the eryaspase treatment schedule or collection of the samples for pharmacokinetic parameters. The importance of asparagine concentration in the CSF and its influence on the patient's relapse risk remains to be elucidated during asparaginase treatment and should be further investigated in large cohorts.^{30,31} A study found that asparagine levels in CSF were reduced during PEG-asparaginase treatment, but complete depletion was observed in a minority of patients.³⁰ Thus, the impact of eryaspase on CSF asparagine levels warrants further investigation in a setting with more samples collected after each administration and accompanied by AEA measurements.

The chemotherapy regimens used in the ALLTogether and NOPHO ALL2008 protocols are quite similar, but the schedule of asparaginase administration differs. Under the NOPHO ALL2008 protocol, asparaginase treatment was scheduled at 2- and 6-week intervals for all non-high-risk patients, whereas the ALLTogether protocol only used 2-week intervals. Eryaspase displayed a favourable safety profile in both treatment regimens; however, more patients following the NOPHO ALL2008 regimen had hypersensitivity, which may have been caused by the 6-week asparaginase-free intervals.

Eryaspase was well tolerated and most patients completed asparaginase treatment. Most patients have AEA levels above the therapeutic target after the first to fourth infusions, although very few patients were included in the analyses following the fourth dose. Based on the half-life of eryaspase, our findings confirmed that a 2-week schedule is appropriate when treating patients with ALL. Future work will focus on the clinical efficacy of eryaspase in larger cohorts.

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CONFLICT OF INTERESTS

Birgitte Klug Albertsen: sponsor for the investigator-initiated study, the author has no financial conflict of interests. Speaker and/or advisory board honoraria from Erytech (2020) and Servier (2021). Kjeld Schmiegelow: speaker and/or advisory board honoraria from Illumina (2021), Jazz Pharmaceuticals (2020, 2021) and Servier (2020, 2021); speaker fee from Amgen (2020, 2021) and Medscape (2020, 2021); educational grant from Servier (2020, 2021);

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AUTHOR CONTRIBUTIONS

Line Stensig Lynggaard, conception and design, served as national co-investigator and study coordinator, recruited patients, collected, analysed and interpreted data and drafted the manuscript; Birgitte Klug Albertsen, conception and design, served as sponsor and national investigator, recruited patients, collected and interpreted data and edited the manuscript; Goda Vaitkeviciene, Anne Kristine Lehmann, Cecilia Langenskiöld, Päivi M. Lähdenmäki and Kristi Lepik served as national principal investigators, recruited patients and collected data and edited the manuscript; Iman El Hariry, conception and design; Kjeld Schmiegelow, conception and design of the study, served as principal investigator of the NOPHO ALL2008 protocol and edited the manuscript. All authors revised the manuscript critically and approved the final version.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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