Efficacy and safety of recombinant *E. coli*-asparaginase in infants (less than one year of age) with acute lymphoblastic leukemia

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ABSTRACT

The pharmacokinetics, pharmacodynamics, efficacy and safety of a new recombinant *E. coli*-asparaginase preparation were evaluated in infants (<1 year of age) with *de novo* acute lymphoblastic leukemia. Twelve patients were treated according to the INTERFANT-06 protocol and received up to 10,000 U/m^2 recombinant asparaginase as intravenous infusions on days 15, 18, 22, 25, 29 and 33 of remission induction treatment. The asparaginase dose was individually adjusted by protocol to 67% of the calculated dose for infants <6 months, and to 75% of the calculated dose for infants aged 6-12 months. The trough serum asparaginase activities observed were above 20, 50, and 100 U/L in 86%, 71%, and 51% of measured samples, respectively. Looking only at the data assessed 3 days after asparaginase infusion these percentages were 91%, 84%, and 74%, respectively. Asparagine was completely depleted in serum in all but one patient who was the youngest in the study. No anti-asparaginase antibodies were detected during this treatment phase. Observed adverse reactions are known to be possible and are labeled side effects of asparaginase treatment and chemotherapy. We conclude that the asparaginase dose regimen used in infants is safe and provides complete asparagine depletion for the desired time period in nearly all patients. Measured asparaginase trough serum levels justify the higher doses used in infants compared to in older children and show that 3-day intervals are preferred over 4-day intervals. (*This trial was registered at www.clinicaltrialsregister.eu as EudraCT number 2008-006300-27*).

Introduction

Acute lymphoblastic leukemia (ALL) in infants (<1 year of age) is uncommon (accounting for 2-5% of cases of childhood ALL), biologically distinct from the disease in older pediatric patients, and associated with a relatively poor prognosis. The pro-B, CD10-negative immunophenotype is typically found in infant acute leukemia, and the most common genetic alteration is rearrangement of the *MLL* gene (observed in up to 80% of infants with ALL).[1-3]

In 1999, a large international collaborative study, INTERFANT-99, was launched. Four hundred and eighty-two patients were recruited into this trial from the major ALL study groups in the world (AIEOP, BFM, COALL, DCOG, DFCI, FRALLE, NOPHO, SJCRH, UKCCSG, and others). Ninety-four percent of the patients achieved a complete remission at the end of induction treatment and the early death rate was 3.8%. At 4 years, event-free survival and overall survival rates were 47% and 55%, respectively.[2] Native *E.coli*-asparaginase (ASNase) was a major part of the induction and consolidation treatment within this protocol. Patients received six doses of 5 000 U/m^2 ASNase during the induction phase and another two administrations of the same dose during consolidation treatment (MARAM protocol) with an age-dependent dose reduction to 67% of dose for infants <6 months and to 75% for those aged between 6 and 12 months.

This treatment protocol is used as the standard for continuing international collaborative studies that aim to further improve outcomes of infants with ALL. A subsequent trial (INTERFANT 06; EudraCT number: 2005-004599-19) was launched in 2006. Within this new protocol, ASNase treatment was intensified by increasing the dose from 5 000 to 10 000 U/m^2 during induction treatment including the same age-dependent dose reductions as mentioned above, replacing the two doses of native *E.coli*-ASNase during consolidation by one dose of pegylated *E.coli*-ASNase 2 500 U/m^2 (Oncaspar®) at the end of the MARAM protocol (now called MARMA)

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and adding another dose of pegylated *E.coli*-ASNase into the re-induction course (OCTADAD).

Within this trial, a newly developed recombinant asparaginase (rASNase) preparation was used by nine participating centers in the Netherlands (n=3) and Germany (n=6). rASNase has similar pharmacokinetic and pharmacodynamic properties as other *E.coli*-ASNase preparations used in Europe (e.g. Asparaginase medac®, Paronal®) but is produced by optimized fermentation and purification techniques and is therefore - in contrast to the currently approved ASNase products - nearly free of ASNase aggregates. For the production of rASNase a genetically engineered *E.coli* strain is used as opposed to the non-recombinant *E.coli* strain used for production of commercially available *E.coli*-ASNase preparations such as Asparaginase medac®. The genetically modified *E.coli* strain used for recombinant expression of rASNase has been transformed with a circular DNA vector (i.e. plasmid) that bears the coding sequence for ASNase. High levels of expression of rASNase in the microbial fermentation can, therefore, be induced via a suitable promoter gene sequence allowing for much higher ASNase yields per cell. Nevertheless, at the protein level the expressed rASNase and *E.coli*-ASNase share the identical amino acid sequence. In a preceding trial, rASNase was shown to be pharmacokinetically bioequivalent to Asparaginase medac® in pediatric patients aged 1-15 years and led to complete asparagine depletion in serum in all patients for the desired time period during remission induction treatment.[4]

The aim of this trial was to show that the rASNase dose regimen used in the current INTERFANT-06 protocol is safe in infants, provides sufficient ASNase activity during induction treatment and leads to complete asparagine depletion in serum for the desired time period. This is the first prospective trial investigating the pharmacokinetics and pharmacodynamics of an ASNase preparation in infants with *de novo* ALL.

Methods

Recombinant asparaginase

rASNase was developed by medac and Wacker Biotech GmbH (formerly ProThera GmbH) Jena, Germany. The final formulation of this investigational drug product was designed by medac and is available in vials with 10 000 U dry substance.

Patients and treatment

It was planned that 12 patients would be included in this trial and they were recruited between July 2009 and May 2010 by three participating centers in the Netherlands (Groningen, Rotterdam Utrecht) and six centers in Germany (Berlin, Erlangen, Frankfurt a.M., Freiburg, Hamburg, Stuttgart).

All children participated in the induction therapy of trial INTERFANT-06 and received combination chemotherapy treatment consisting of a prednisone pre-phase (60 mg/m^2/day; days 1-7), dexamethasone (6 mg/m^2/day days 8-28, followed by 1 week tapering off), vincristine (1.5 mg/m^2/day; days 8, 15, 22, and 29), cytarabine (75 mg/m^2/day; days 8-21), daunorubicin (30 mg/m^2/day; days 8 and 9), rASNase (10 000 U/m^2/day; days 15, 18, 22, 25, 29, and 33), plus intrathecal injections with methotrexate/prednisolone and cytarabine/prednisolone.

The chemotherapy and rASNase dose were individually adjusted to the specific age of the patient at the start of the respective treatment phase. Children <6 months of age received 67% of the calculated dose based on body surface area whereas children aged 6 through 12 months received 75% of the calculated dose and children >12 months of age received the full dose.

The study protocol was approved by institutional review boards at the participating institutions. Parents or guardians provided informed consent. The study was conducted in accordance with the basic principles of the Declaration of Helsinki.

Blood sample processing guidelines and analytical assays

Blood sample processing guidelines and analytical assays to measure asparaginase, amino acids, and anti-ASNase-antibody levels in serum are described in the *Online Supplementary Methods* section.

Pharmacokinetic/pharmacodynamic and efficacy assessment

Trough ASNase activity and amino acid levels in serum were determined prior to administration of rASNase infusion 1 (day 15; baseline value), 2 (day 18), 4 (day 25), and 6 (day 33) during remission induction treatment.

The efficacy of treatment was determined by evaluating the complete remission rate and minimal residual disease (MRD) status at protocol day 33.

Complete remission was defined on morphological grounds by the presence of <5% leukemic blasts in bone marrow (M1 marrow), no leukemic blasts in peripheral blood or cerebrospinal fluid, no other documented extramedullary leukemia with the exception of testicular enlargement, and regenerating hematopoiesis.

The *MLL* genomic breakpoint and/or Ig/TcR rearrangement were used as MRD-polymerase chain reaction (PCR) targets. According to INTERFANT-06 the *MLL* breakpoint fusion region was used as the main MRD-PCR target. In cases without *MLL* gene translocations or cases with a limited quantitative range of *MLL* targets, Ig/TcR gene rearrangements could be used as MRD-PCR targets.

Safety

Toxicity data were graded according to the CTCAE criteria version 3.0. Before and during rASNase therapy bilirubin, aspartate and alanine transaminases, creatinine, fibrinogen, and antithrombin III levels were assessed.

Statistics

It was planned that a total of 12 patients would be enrolled in this trial. With this sample size a minimum amount of data was available to describe pharmacokinetic and pharmacodynamic parameters taking into account the feasibility of the trial within a rare indication with an extremely low number of available infants with ALL.

Given the non-controlled study design statistical analysis consisted of calculating point- and interval estimates for the parameters of interest. The statistical analysis was performed using SAS (SAS Institute Inc., Cary, NC, USA) version 9.1.3 on a Windows platform.

Results

Patients' characteristics

Altogether 12 infants, seven males and five females, with a diagnosis of ALL participated in this study. All patients completed their full course of rASNase treatment and could be analyzed for efficacy and safety. At diagnosis, six patients were aged below 6 months whereas the other six

patients were between 6 and 12 months old. Further characteristics of the patients are shown in Table 1.

Asparaginase treatment intensity

All 12 patients completed their full course of treatment consisting of six infusions of rASNase. The dose per infusion administered to the six patients below 6 months of age was 6 700 U/m^2. The five patients aged 6 up to 12 months received 7 500 U/m^2 per infusion. The patient 12.2 months of age at first rASNase infusion was given 10 000 U/m^2. All patients except one received all rASNase infusions at the scheduled intervals defined in the protocol. In one patient (number 3) the fifth rASNase infusion was postponed for 1 day due to grade III febrile neutropenia.

Asparaginase trough activity levels in serum during induction treatment for acute lymphoblastic leukemia

The goal of ASNase treatment is to achieve serum activities higher than 100 U/L for a desired period which guarantees complete asparagine depletion.[5] The observed trough serum ASNase activities were ≥20, ≥50, or ≥100 U/L in 86%, 71%, and 51% of all measured samples, respectively. Four patients had an ASNase activity <20 U/L at some time points during induction treatment. Individual data are shown in Table 2.

The trough ASNase activity levels on day 33 were considerably lower than those on day 18 and day 25 which was due to the fact that the latter levels were assessed 3 days after the rASNase infusion while the assessment on day 33 was performed 4 days after the last rASNase infusion. Looking only at the data from days 18 and 25, the observed trough serum ASNase activities were ≥20, ≥50, and ≥100 U/L in 91%, 87%, and 74% of measured samples, respectively.

Age seems to have no influence on ASNase serum activity levels although younger patients received reduced doses of rASNase (<6 months of age: 67% of the calculated dose based on body surface area; 6-12 months of age: 75% of the calculated dose). Pearson's correlation coefficient for age and ASNase serum activity on day 25 is 0.05 (P=0.87).

The youngest infant (n. 6) had a very low level of ASNase activity before the fourth ASNase infusion and no activity was found before the last rASNase administration.

Pharmacodynamic results

Mean asparagine concentrations in serum dropped from the mean predose concentration of 45 μmol/L (90% CI: 38-54) to below the lower limit of quantification of the bioanalytical method (<0.5 μmol/L) in all patients at all time points measured except in patient n. 6 on day 33. The data are shown in Table 3.

As a result of continued cleavage of asparagine, mean serum levels of the amino acid aspartate increased from 2.8 μM (range, 1.2 - 4.9 μM) at baseline to 12.4 μM (range, 5.10 - 23.2 μM), 13.1 μM (range, 4.9 - 40.1 μM), and 8.9 μM (range, 3.7 - 44.2 μM) on days 18, 25, and 33, respectively.

Table 1. Patients' characteristics.

Median age at first rASNase infusion, months; range	6; 0.5-12.2
Median weight, g; range	6,982; 3,100-9,870
Median body surface area, m^2; range	0.36; 0.19-0.45
Sex, male / female	7 / 5
Immunophenotype, n.	
Pro-B-ALL	9
Common ALL	1
Pre-B-ALL	2
Genetics, n.	
MLL-AF4	5
MLL-AF9	1
MLL-ENL	2
t (1,14)	1
No aberrations	3

Table 2. Descriptive statistics of serum trough ASNase concentration (U/L) *versus* day of induction.

Patient	Age* (months)	Genetics	rASNase dose (U/m^2)	Day 18 (3 days after 1st rASNase infusion)	Day 25 (3 days after 3rd rASNase infusion)	Day 33 (4 days after 5th rASNase infusion)
6	0.5	*MLL-AF4*	6,700	NS	6	BLLQ
3	1.0	*MLL-ENL*	6,700	260	140	18
11	1.0	*MLL-AF4*	6,700	280	424	58
8	2.0	*MLL-AF4*	6,700	320	81	20
9	3.4	*MLL-AF9*	6,700	42	50	23
2	5.0	-	6,700	302	368	98
5	7.0	t 1,14	7,500	209	17	29
7	8.0	*MLL-AF4*	7,500	67	145	56
1	9.0	-	7,500	187	233	129
10	9.0	*MLL-ENL*	7,500	118	111	14
4	9.2	*MLL-AF4*	7,500	120	120	36
12	12.2	-	10,000	330	240	64
geometric mean (90% CI)	6**	NA	NA	171 (117-249)	99 (52-191)	29 (15-55)

**at first infusion of rASNase; **median; BLLQ: below lower limit of quantification (2 U/L); NS: no sample; NA: not applicable.*

Influence of asparaginase treatment on glutamine levels

Besides asparagine, asparaginase is also able to cleave the amino acid glutamine to glutamic acid and ammonia, however with much less efficiency. Whereas rASNase completely depleted serum of asparagine, glutamine levels were only moderately affected with a median maximum decline of 26% on day 33 (Table 4). This was accompanied by a moderate rise of the cleavage product glutamic acid on days 18 and 25 which however returned to baseline on day 33.

Anti-ASNase antibodies

None of the infants included in this trial developed anti-ASNase antibodies during the observation period.

Remission and minimal residual disease status

All patients achieved a complete remission by protocol day 33. MRD data were available from 10 patients. Only one of these patients achieved MRD negativity on day 33.

After induction treatment, four patients were allocated to each risk group (standard risk, medium risk, high risk).

Safety

No patient developed a clinical allergic reaction to rASNase treatment.

Three of the 12 patients developed ASNase-typical adverse drug reactions [hemorrhage (nose bleeding); increased alanine aminotransferase CTC grade III; thrombosis of the superior vena cava). In another patient, the rASNase infusion had to be postponed for 1 day because of grade III febrile neutropenia.

Laboratory safety parameters showed a wide variability; changes in liver and kidney function parameters, blood cell counts and coagulation parameters were observed due to the underlying disease, general condition and chemotherapy. Changes in bilirubin, aspartate and alanine transaminases, creatinine, fibrinogen, and antithrombin III levels during induction treatment with rASNase are shown in Table 5. With regards to laboratory parameters, the only CTCAE grade III/IV adverse events observed were hypofibrinogenemia (in four patients: grade III in two and grade IV in the other two) and increased alanine transaminase (one patient with grade III).

Discussion

Due to the rarity of ALL in infants <12 months of age, only a limited number of patients were recruited into this trial, as planned. Nevertheless, some important conclusions can be drawn with respect to the efficacy and safety of treatment with a native *E.coli*-ASNase preparation during induction treatment of *de novo* ALL which was prospectively evaluated for the first time in this age group.

The treatment regimen of ASNase used in the INTERFANT-06 protocol is adequate and led to a complete depletion of asparagine for the desired time period in all patients but one. This patient had very low serum trough ASNase levels and asparagine depletion was not complete on day 33 (but on day 25). The patient was the youngest in the study: the diagnosis was made 3 days after birth.

Furthermore, this trial confirms data from another trial[4] that ASNase serum trough activity levels need not necessarily exceed the often cited cut-off level of 100 U/L to achieve complete asparagine depletion. Trough levels as low as 20 U/L seem to be sufficient for that purpose.

The trough serum ASNase activity levels observed in this trial varied widely between the patients and did not show any correlation with the administered dose or age of the individual patient. Patel *et al.* observed that the enzyme

Table 3. Patients with complete asparagine depletion during induction treatment.

Time point(s)	N. (%)	Exact 90% CI*
Day 18 (3 days after 1st rASNase infusion)**	11 (100%)	76-100%
Day 25 (3 days after 3rd rASNase infusion)	12 (100%)	78-100%
Day 33 (4 days after 5th rASNase infusion)	11 (92%)	66-100%
Day 18, day 25 and day 33	11 (92%)	66-100%

**Pearson-Clopper confidence interval. **Patient n. 6 excluded due to missing value.*

Table 4. Serum levels of glutamine and glutamic acid; geometric mean (90% CI) .

Protocol day	Glutamine (μM)	Glutamic acid (μM)
15	468 (427-514)	78 (59-103)
18	393 (339-455)	98 (74-130)
25	398 (348-454)	89 (66-120)
33	347 (304-396)	76 (58-99)

Serum levels were measured before rASNase infusions 1 (day 15 = baseline), 2, 4, and 6.

Table 5. Influence of rASNase treatment on laboratory parameters; median (Q1; Q3).

Parameter	Baseline	Day 15-21	Day 22-28	Day 29-33	Day 34-39
Bilirubin, μM	6.8 (5.1; 14.0)	7.4 (5.1; 12.0)	10.3 (5.6; 12.0)	10.0 (6.8; 15.3)	7.0 (3.4; 10.0)
AST, U/L	39.0 (31.5; 75.5)	31.0 (26.0; 34.0)	48.0 (25.0; 59.0)	42.5 (32.5; 61.5)	40.5 (24.0; 70.0)
ALT, U/L	96.0 (47.0; 132.5)	73.5 (46.0; 101.0)	54.0 (29.0; 117.0)	94.5 (45.5; 196.0)	66.0 (47.0; 106.5)
Serum creatinine, μM	17.7 (11.1; 17.8)	20.8 (16.3; 30.0)	17.7 (15.1; 23.0)	17.7 (14.1; 23.9)	17.2 (14.0; 18.3)
Fibrinogen, g/L	1.8 (1.1; 2.7)	1.3 (0.9; 1.9)	0.9 (0.8; 1.8)	1.8 (0.8; 3.5)	2.0 (1.0; 3.2)
Antithrombin III, %	113.7 (102.0; 132.6)	85.0 (77.0; 87.0)	62.0 (56.0; 70.0)	71.7 (56.0; 83.0)	73.5 (68.0; 81.2)

asparaginyl endopeptidase degrades ASNase and is very variably expressed in children with ALL, with the greatest activity observed in high-risk patients. This could be an explanation for the observed variability of ASNase trough activity levels in this trial, bearing in mind that no anti-ASNase antibodies could be detected in any of the patients.[6]

The observed ASNase activity serum levels 3 days after ASNase infusion were comparable with those recorded in older children (1 - 14 years) with *de novo* ALL treated with 5 000 U/m² rASNase every third day during induction treatment of *de novo* ALL.[4] This means that rASNase doses between 5 000 and 10 000 U/m² will lead to comparable trough ASNase serum activity levels in children.

Measured ASNase trough serum levels justify the higher doses used in infants than in older children and show that 3-day intervals are preferred over 4-day intervals.

Glutamine is another substrate for ASNase treatment but its serum levels were only slightly and temporarily reduced during treatment with rASNase. Glutamine cleavage is not, therefore, an important factor in the anti-leukemic effect of *E.coli*-ASNase.

All 12 patients were in complete remission after induction treatment, which is consistent with the results obtained in the INTERFANT-99 study (445 patients of 482 patients were in complete remission after 5 weeks of induction treatment). Treatment with rASNase was well tolerated by most patients. None of the patients developed an allergic reaction or anti-ASNase antibodies during treatment with rASNase. Antibody formation can also occur after the induction phase. We only studied the induction phase, so information on anti-ASNase antibody formation or allergies to (PEG)asparaginase in later phases of treatment are not available.

No suspected unexpected serious adverse reactions occurred within this study. All observed adverse reactions were known as possible and labeled side effects of asparagine treatment and chemotherapy.

We conclude that the asparaginase dose regimen used in infants is safe and provides complete asparagine depletion for the desired time period in nearly all patients. Measured ASNase trough serum levels justify the higher doses used in infants than in older children and show that 3-days intervals are preferred over 4-day intervals.

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Authorship and Disclosures

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