

RESEARCH ARTICLE

Efficacy and safety of recombinant *E. coli* asparaginase in children with previously untreated acute lymphoblastic leukemia: A randomized multicenter study of the Dutch Childhood Oncology Group

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Funding information

Grant sponsor: medac GmbH

Abstract

Background: The efficacy and safety of recombinant *Escherichia coli*-asparaginase (rASNase) was compared to native *E. coli* asparaginase (Asparaginase medac).

Methods: One hundred and ninety-nine children with newly diagnosed acute lymphoblastic leukemia were randomized to receive one of both agents at a dose of 5,000 U/m² during induction (eight doses) and 10,000 U/m² during the postinduction phase (only high-risk patients; standard- and medium-risk patients received pegaspargase).

Results: Median trough serum asparaginase activity levels were comparable between both groups; they ranged from 143 to 182 U/l during induction and were above the target value of 100 U/l. Complete asparagine depletion in serum was achieved in 97.9% of patients, with no significant differences between both groups. On day 33 (end of induction), only two (2%) evaluable patients in each group had measurable asparagine serum levels, and complete asparagine depletion in the cerebrospinal fluid was achieved in 98.8% and 93.6% of the patients with rASNase and Asparaginase medac, respectively. During induction, 2.1% and 5% of patients developed an allergic reaction to rASNase or Asparaginase medac, respectively. Approximately 41% of the patients in both groups had a clinical allergy or enzyme inactivation to the first dose of any asparaginase preparation in postinduction. A comparable proportion of patients in both groups developed anti-asparaginase antibodies (57%) during repeated administration of asparaginase. Minimal residual disease levels at the end of induction, 5-year event-free survival, and 5-year cumulative incidence of relapse did not differ between both groups.

Conclusion: The efficacy, safety, and immunogenicity of both asparaginase preparations are comparable. This trial was registered at www.clinicaltrials.gov as #NCT00784017; EudraCT number 2006-003180-31.

KEYWORDS

efficacy, pediatric acute lymphoblastic leukemia, randomized trial, recombinant asparaginase, toxicity

1 | INTRODUCTION

Asparaginase (ASNase) represents an essential treatment component for patients with acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma.^{1–4} During the last decades, a shortage of supply of this essential drug has occasionally occurred in several countries.⁵ Recombinant *Escherichia coli*-ASNase (rASNase) was developed to ensure the long-term supply of this important drug. The amino acid sequence and *in vitro* enzymatic characteristics of rASNase are identical to other approved *E.coli*-ASNase preparations such as Asparaginase medac or ElsparTM. In contrast to other ASNase preparations, the specific formulation of rASNase results in an isotonic preparation with negligible amounts of higher aggregates which might result in less hypersensitivity reactions. ASNase is a foreign protein of bacterial origin and therefore anti-ASNase antibodies (AAAs) may develop during repeated administration. This causes hypersensitivity reactions, which are important problems associated with the administration of ASNase. Such hypersensitivity reactions may or may not be accompanied by clinical symptoms. Sometimes, patients develop AAAs without clinical symptoms but show accelerated ASNase inactivation/elimination. This is called silent inactivation.⁶ On the other hand, patients may show allergic-like reactions without having developed detectable AAAs.^{7,8} In a phase 2 study, pharmacokinetic bioequivalence between rASNase and Asparaginase medac could be demonstrated.⁹ The current study aimed to demonstrate the pharmacodynamic equivalence of both ASNase preparations, and to provide a deeper insight into the efficacy and safety (especially immunogenicity) of rASNase.

The optimal therapeutic effects of ASNase depend on a complete and sustained depletion of asparagine (ASN) levels in serum.¹⁰ Therefore, this parameter was used as the primary study endpoint. The trial was embedded into the ALL-10 protocol of the Dutch Childhood Oncology Group (DCOG).¹¹

2 | METHODS

2.1 | Patients and treatment

rASNase was developed by medac GmbH (Wedel, Germany) and Wacker Biotech GmbH (Jena, Germany). The final formulation was designed by medac. rASNase (Spectrila[®]) has recently been approved by the European Medicines Agency for the treatment of ALL in patients of all age groups. Asparaginase medac is produced by Kyowa-Hakko Kirin, Tokyo, Japan.

One hundred and ninety-nine consecutive children with previously untreated ALL participated in the study between October 2008 and February 2012 at seven hospitals in the Netherlands. The Institutional Review Board approved this study before patient enrollment. Informed consent was obtained from the parents or guardians and from patients ≥ 12 years of age. This study was conducted in accordance with the Declaration of Helsinki.

All children participated in the DCOG ALL-10 trial and received induction chemotherapy consisting of prednisolone (60 mg/m²; days 1–29, tapering off till day 36), vincristine (1.5 mg/m²; days 8, 15, 22,

and 29), daunorubicin (30 mg/m²; days 8, 15, 22, and 29), *E.coli*-ASNase (5,000 U/m²; days 12, 15, 18, 21, 24, 27, 30, and 33), and intrathecal therapy.¹¹

Patients who fulfilled the inclusion/exclusion criteria were randomized to rASNase or Asparaginase medac in a double-blind fashion prior to the first ASNase administration. Both drugs were administered intravenously over 1–2 hr. During postinduction treatment, only patients assigned to the high-risk group received further infusions of rASNase or Asparaginase medac according to their primary randomization at a dose of 10,000 U/m² on days 22, 25, 29, and 32 of blocks HR1, HR2, HR4, and HR5, and days 8, 11, 15, and 18 of protocol II.

Patients in the standard- and medium-risk group received one and 15 infusions of pegaspargase (2,500 U/m²/dose), respectively, during postinduction treatment.¹¹

If a patient experienced a severe (Common Terminology Criteria for Adverse Events Grade III/IV) systemic allergic reaction to rASNase or Asparaginase medac, treatment with both ASNase preparations had to be stopped. Treatment could be continued with pegaspargase or *Erwinia*-ASNase. ASNase activity levels were not measured in real time; as a consequence, switching preparation in patients with silent inactivation was not possible.

Before the first (baseline) and every second administration of rASNase or Asparaginase medac in induction and postinduction, blood samplings were performed and analyzed for serum levels of ASNase activity, amino acids (ASN, glutamine [GLN], aspartic acid [ASP], and glutamic acid [GLU]), as well as AAAs. In standard- and medium-risk groups, blood samples were drawn before and 7 or 14 days after the first dose of pegaspargase during postinduction treatment. At days 1 and 33 of induction treatment, cerebrospinal fluid (CSF) samples were drawn for determination of amino acid levels.

2.2 | Blood and CSF sample processing guidelines

Blood and CSF sample processing has been described in detail previously.⁹ In brief, samples were immediately put on ice and transferred to the laboratory for further processing. Samples for amino acid determination were deproteinized with sulphosalicylic acid to stop *ex vivo* enzymatic activity. The tubes with the processed samples were stored at -80°C until analysis.

2.3 | Analytical assays

The determination of ASNase activity and amino acids in serum and CSF was carried out by CRS Clinical Research Services Mannheim GmbH (Grünstadt, Germany).

Serum and CSF levels of ASNase activity were determined with a sensitive microplate reader-based method (aspartic acid β -hydroxamate (AHA)-assay; lower limit of quantification [LLQ] 2.5 U/l).¹²

Serum and CSF levels of the amino acids ASN, ASP, GLN, and GLU were analyzed by using an RP-HPLC method. The LLQ of this assay for ASN in serum and CSF samples was 0.5 μM .

AAAs (IgG/IgM) were measured by two indirect ELISAs using the drugs Asparaginase medac or rASNase immobilized to the solid

phase.¹³ The assays quasiquantitatively detect the presence of AAAs in arbitrary units per milliliter (AU/ml). AAAs against rASNase and Asparaginase medac were determined during induction and postinduction treatment.

A blood sample was considered AAA-positive if at least one of the two values (antibody against rASNase or Asparaginase medac) was 6.25 AU/ml or above. This “worst-case” approach was chosen to increase the sensitivity of AAA evaluation.

If enough serum was available, the AAA-positive samples were tested for their enzyme-neutralizing capacity. The analytical assay is described in the Supplementary Methods.

2.4 | Efficacy assessment

The efficacy of treatment was determined by evaluating the complete remission (CR) rate and minimal residual disease (MRD) status at protocol day 33 (after induction phase IA), as well as relapse-free and event-free survival (EFS) (definitions in the Supplementary Methods).

2.5 | Statistics

The primary objective of this trial was to demonstrate the noninferiority of rASNase compared to Asparaginase medac, with respect to the rate of patients with complete ASN depletion in serum during induction treatment.

Complete ASN depletion was defined as ASN level below the lower LLQ (0.5 μm) in each measurement (always immediately before ASNase infusions 2, 4, 6, and 8). If a treatment delay between two consecutive ASNase infusions of more than 4 days occurred, the blood sample taken directly prior to the delayed ASNase infusion was not considered to be evaluable. The primary endpoint was evaluable if ASN levels were available for at least three of the four scheduled time points.

For confirmatory analysis, the exact two-sided 95% confidence interval (CI) based on Chan and Zhang for the difference between complete ASN depletion rates (rASNase minus Asparaginase medac) was provided. rASNase was deemed to be not less effective than Asparaginase medac if the lower limit of this CI was greater than the prespecified noninferiority margin of -10% . The unconditional exact test of noninferiority for binomial differences based on restricted maximum likelihood estimates was applied to yield the one-sided P -value. Statistical significance was claimed if the resulting P -value was less than the prespecified significance level of 0.025.

AAAs during the repeated administration of ASNase were described using standard methods for descriptive data analysis.

EFS was computed from diagnosis to the first event or last follow-up. Events were defined as relapse, secondary malignancy, or death. The Kaplan–Meier methodology was used to estimate EFS. The cumulative incidence of relapse (CIR) was computed using a competing-risk model, with death as the competing event. EFS and CIR analyses were done by intention-to-treat. EFS and CIR curves were compared using the log-rank test.

SAS (SAS Institute Inc., Cary, NC, USA) Version 9.2 on Windows platform was used for statistical analysis. Unconditional exact CIs

TABLE 1 Patient characteristics

Parameter	rASNase	Asparaginase medac
Number of patients	98	101
Median age, years; range	5; 1–17	4; 1–16
Median body surface area, m ² ; range	0.85; 0.4–2.1	0.73; 0.4–1.9
Sex: male/female	54/44	53/48
At diagnosis		
Median white blood cells ($\times 10^9/\text{l}$); range	11.6; 1.1–573	12.9; 0.1–695
Median peripheral blasts (%); range	34.5; 0–97	57.5; 0–96
Median marrow blasts (%); range	90; 26–99	91; 28–100
Immunophenotype (%)		
Pro-B-ALL	1.0	3.0
Common ALL	63.3	57.4
Pre-B-ALL	22.4	25.7
T-ALL	12.2	13.9
MPAL ^a	1.0	0
Genetics (%)		
No aberrations or not yet defined	45.9	45.5
Hyperdiploid	28.6	27.7
TEL-AML 1	16.3	14.9
TEL-AML 1, hyperdiploid	1.0	1.0
BCR-ABL	0	1.0
MLL-AF4	0	1.0
Other	8.2	8.9
Risk group at start of postinduction (%)		
Standard risk	24.5	23.8
Medium risk	61.2	62.4
High risk	8.2	7.9
Not applicable ^b	6.1	5.9

^aMPAL, Mixed-phenotype acute leukemia

^bPatients withdrawn from study before postinduction treatment.

were calculated with StatXact Version 9.0 (Cytel software Corporation, Cambridge).

3 | RESULTS

3.1 | Patients characteristics

One hundred and ninety-nine children with ALL (107 male and 92 female) participated in this study. 192 patients completed induction treatment and 187 patients entered the postinduction phase. Further patient characteristics are shown in Table 1.

3.2 | ASN depletion in serum and CSF in induction

rASNase was shown to be significantly noninferior ($P = 0.0019$) to the reference ASNase (Asparaginase medac) with respect to the complete

ASN depletion rate in serum during the induction phase (Table 2). Only two patients in each group had measurable ASN levels in serum at least at one time point. This was due to the development of AAAs and silent ASNase inactivation in three patients, but in one patient (no AAAs and sufficient ASNase activity) the reason was unclear. Four patients in the reference group and five patients in the rASNase group were not evaluable. Complete ASN depletion in CSF on day 33 of the induction phase was achieved in most patients of both groups (Table 2). More patients (6.4% vs. 1.2%) in the reference group had incomplete ASN depletion on day 33 in CSF.

3.3 | ASNase activity levels in serum

Median trough ASNase activity levels during induction were comparable between both groups; they ranged from 143 to 182 U/I and were, therefore, always above the target value of 100 U/I (Table 2).

A comparable number of patients had ASNase activities ≥ 100 U/I in all serum samples taken from day 15 to day 33 during induction treatment (65.9% in the reference group and 62.2% in the rASNase group). Table 2 shows the number and proportion of patients with any ASNase activity in serum lower than 100 U/I during the induction phase, from day 15 to day 33.

Since only two patients in each group had measurable ASN values, these data suggest that ASNase activities in serum lower than 100 U/I are sufficient to achieve a complete ASN depletion.

The median ASNase activity in CSF on day 33 was below the lower LLQ in both treatment groups. Only three samples (two in the reference group and one in the rASNase group) contained measurable amounts of ASNase activity.

Median trough serum ASNase activity levels in high-risk patients during postinduction were slightly higher compared to induction treatment, probably due to the higher dose (10,000 vs. 5,000 U/m², Supplementary Table S1).

3.4 | Influence of ASNase treatment on levels of other amino acids

The depletion of ASN in serum is the desired pharmacodynamic effect of ASNase treatment. As a result, levels of the amino acid ASP increased accordingly (approximately four-fold). Besides ASN, ASNase is also able to cleave the amino acid GLN to GLU and ammonia, although with much less efficiency.

GLN serum levels did not change remarkably after treatment with rASNase. Nevertheless, a continuous turnover of GLN occurred during treatment as indicated by a rise of the cleavage product GLU (Table 2).

CSF levels of the amino acids ASP, GLN, and GLU did not change significantly during treatment with both ASNase preparations.

3.5 | Response after induction phase

In each of the groups, two patients (2.0%) were not in CR. The MRD status after the induction phase was negative in about 30% of patients in both groups (Supplementary Table S2).

TABLE 2 Asparagine levels in serum and CSF, and serum asparaginase/glutamine/glutamic acid levels during induction treatment

	rASNase	Asparaginase medac
<i>Asparagine levels</i>		
Serum		
Number of evaluable patients	93 (100%)	97 (100%)
Mean (SD) ASN level at baseline, μm	44.5 (9.8)	43.6 (11.6)
Complete ASN depletion (ASN < 0.5 μm)		
Yes	91 (97.8%)	95 (97.9%)
No	2 (2.2%)	2 (2.1%)
Difference (95% CI ^a)	-0.1 (-5.67%; 5.31%)	
P-value ^b	0.0019	
CSF		
Number of evaluable patients	82 (100%)	94 (100%)
Mean (SD) ASN level at baseline, μm	4.14 (1.38)	3.99 (1.18)
Complete ASN depletion (ASN < 0.5 μm)		
Yes	81 (98.8%)	88 (93.6%)
No	1 (1.2%)	6 (6.4%)
Difference (95% CI ^a)	5.2% (-0.87%; 12.19%)	
P-value ^c	0.086	
<i>Asparaginase levels^d</i>		
Day 15	162 (115; 258)	143 (96; 231)
Day 21	177 (115; 268)	167 (108; 249)
Day 27	177 (97; 256)	182 (132; 261)
Day 33	173 (95; 255)	180 (118; 287)
Number of evaluable patients, n (%)	90 (100)	91 (100)
<100 U/l	34 (37.8)	31 (34.1)
<50 U/l	11 (12.2)	7 (7.7)
<25 U/l	8 (8.9)	4 (4.4)
<i>Glutamine, μm^{e}</i>		
Baseline	432 (97)	411 (88)
Day 15	384 (96)	364 (90)
Day 21	375 (106)	363 (115)
Day 27	410 (143)	421 (146)
Day 33	416 (150)	403 (118)
<i>Glutamic acid, μm^{e}</i>		
Baseline	42 (23)	42 (19)
Day 15	57 (28)	57 (27)
Day 21	59 (30)	59 (49)
Day 27	67 (56)	65 (33)
Day 33	68 (34)	75 (45)

^aUnconditional exact confidence interval based on Chan and Zhang.

^bUnconditional exact test of noninferiority for binomial differences based on restricted maximum likelihood estimates.

^cUnconditional exact test for binomial differences.

^dMedian (25%; 75% percentile).

^eMean (SD).

SD, standard deviation.

TABLE 3 Patients with allergic reactions during induction treatment

Patient number	Day of protocol	Clinical symptoms	CTCAE grade	AAA during induction	Action taken ^a	Observations in postinduction phase
rASNase treatment						
137	24	Flushing	I	Negative	ASNase infusion temporarily interrupted	Silent inactivation after first pegaspargase dose; AAA-positive
145	30, 33	Rash, flushing, urticaria, dyspnea	II	Positive (day 33)	ASNase infusion on day 33 stopped	Silent inactivation after first pegaspargase dose; AAA-positive
Asparaginase medac treatment						
104	15	Flushing, dyspnea	II	Negative	ASNase infusion stopped; switch to pegaspargase at day 18	No further data available
123	33	Urticaria, hypotension	II	Negative	ASNase infusion stopped	Silent inactivation after first pegaspargase dose; AAA-positive
159	24	Rash	II	Negative	None	Weakly AAA-positive after first pegaspargase dose, but sufficient ASNase activity
193	12	Dyspnea	II	Negative	Switch to pegaspargase at day 15	No further data available
205	27	Flushing, rash	II	Negative	ASNase infusion temporarily interrupted	No silent inactivation after first pegaspargase dose; AAA-negative

^aGrade II allergic reactions were usually treated with clemastine/hydrocortisone. CTCAE, Common Terminology Criteria for Adverse Events.

3.6 | CIR and EFS

Median follow-up was 56 months in the rASNase group and 57 months in the Asparaginase medac group. Eight first events occurred in the rASNase group (three deaths in induction, one death in CR, three relapses, and one secondary malignancy) and four events occurred in the Asparaginase medac group (one death in CR and three relapses).

Kaplan–Meier estimates of EFS at 5 years were 91.0% (standard error (SE) 3.2) in the rASNase group and 97.0% (SE 1.7) in the Asparaginase medac group ($P = 0.228$). Estimates of 5-year CIR were 4.1% (SE 2.4) in the rASNase group and 2.0% (SE 1.4) in the Asparaginase medac group ($P = 0.996$) (Supplementary Figure S1).

3.7 | Immunogenicity

During induction treatment, two patients (2.1%) in the rASNase group and five patients (5.0%) in the reference group experienced an allergic reaction during or up to 12 hr after ASNase infusion. One patient experienced an allergic reaction to the first ASNase dose. The other six patients had sufficient enzyme activity during induction treatment up to the event. AAAs were detected in only one of these seven patients. However, at least four of these patients developed AAAs prior to the first dose of pegaspargase in postinduction treatment, accompanied by insufficient ASNase activity with or without allergic reactions (Table 3).

Before induction treatment, four of 101 (4.0%) patients in the Asparaginase medac group and one of 97 (1.0%) patients in the rASNase group were weakly AAA-positive (maximum 35.2 AU/ml). These weakly positive AAA levels at baseline did not have an impact on ASNase efficacy during induction treatment. Two of these five patients had increased AAA levels at baseline before postinduction treatment, followed by complete enzyme inactivation.

Up to day 33 of induction treatment, about 10% of patients were measured weakly positive for AAAs at least at one time point (nine

patients in the ASNase medac group and 10 patients in the rASNase group). From these 19 patients, three had an incomplete ASN depletion on day 33 of induction treatment, 16 (84.2%) were AAA-positive at baseline before postinduction treatment, and 15 (78.9%) had no or very low ASNase serum activity after administration of the first ASNase dose in the postinduction phase.

Most antibodies to the ASNase protein developed in the interval between day 33 of induction treatment and the first ASNase dose in the postinduction phase. No differences were detected between both treatment arms.

Hypersensitivity reactions to the first dose of any ASNase preparation during postinduction were observed in 36 evaluable patients (41.9%) receiving rASNase, and in 37 patients (41.1%) receiving Asparaginase medac. Hypersensitivity reactions were defined as any clinical allergic reaction occurring during or up to 12 hr after the first ASNase infusion during the postinduction phase or the silent inactivation of ASNase activity. A total of 69 of all 73 hypersensitive patients were also tested positive for AAAs. Of these, 65 patients were already AAA-positive before administration of the first ASNase dose during the postinduction phase.

Additionally, 22 patients in the rASNase group and 16 patients in the reference group were AAA-positive before and/or after the first ASNase dose in postinduction treatment, but did not show signs of hypersensitivity (allergy or silent ASNase inactivation) or were not evaluable for hypersensitivity status. However, these patients had lower AAA levels compared to the hypersensitive patients (Table 4) and showed no or less increase in AAA reactivity after the first dose.

Overall, 234 serum samples in a total of 113 patients were tested positive for AAAs within this clinical trial. If possible (sufficient serum still available), these samples were additionally tested for their enzyme-neutralizing capability. About two-thirds of the AAA-positive samples contained ASNase-neutralizing antibodies and this did not differ between both groups (Table 5).

TABLE 4 Median antibody levels (AU/ml) in AAA-positive patients before and after the first ASNase dose in postinduction

Group	rASNase			Asparaginase medac		
	N	Before	After	n	Before	After
Standard risk						
Hypersensitive	9	52	1,409	10	197	1,930
Not hypersensitive	5	<6.25	31	3	13	114
Medium risk						
Hypersensitive	26	97	297	22	100	338
Not hypersensitive	14	27	23	11	30	19
High risk						
Hypersensitive	1	40	No data	5	75	No data
Not hypersensitive	1	12	<6.25	1	40	<6.25

3.8 | Adverse events

The number of patients with predefined ASNase-typical adverse events was documented. The results of induction treatment are shown in Table 6. No significant differences in the frequency of these events between both treatment groups could be detected. None of the patients truncated their ASNase treatment because of adverse events other than hypersensitivity.

Both ASNase preparations showed a comparable change in coagulation parameters. As expected, fibrinogen and antithrombin III were significantly reduced during treatment with ASNase. Serum bilirubin levels slightly increased during treatment with ASNase. Mean serum levels of transaminases were already above the upper limit of normal (ULN) at baseline and did not deteriorate further during ASNase treatment. Mean serum levels of amylase and creatinine were always below ULN. Mean serum glucose and protein levels slightly decreased during ASNase treatment (Supplementary Table S3).

4 | DISCUSSION

Both ASNase preparations led to a complete ASN depletion in serum in nearly all the patients during induction treatment. Noninferiority of rASNase versus the reference ASNase could be demonstrated with a highly significant *P*-value of 0.0019, with an absolute difference in complete ASN depletion in serum of only −0.1% and an associated 95% CI of −5.67 to 5.31%. Also, the efficacy of both the products did not differ, as shown by comparable MRD levels at the end of induction and comparable long-term EFS.

Furthermore, most patients achieved a complete ASN depletion in CSF on day 33 of induction treatment. It has been suggested that this is one advantage compared to pegaspargase, which often shows incomplete ASN depletion in this compartment, but large studies are lacking.^{14,15}

Serum trough levels of ASNase activity higher than 100 U/l have been suggested to correlate with a complete ASN depletion in serum and CSF. This threshold goes back to an early paper by Riccardi et al., in which it was demonstrated in rhesus monkeys and seven patients that this level always correlated with complete ASN depletion in CSF.¹⁶ Therefore, this threshold is based on a very limited sample size and no statistical calculations. Nevertheless, most study groups have used this threshold for the development of dose regimens for various ASNase preparations between the years 1980 and 2000, mostly referencing to this publication. Our clinical trial has confirmed that all serum samples which contain more than 100 U/l ASNase activity correlated with a complete ASN depletion in serum and CSF. However, our data also show that a complete ASN depletion in serum and CSF can be even obtained with serum trough levels as low as 10 or 20 U/l ASNase activity. This has also been confirmed by other study groups.^{17–19} From the results of the Italian AIEOP ALL 95 study, Rizzari et al. concluded that ASN levels may also be undetectable in patients with ASNase trough activity levels < 50 U/l, challenging the opinion that an activity level of at least 100 U/l is needed to obtain ASN depletion.²⁰ Nevertheless, a serum trough activity of 100 U/l should always be targeted to be on the safe side.

The results of our study confirm that a dose of 5,000 U/m² of rASNase or Asparaginase medac administered every third day is sufficient to guarantee a complete ASN depletion in serum and CSF. Whether the empirical use of a higher dose (10,000 U/m²) in postinduction treatment is really necessary is, at least, questionable. However, due to the small number of high-risk patients treated with this dose in this trial, no final conclusions can be drawn.

GLN is another substrate for ASNase. With the dose regimen of 5,000 U/m² ASNase every third day, no significant reduction of GLN serum levels were seen. These data suggest that the desired ASN-depleting effect of ASNase in serum and CSF can be achieved without significant GLN reduction.

ASNase can induce both neutralizing and non-neutralizing antibodies, and antibodies of different immunoglobulin classes (IgG, IgM). These antibodies may induce allergic reactions, inactivate the enzyme, or accelerate its elimination.^{7,21} Only 19 of 199 patients were positive for AAAs during induction treatment. However, most of these patients showed a strong increase in AAA levels before the start of

TABLE 5 Proportion of AAA-positive samples/patients with ASNase-neutralizing capability

Treatment group	Samples		Patients	
	rASNase	ASNase medac	rASNase	ASNase medac
N	110	124	58	55
Neutralizing AAAs (%)	69.1	65.3	91.4	87.3
Non-neutralizing AAAs (%)	25.5	29.8	8.6	9.1
No data (%)	5.5	4.8	–	3.6

TABLE 6 Frequency of patients with ASNase-typical adverse events during induction treatment

Study group	rASNase	Asparaginase medac	P-value ^b
Number of patients, n (%)	97 (100)	101 (100)	
Patients with any ASNase-typical adverse events	47.4%	50.5%	0.7191
Difference rASNase – ASNase medac (95% CI ^a)		–3.1% (–17.10; 11.01)	
Allergic reaction within 12 hr after ASNase infusion	2.1%	5.0%	
Liver enzymes ≥ CTCAE Grade III			
Any event	44.3%	39.6%	0.5322
Alanine aminotransferase (ALT) increased	43.3%	35.6%	0.2864
Hyperbilirubinemia	10.3%	10.9%	0.9289
Aspartate aminotransferase (AST) increased	7.2%	6.9%	0.9954
Pancreatitis ≥ CTCAE Grade II	–	1.0%	
Hemorrhage or thromboembolism ≥ CTCAE Grade II			
Any event	2.1%	4.0%	0.5322
Thrombosis/thrombus/embolism	2.1%	2.0%	1.0000
CNS hemorrhage	–	1.0%	
Hematoma	–	1.0%	
Neurotoxicity ≥ CTCAE Grade III			
Any event	4.1%	5.9%	0.5997
Syncope (fainting)	3.1%	1.0%	0.3239
Seizure	–	2.0%	
Dizziness	1.0%	–	
Involuntary movement	–	1.0%	
Mood alteration (agitation)	1.0%	–	
Neuropathy-motor	–	1.0%	
Somnolence	–	1.0%	

^aUnconditional exact confidence interval based on Chan and Zhang.^bUnconditional exact Barnard test.

CTCAE, Common Terminology Criteria for Adverse Events.

postinduction ASNase treatment. Furthermore, 15 (79%) of these patients had silent ASNase inactivation after administration of the first ASNase dose in the postinduction phase, in contrast to 38.7% for the total study population (65 of 168 evaluable patients). These data show that patients with AAA during induction treatment are at a higher risk of developing increased AAA levels prior to postinduction treatment. These increased AAA levels come along with the early inactivation of ASNase activity after the next dose of ASNase.

Most AAAs developed in the ASNase-treatment-free time gap between day 33 (week 5) of induction treatment until the start of postinduction ASNase treatment in week 13 (high-risk group) or 20 (standard- and medium-risk group). To overcome this problem, a possible approach could be to avoid ASNase-free treatment periods. This strategy is currently being explored for medium-risk patients in the ongoing ALL-11 protocol of the DCOG (EudraCT 2012-000067-25).

Our antibody assays were able to detect nearly all patients who experienced an allergic reaction or silent inactivation of ASNase activity. Sixty-five of the 73 (89%) patients with hypersensitivity reactions to the first postinduction ASNase infusion were already AAA-positive before the start of postinduction treatment. An additional 38 patients (11 standard-risk patients and 27 medium-risk patients) were tested weakly positive for AAAs at this time point but did not show an aller-

gic reaction or silent ASNase inactivation. AAA measurements in these patients could be considered as false positive; however, it has to be noticed that the pegaspargase that has been used in these patients during this treatment phase may be protected against the binding of AAAs. The data (Table 4) suggest that higher AAA titers are necessary to overcome the effect of the protective poly(ethylene glycol) shield in the pegaspargase molecule. Of these 38 patients, 22 could fulfill their complete course of pegaspargase treatment, but 16 patients switched to *Erwinia*-ASNase later because of clinical allergies.

Both ASNase preparations were similarly effective with respect to CR rate and MRD status at the end of induction treatment. The ASNase trough activities in serum were not significantly different between patients with negative and positive MRD status. Furthermore, relapse- and event-free survival was comparable in both groups. Both preparations show pharmacodynamic equivalence, and comparable toxicity and efficacy. Therefore, there is no clinical advantage of one of the preparations. However, the long-term supply of ASNase will be ensured with the new manufacturing process of rASNase.

In conclusion, the pharmacodynamic equivalence of rASNase and Asparaginase medac could be demonstrated in this clinical study. The applied dose regimen produced sufficient ASNase activities in serum and was accompanied with a complete ASN depletion in serum and

CSF in the majority of patients. Most of the hypersensitive patients could be detected early with the antibody assays. The majority of AAA-positive samples had enzyme-neutralizing effects. MRD levels and long-term EFS and CIR did not differ between both treatment groups.

ACKNOWLEDGMENTS

This work was supported by a grant from medac GmbH (Wedel, Germany), the sponsor of this clinical protocol. The authors would like to thank CRS Clinical Research Services Mannheim GmbH, Grönstadt, Germany for the measurement and analysis of pharmacokinetic/pharmacodynamic data.

CONFLICT OF INTEREST

R.P., I.v.d.S., M.t.L., W.J.E.T., C.v.d.B., G.J.L.K., M.B., W.J.W.K., and H.d.G.-K. declare no competing financial interests. H.-J.K., U.P., and T.K. are employees of medac GmbH.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: van der Sluis I, de Groot-Kruseman H, te Loo M, et al. Efficacy and safety of recombinant *E. coli* asparaginase in children with previously untreated acute lymphoblastic leukemia: a randomized multicenter study of the Dutch Childhood Oncology Group. *Pediatr Blood Cancer*. 2018;e27083. <https://doi.org/10.1002/pbc.27083>.

SUPPLEMENTARY FILE

SUPPLEMENTARY METHODS

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The NAb assay cut-point was determined using 50 drug-naïve baseline study sera (applying outlier exclusion and statistical calculations allowing about 5% false-positive results). Samples showing an ASNase activity reduction at least to the cut-off value were assessed as neutralizing, such with a lower reduction as non-neutralizing. Hemolytic samples assessed as neutralizing had to be excluded from NAb evaluation due to observed inhibitory effects of hemolysis on ASNase activity. Lipemic samples were found to decrease NAb assay sensitivity due to their turbidity causing elevated absorption. NAb detection with this assay was also hampered by the presence of active drug. Due to the high immunological cross-reactivity between rASNase and Asparaginase medac the NAb assay is capable of detecting both neutralizing anti-rASNase and anti-Asparaginase medac antibodies. By means of titration (serial 1:2 dilution steps in sample

diluent) the neutralizing capacity of those samples containing neutralizing antibodies was determined in a second test run (NAb endpoint titer = last dilution assessed neutralizing).

Definitions complete remission and MRD negativity

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 and CSF, no other documented extramedullary leukemia with the exception of testicular enlargement, and regenerating hematopoiesis.

MRD status was determined by clonal immunoglobulin H (IgH), T-cell receptor delta (TCRdelta), and TCRgamma gene rearrangements with polymerase chain reaction (PCR) on day 33. MRD negativity was defined as MRD < 10^{-4} with two MRD-PCR targets.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table S1. ASNase activity in serum during post-induction treatment in high-risk patients

Time point	rASNase	Asparaginase medac
HR block 1		
<i>Before 2nd ASNase dose</i>		
Number of patients	7	5
ASNase activity < 2.5 U/L	0	2
Median (range) ASNase activity*, U/L	229 (60-471)	257 (256-306)
<i>Before 4th ASNase dose</i>		
Number of patients	6	4
ASNase activity < 2.5 U/L	0	2
Median (range) ASNase activity*, U/L	158 (80-476)	157 (134-180)
HR block 2		
<i>Before 2nd ASNase dose</i>		
Number of patients	6	2
ASNase activity < 2.5 U/L	0	1
Median (range) ASNase activity*, U/L	200 (123-491)	84
<i>Before 4th ASNase dose</i>		
Number of patients	6	3
ASNase activity < 2.5 U/L	0	1
Median (range) ASNase activity*, U/L	68 (39-348)	146 (143-149)
* calculated for patients with ASNase activity levels > 2.5 U/L only		

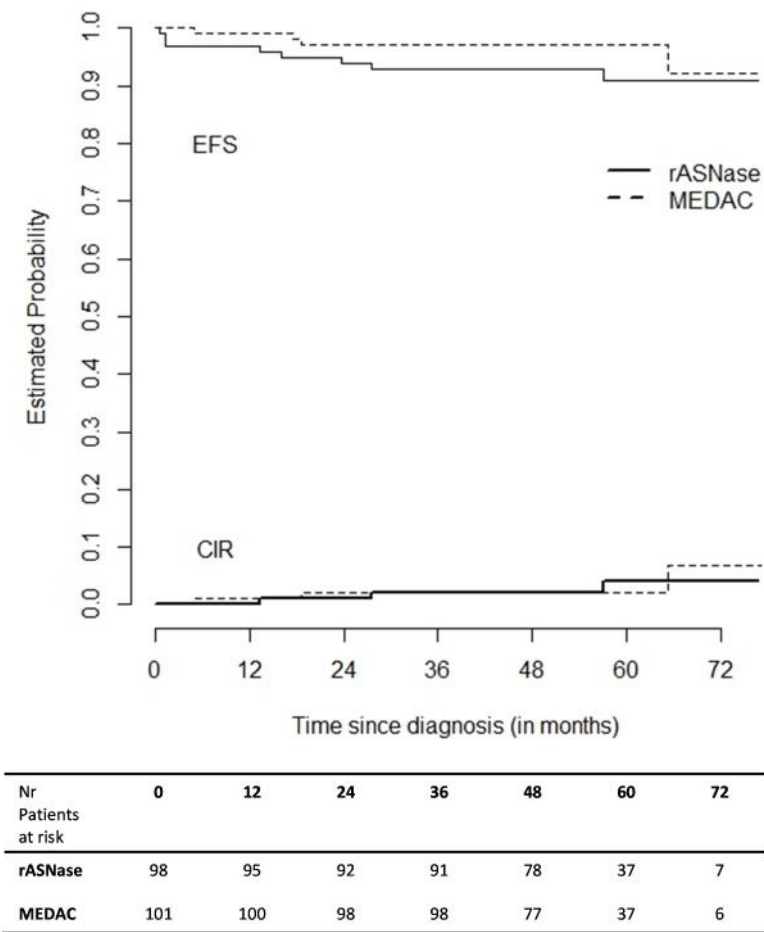
Supplementary Table S2 . CR and MRD status at end of induction treatment

Treatment group	rASNase	Asparaginase medac
Complete remission		
<i>Number of evaluable patients</i>	92	99
<i>Yes</i>	90 (97.8%)	97 (98.0%)
<i>No</i>	2 (2.2%)	2 (2.0%)
MRD status		
<i>Number of evaluable patients</i>	92	92
<i>MRD negative</i>	29 (31.5%)	32 (34.8%)
<i>MRD positive</i>	63 (68.5%)	60 (65.2%)

Supplementary Table S3. Laboratory parameters during induction treatment

Parameter	Baseline	Week 2	Week 3	Week 4	Week 5	Week 6
Fibrinogen, N-fold lower limit of normal; mean $\pm$ SD						
rASNase	1.00 $\pm$ 0.47	0.73 $\pm$ 0.52	0.54 $\pm$ 0.31	0.53 $\pm$ 0.34	0.67 $\pm$ 0.57	2.42 $\pm$ 1.00
ASNase medac	0.93 $\pm$ 0.51	0.62 $\pm$ 0.30	0.60 $\pm$ 0.43	0.55 $\pm$ 0.48	0.67 $\pm$ 0.58	2.45 $\pm$ 1.14
Antithrombin III, N-fold lower limit of normal; mean $\pm$ SD						
rASNase	1.68 $\pm$ 0.28	1.20 $\pm$ 0.24	0.97 $\pm$ 0.22	0.90 $\pm$ 0.24	0.86 $\pm$ 0.26	1.36 $\pm$ 0.33
ASNase medac	1.67 $\pm$ 0.29	1.18 $\pm$ 0.26	0.92 $\pm$ 0.26	0.87 $\pm$ 0.32	0.88 $\pm$ 0.33	1.35 $\pm$ 0.34
Bilirubin total, N-fold upper limit of normal; mean $\pm$ SD						
rASNase	0.66 $\pm$ 0.37	0.99 $\pm$ 0.63	1.13 $\pm$ 0.80	1.06 $\pm$ 0.91	1.51 $\pm$ 1.69	0.86 $\pm$ 0.73
ASNase medac	0.61 $\pm$ 0.43	0.99 $\pm$ 0.74	1.21 $\pm$ 0.86	1.34 $\pm$ 2.01	1.28 $\pm$ 1.10	0.91 $\pm$ 1.01
AST, N-fold upper limit of normal; mean $\pm$ SD						
rASNase	1.40 $\pm$ 1.46	0.76 $\pm$ 0.61	0.72 $\pm$ 0.61	1.12 $\pm$ 2.20	1.32 $\pm$ 2.78	1.72 $\pm$ 2.09
ASNase medac	1.39 $\pm$ 1.84	0.76 $\pm$ 0.76	0.67 $\pm$ 0.62	0.99 $\pm$ 2.56	0.94 $\pm$ 0.71	1.95 $\pm$ 2.62
ALT, N-fold upper limit of normal; mean $\pm$ SD						
rASNase	4.94 $\pm$ 5.77	4.32 $\pm$ 4.33	2.44 $\pm$ 2.24	3.07 $\pm$ 5.75	2.55 $\pm$ 3.45	3.65 $\pm$ 2.09
ASNase medac	4.22 $\pm$ 4.88	3.54 $\pm$ 3.72	2.15 $\pm$ 2.19	2.69 $\pm$ 6.79	2.44 $\pm$ 3.49	3.33 $\pm$ 5.59
Amylase, N-fold upper limit of normal; mean $\pm$ SD						
rASNase	0.61 $\pm$ 0.32	0.67 $\pm$ 0.56	0.70 $\pm$ 0.34	0.66 $\pm$ 0.30	0.56 $\pm$ 0.27	0.57 $\pm$ 0.27
ASNase medac	0.64 $\pm$ 0.33	0.63 $\pm$ 0.25	0.66 $\pm$ 0.27	0.71 $\pm$ 0.70	0.55 $\pm$ 0.33	0.55 $\pm$ 0.29
Creatinine, N-fold upper limit of normal; mean $\pm$ SD						
rASNase	0.46 $\pm$ 0.22	0.50 $\pm$ 0.26	0.48 $\pm$ 0.22	0.47 $\pm$ 0.23	0.41 $\pm$ 0.20	0.38 $\pm$ 0.19
ASNase medac	0.45 $\pm$ 0.19	0.47 $\pm$ 0.20	0.46 $\pm$ 0.20	0.52 $\pm$ 0.79	0.40 $\pm$ 0.20	0.37 $\pm$ 0.18
Glucose in serum, mM; mean $\pm$ SD						
rASNase	6.18 $\pm$ 1.39	6.30 $\pm$ 5.36	5.32 $\pm$ 1.52	5.45 $\pm$ 1.68	4.72 $\pm$ 1.58	5.23 $\pm$ 1.62
ASNase medac	6.82 $\pm$ 2.37	5.93 $\pm$ 3.04	5.70 $\pm$ 2.78	5.96 $\pm$ 3.13	4.58 $\pm$ 1.59	5.08 $\pm$ 0.88
Protein in serum, g/L; mean $\pm$ SD						
rASNase	65.7 $\pm$ 6.0	59.3 $\pm$ 5.0	52.9 $\pm$ 4.8	49.4 $\pm$ 5.5	48.6 $\pm$ 5.4	53.9 $\pm$ 7.3
ASNase medac	64.9 $\pm$ 5.6	57.5 $\pm$ 5.6	51.0 $\pm$ 5.7	47.9 $\pm$ 6.4	47.5 $\pm$ 6.3	53.5 $\pm$ 6.6

Supplementary Figure S1. Event Free Survival (EFS) and Cumulative Incidence of Relapse (CIR) of recombinant E.coli asparaginase (rASNase) and Asparaginase Medac (MEDAC).



SUPPLEMENTARY FILE

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