

Individualized Asparaginase Dosing in Childhood Acute Lymphoblastic Leukemia

Robin Q.H. Kloos, MD¹; Rob Pieters, PhD²; Florine M.V. Jumelet, BS¹; Hester A. de Groot-Kruseman, PhD^{2,3}; Cor van den Bos, MD, PhD^{2,4}; and Inge M. van der Sluis, MD, PhD^{1,2}

abstract

PURPOSE In the DCOG ALL-11 protocol, polyethylene glycol–conjugated *Escherichia coli* asparaginase (PEGasparaginase) and *Erwinia* asparaginase treatment of pediatric acute lymphoblastic leukemia are individualized with therapeutic drug monitoring (TDM). The efficacy of TDM and its effect on asparaginase-associated toxicity are reported.

PATIENTS AND METHODS After induction with 3 fixed intravenous doses of 1,500 IU/m² PEGasparaginase, medium-risk patients (n = 243) received 14 individualized doses that targeted trough levels of 100–250 IU/L, standard-risk patients (n = 108) received 1 individualized dose, and high-risk patients (n = 18) received 2–5 fixed administrations (1,500 IU/m²). After a neutralizing hypersensitivity reaction, patients were started with 20,000 IU/m² *Erwinia* asparaginase 3 times per week, and L-asparagine was measured to monitor asparaginase efficacy. Several asparaginase-associated toxicities were studied.

RESULTS The final median PEGasparaginase dose was lowered to 450 IU/m². Overall, 97% of all trough levels of nonallergic patients were > 100 IU/L. Asparagine was < 0.5 μM in 96% and 67% of the PEGasparaginase and *Erwinia* asparaginase levels > 100 IU/L, respectively. Ten percent developed a neutralizing hypersensitivity reaction to PEGasparaginase, of which 40% were silent inactivations. The cumulative incidence of grade 3–4 pancreatitis, central neurotoxicity, and thromboses was 12%, 4%, and 6%, respectively, and not associated with asparaginase activity levels. During medium-risk intensification, 50% had increased ALT and 3% hyperbilirubinemia (both grade 3/4 and correlated with asparaginase activity levels), and 37% had grade 3/4 hypertriglyceridemia. Hypertriglyceridemia occurred less in intensification compared with ALL-10 (37% v 47%), which is similar to ALL-11 but with higher asparaginase levels during intensification.

CONCLUSION TDM of asparaginase results in a significant reduction of the PEGasparaginase dose with adequate asparaginase activity levels and sufficient asparagine depletion. In addition, with TDM, silent inactivation and allergic-like reactions were identified. However, the effect of reduced asparaginase activity levels on toxicity is limited.

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INTRODUCTION

Asparaginase is essential for pediatric acute lymphoblastic leukemia (ALL) treatment.^{1–7} The drug starves leukemic cells by converting extracellular asparagine, which is essential for these cells.⁸ Asparaginase activity > 100 IU/L is considered to be sufficient for complete asparagine depletion.^{9–16}

Hypersensitivity reactions to asparaginase occur with or without clinical symptoms of an allergy, the latter, called silent inactivation (SI), neutralizing the drug completely and requiring a switch from *Escherichia coli* asparaginase to *Erwinia* asparaginase.¹⁷ Patients may also develop atypical allergies without inactivation (allergic-like reactions), which do not require a switch in formulation to ensure adequate treatment.¹⁸ Currently,

in most countries, polyethylene glycol–conjugated *E coli* asparaginase (PEGasparaginase) is used as a first-line formulation and *Erwinia* asparaginase (Erwinase; Jazz Pharmaceuticals, Dublin, Ireland) as second line.

In addition, other asparaginase-associated toxicity hampers asparaginase treatment, which possibly results in worse outcomes.¹⁹ We showed that a fixed PEGasparaginase dose of 2,500 IU/m² results in high trough asparaginase activity levels, which possibly cause toxicity.²⁰ Furthermore, asparaginase activity levels show large inter- and inpatient variability.^{21,22} Therefore, therapeutic drug monitoring (TDM) was implemented in the Dutch Childhood Oncology Group (DCOG) ALL-11 treatment protocol. In this way, SI also is detected, and allergic-like reactions are identified. Here, the efficacy of the ALL-11 TDM

ASSOCIATED CONTENT

Data Supplement Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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program and the effect on asparaginase-associated toxicity were studied.

PATIENTS AND METHODS

Patients and Therapy

Patients treated according to ALL-11 between April 2012 and December 2016 were included. Asparaginase-associated toxicity (hypersensitivity reactions, pancreatitis, central neurotoxicity, thrombosis, and infections) were nationally registered by DCOG. In 50 patients treated in the Erasmus MC Rotterdam or Amsterdam University Medical Center, hepatotoxicity, hypertriglyceridemia, the number of transfusions, and hyperglycemia were also assessed. Informed consent was signed by patients and parents according to Dutch law. The study (CCMO register identifier: NL50250.078.14) was approved by institutional review boards according to the Declaration of Helsinki.

The ALL-11 protocol is described and TDM presented in the Data Supplement (online only). In induction, patients were treated with 3 intravenous PEGasparaginase doses (1,500 IU/m² biweekly). Thereafter, patients were stratified as standard risk (SR), medium risk (MR), or high risk (HR) on the basis of treatment response (minimal residual disease [MRD] levels and prednisone response), MLL-AF4 rearrangements, and CNS/testes involvement. After an interval of approximately 12 weeks, SR patients were treated with 1 individualized dose during protocol IV; MR patients were treated with 14 individualized doses biweekly during intensification. The algorithm of dose reductions is described in the Data Supplement. HR patients were treated with 2-5 doses of 1,500 IU/m² with intervals of approximately 7 weeks.

For the nationwide TDM program, trough serum asparaginase activity levels were measured for dose adjustments,

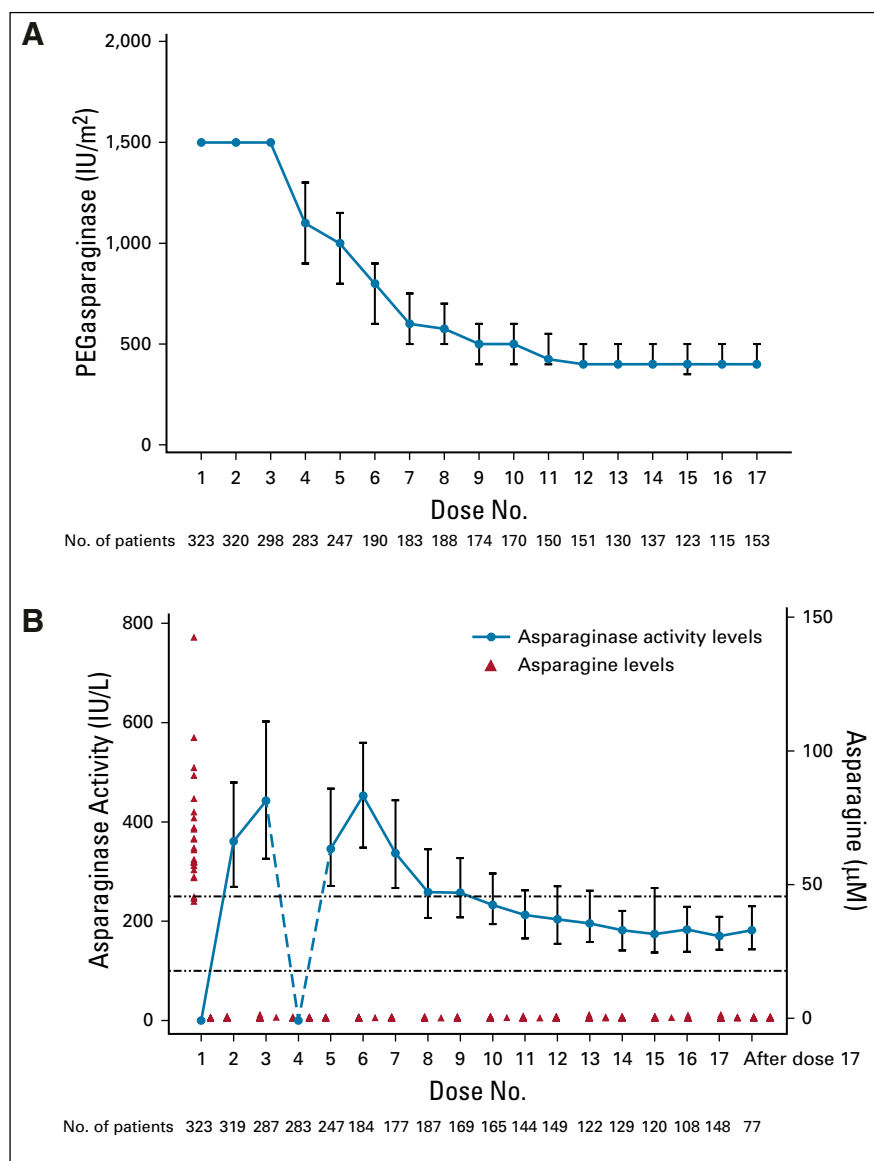


FIG 1. Therapeutic drug monitoring of polyethylene glycol-conjugated *Escherichia coli* asparaginase (PEGasparaginase). (A) The PEGasparaginase dose (median and interquartile range [IQR]) and (B) the trough asparaginase activity (median and IQR) and corresponding asparagine levels during the different PEGasparaginase doses are shown. The first 3 doses had a fixed dose of 1,500 IU/m², after which the doses were individualized. Because of the interruption of asparaginase treatment after induction, the trough level previous to dose 4 was 0. The asparagine comprised both L- and D-asparagine because L-asparagine was measured in only 50 patients. This figure only includes patients without a neutralizing hypersensitivity reaction.

TABLE 1. Asparaginase Activity Levels of Patients Without a Hypersensitivity Reaction

		Trough Asparaginase Activity Level (IU/L), No. (%)			
Patient Group	No. of Patients	< 50	50-99	100-250	> 250
All patients					
After dose 1	319	1 (< 0.5)	7 (2)	61 (19)	250 (79)
After dose 2	287	4 (1.0)	10 (4)	26 (9)	247 (86)
After dose 3 ^a		—	—	—	—
SR + MR patients					
After dose 4	247	6 (2.0)	3 (1)	34 (14)	204 (83)
MR patients only					
After dose 5	184	2 (1.0)	2 (1)	17 (9)	163 (89)
After dose 6	177	1 (1.0)	2 (1)	37 (21)	137 (77)
After dose 7	187	1 (1.0)	1 (1)	84 (45)	101 (54)
After dose 8	169	0 (0.0)	3 (2)	77 (46)	89 (53)
After dose 9	165	0 (0.0)	2 (1)	96 (58)	67 (41)
After dose 10	144	0 (0.0)	5 (4)	95 (66)	44 (30)
After dose 11	149	0 (0.0)	3 (2)	101 (68)	45 (30)
After dose 12	122	0 (0.0)	4 (3)	84 (69)	34 (28)
After dose 13	129	0 (0.0)	7 (5)	105 (82)	17 (13)
After dose 14	120	1 (1.0)	7 (6)	96 (80)	16 (13)
After dose 15	108	0 (0.0)	6 (6)	83 (77)	19 (17)
After dose 16	148	0 (0.0)	5 (3)	129 (87)	14 (10)
After dose 17	77	0 (0.0)	4 (5)	60 (78)	13 (17)

NOTE. The number of asparaginase activity levels drops after several doses because levels were measured every 4 weeks when stable levels had been reached.

Abbreviations: MR, medium risk; SR, standard risk.

^aThere was no asparaginase activity level measured after the third polyethylene glycol-conjugated *Escherichia coli* asparaginase dose. Just before that dose, a trough level was measured on which the first dose adjustment of the first dose in intensification was based.

which targeted 100-250 IU/L. Weekly levels were measured after the first PEGasparaginase dose or the first dose after an asparaginase-free interval for early detection of SI. When asparaginase activity levels were stable within the target range, trough levels were measured every 4 weeks. In HR patients, weekly levels were measured after each dose to detect SI. The asparaginase level measurements and formulation of dosing adjustments were performed centrally.

In case of a neutralizing hypersensitivity reaction, patients were switched to intravenous Erwinase 20,000 IU/m², starting 3 times a week for 2 weeks. Each PEGasparaginase dose was substituted with 2 weeks of Erwinase treatment. After 2 weeks, the dose and/or dosing schedule was adjusted to ensure Erwinase activity levels > 100 IU/L. On the basis of the 48- and 72-hour levels, the decision was made to increase the dose or change the dosing frequency.

Efficacy

PEGasparaginase doses and trough asparaginase activity levels, which targeted 100-250 IU/L, were analyzed. For TDM of Erwinase, the adjusted dosing intervals also were analyzed. Asparagine and glutamine concentrations were measured in serum samples collected at Erasmus MC. Beside total asparagine levels, the subtype L-asparagine (the type of asparagine incorporated in proteins and hydrolyzed by L-asparaginase) was measured as this²³ (Data Supplement).

Toxicity

Asparaginase-associated toxicity (< 2 weeks after asparaginase administration) was graded according to the Common Terminology Criteria for Adverse Events (version 4.03). Hypersensitivity reactions (neutralizing allergies, allergic-like reactions, or SI), grade ≥ 3 central neurotoxicity (ataxia, somnolence, depressed level of consciousness, agitation, seizures, and posterior reversible encephalopathy syndrome [PRES]), grade ≥ 3 pancreatitis that fulfilled the criteria described by Schmiegelow et al,²⁴ the number of patients with at least 1 infection (grade ≥ 2), and grade ≥ 3 thrombosis without central line thromboses were registered by the DCOG. Hepatotoxicity, hypertriglyceridemia, number of transfusions, and hyperglycemia were analyzed in a subset of 50 patients.

Allergic reactions were defined as symptomatic reactions that resulted in neutralization of asparaginase. SI is neutralization of asparaginase in the absence of symptoms, and allergic-like reactions are symptomatic allergic reactions without neutralization of asparaginase.²⁴

Toxicity was correlated with asparaginase levels and compared with patients treated according to the ALL-10 protocol, which is similar to ALL-11 but with native *E coli* asparaginase treatment during induction (8 × 5,000 IU/m²; trough levels, 143-182 IU/L) and PEGasparaginase during intensification (2,500 IU/m²; mean trough level, 899 IU/L)^{20,25} (Table 3).

Measurements and Statistical Analysis

The measurements of asparaginase activity levels, asparagine and glutamine concentrations, and antibodies as well as the statistical analysis are described in the Data Supplement.

RESULTS

Patient Characteristics

Three hundred eighty-two patients were included, with a median age of 5.3 years (interquartile range [IQR], 3.3-10.3 years); the median age of patients in whom the extra laboratory measurements were performed was 4.7 years (IQR, 3.4-7.7 years). After induction, 108 patients were treated as SR, 243 as MR, and 18 as HR. In total, 13

patients were treated with asparaginase in induction only because of asparaginase-related toxicity or relapse, after which they were switched to relapse treatment protocols.

Efficacy

Figure 1 shows the PEGasparaginase doses and corresponding trough PEGasparaginase levels of nonallergic patients. The first 3 doses were fixed at 1,500 IU/m². Thereafter, doses were reduced in all patients to target trough levels of 100-250 IU/L. Overall, 97% of the trough levels were $\geq$ 100 IU/L. After the 10th administration, stable asparaginase levels within the target range were reached, with a median dose of 450 IU/m² (IQR, 450-500 IU/m²). There were no PEGasparaginase levels < 50 IU/L at that point; 68%-87% of the trough levels were within the target range of 100-250 IU/L (Table 1). MRD levels at the end of induction (day 33) were similar to DCOG ALL-10.

Thirty-seven patients started with 20,000 IU/m² Erwinase 3 times a week for 2 weeks. In 26 patients, the dose and/or dosing frequency was adjusted. During the first 2 weeks, 89 (76%) of 117 48-hour levels and 16 (24%) of 67 72-hour levels were > 100 IU/L. Thereafter, the Erwinase dose varied between 15,000 and 40,000 IU/m². Thirteen patients (57%) were treated every other day (72% of the 48-hour levels [120 of 166 levels] > 100 IU/L), and 3 patients (13%) were treated 2 times a week (63% of the 72-hour levels [10 of 19] and 44% of the 96-hour levels [7 of 16] > 100 IU/L). Of the 10 patients who continued treatment 3 times a week after the first 2 weeks, 76% of the 48-hour levels (99 of 131) and 34% of the 72-hour levels (36 of 105) were > 100 IU/L.

Asparagine and glutamine were measured in 754 samples (PEGasparaginase, 637 samples [asparagine concentrations shown in Fig 1]; Erwinase, 117 samples) in 110 patients. Median baseline asparagine and glutamine levels were 68.52 and 557 μ M, respectively (n = 12). During asparaginase treatment, asparagine varied between 0.03 and 1.26 μ M. Of the samples > 100 IU/L, asparagine was < 0.2 μ M in 45% and < 0.5 μ M (< 1% of baseline asparagine concentration) in 96% for PEGasparaginase but in 11% and 67%, respectively, for Erwinase.

The median L-asparagine concentration in PEGasparaginase samples (n = 50; asparaginase activity level, 11-752 IU/L) was 0.15 μ M (IQR, 0.08-0.24 μ M), and 0.10 μ M (IQR, 0.05-0.15 μ M) in Erwinase samples (n = 20; asparaginase activity level, 13-530 IU/L; Data Supplement). Overall, 76% of the samples with an asparaginase activity level > 100 IU/L had an L-asparagine level < 0.2 μ M. The mean glutamine level was 571 μ M (standard deviation [SD], 159 μ M) during PEGasparaginase and 600 μ M (SD, 192 μ M) during Erwinase treatment, which was not lower than the baseline of 557 μ M.

Toxicity

Hypersensitivity reactions. Ten percent of patients (n = 40) had a neutralizing hypersensitivity reaction (allergy and SI) to PEGasparaginase (allergy, 6% [n = 22]; SI, 4% [n = 18]; Table 2). The incidence was 3% (n = 13) during induction and 7% (n = 27) during MR intensification. SI and allergies during induction occurred after all doses; during intensification, SI only occurred after the first dose. Of the 8 patients with an allergy during MR intensification, 5 (62%) were during the first dose and 3 (38%) during the second dose.

In none of the patients with a reaction during induction, PEGasparaginase or native *E coli* asparaginase antibodies could be detected, although the PEGasparaginase antibody assay had limitations, which is explained in the Discussion section. Of the reactions during the first dose of intensification, 33% were accompanied by PEGasparaginase antibodies and 11% by antinative *E coli* asparaginase antibodies. The 3 patients with an allergy during the second dose of intensification were positive for both antibodies.

Five patients (1%) had an allergic-like reaction (Table 2). PEGasparaginase was completed in 4 patients. One patient was switched to Erwinase because the reaction was not recognized as an allergic-like reaction.

Of the 37 patients switched to Erwinase, 4 (10%) developed an allergy to (n = 2) or SI of (n = 2) Erwinase (Table 3). Four patients (10%) had an allergic-like reaction; Erwinase was completed in 2 of these 4. In case of allergic-like reactions, administration with decreased infusion rate and/or anti-allergic medication was useful.

Pancreatitis. The cumulative incidence of pancreatitis was 12% (n = 34; Fig 2). The median age at diagnosis was 8.3 years (IQR, 5.0-13.5 years) v 5.2 years (IQR, 3.2-9.8 years) in patients without pancreatitis (*P* = .001). The cumulative incidence during induction was 4% (n = 14). After induction, this was 0% for SR patients, 8% for MR patients, and 6% for HR patients (n = 1). There was no statistically significant correlation between pancreatitis and asparaginase levels. PEGasparaginase was successfully reintroduced in 3 patients. In ALL-10, pancreatitis occurred in 1% of the patients during induction and in 5% of the patients during intensification^{25,26} (Table 3).

Central neurotoxicity. The cumulative incidence of central neurotoxicity was 4% (n = 12; Fig 2), with the majority having PRES (8 of 12 patients). Age at diagnosis of the patients with and without neurotoxicity did not differ. During induction, 4 patients (cumulative incidence, 1%) had central neurotoxicity. After induction, this was 0% for SR patients, 3% for MR patients, and 6% for HR patients (n = 1). There was no statistically significant correlation between central neurotoxicity and asparaginase levels. In all but 1 patient, asparaginase was completed. In ALL-10, central

TABLE 2. Hypersensitivity Reactions

Patient Group	Patients, No. (%)												
	Neutralizing Hypersensitivity Reaction ^a				Neutralizing Allergy			Silent Inactivation			Allergic-Like Reaction (without inactivation)		
	Total	During Induction	After Induction	Total	During Induction	After Induction	Total	During Induction	After Induction	Total	During Induction	After Induction	Total
Hypersensitivity reactions to PEGasparaginase													
Standard risk	108	2 (2)	10 (9)	12 (11)	2 (2)	6 (5)	8 (7)	0 (0)	4 (4)	4 (4)	0 (0)	2 (2)	2 (2)
Medium risk	243	9 (3)	17 (7)	26 (10)	5 (2)	8 (3)	13 (5)	4 (2)	9 (3)	13 (5)	0 (0)	3 (1)	3 (1)
High risk	18	1 (6)	0 (0)	1 (6)	1 (6)	0 (0)	1 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Only induction ^b	13	1 (8)	—	1 (8)	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)
Total	382	13 (3)	27 (7)	40 (10)	8 (2)	14 (4)	22 (6)	5 (1)	13 (3)	18 (4)	0 (0)	5 (1)	5 (1)
Stratified only	369	12 (3)	27 (7)	39 (10)	8 (2)	14 (4)	22 (6)	4 (1)	13 (3)	17 (4)			
Hypersensitivity reactions to Erwinase ^c													
Standard risk	10	1 (10)	1 (10)	2 (20)	0 (0)	1 (10)	1 (10)	1 (10)	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)
Medium risk	26	0 (0)	1 (4)	1 (4)	0 (0)	1 (4)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	4 (15)	4 (15)
High risk	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Only induction ^b	1	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Total	37	2 (5)	2 (5)	4 (10)	0 (0)	2 (5)	2 (5)	2 (5)	0 (0)	2 (5)	0 (0)	4 (10)	4 (10)
Stratified only	36	1 (3)	2 (6)	3 (9)	0 (0)	2 (6)	2 (6)	1 (3)	0 (0)	1 (3)	0 (0)	4 (10)	4 (10)

Abbreviation: PEGasparaginase, polyethylene glycol-conjugated *Escherichia coli* asparaginase.

^aNeutralizing hypersensitivity reactions comprise both neutralizing allergies and silent inactivation.

^bIn some of the patients, only induction therapy could be analyzed because of cessation of asparaginase (n = 7) or mortality (n = 6) during induction.

^cErwinase is manufactured by Porton Biopharma, Salisbury, United Kingdom.

neurotoxicity occurred in 2% of the patients during induction and 10% during intensification^{25,26} (Table 3).

Thrombosis. The cumulative incidence of thromboses was 6% (n = 19; Fig 2) of which 84% were a sagittal sinus thrombosis and 16% a deep venous thrombosis of an extremity. The median age of patients with thrombosis was 9.2 years (IQR, 6.8-11.3 years) v a median age of 5.2 years (IQR, 3.2-9.9 years) of patients without thrombosis ($P = .003$). The cumulative incidence was 4% (n = 14) during induction. After induction, this was 0% in SR and HR patients and 2% in MR intensification. There was no statistically significant correlation between thrombosis and asparaginase activity levels. Asparaginase, with concomitant low-molecular-weight heparin administration, was completed in 18 of the 19 patients. In ALL-10, thrombosis occurred in 2% of patients during induction and 3% during intensification^{25,26} (Table 3).

Infections. The number of patients with at least 1 infection during induction was comparable between ALL-11 and ALL-10: 35% v 37% in protocol 1A and 42% v 41% in protocol 1B. However, fewer patients had an infection during MR intensification in ALL-11: 35% v 46% in the first 19 weeks and 38% v 53% in weeks 20-35⁵ (Table 3). ALL-10 intensification, however, included 6 doses of doxorubicin instead of 4 doses (or none in case of EVT6/RUNX1-positive

patients with leukemia or Down syndrome) in ALL-11 intensification.

Hepatotoxicity. The hepatotoxicity was reflected by ALT and bilirubin concentrations (Data Supplement; Fig 3). Grade 3/4 increased ALT occurred in 26% of the patients during induction and 50% during intensification; grade 3/4 hyperbilirubinemia occurred in 10% of the patients during induction and 3% during intensification. Longitudinal analysis showed that ALT and bilirubin were higher with higher asparaginase activity levels ($P = .004$ and $< .001$, respectively; Data Supplement). In ALL-10, ALT was increased in 36% of the patients, and grade 3/4 hyperbilirubinemia occurred in 11% of the patients during induction^{25,26} (Table 3).

Hypertriglyceridemia. Grade 3/4 hypertriglyceridemia occurred in 12% of the patients during induction and 37% during intensification (Data Supplement) without clinical consequences. Triglycerides were mainly increased before the sixth PEGasparaginase dose (Fig 3), directly after administration of dexamethasone. In ALL-10, 47% of the patients had grade 3/4 hypertriglyceridemia during intensification. We found no correlation between triglyceride concentrations and asparaginase levels.

TABLE 3. Asparaginase-Associated Toxicity During DCOG ALL-10 and DCOG ALL-11

Treatment Phase	DCOG ALL-10 ^{25,26}	DCOG ALL-11
Induction	Native <i>Escherichia coli</i> asparaginase Trough levels: 143-182 IU/L	PEGasparaginase Median trough level: 403 IU/L
Pancreatitis (grade ≥ 3)	Incidence, 1%	Cumulative incidence, 4%
Thrombosis (grade ≥ 3)	Incidence, 2%	Cumulative incidence, 4%
Central neurotoxicity (grade ≥ 3)	Incidence, 2%	Cumulative incidence, 1%
Infections (No. of patients with at least 1 grade ≥ 2 infection)	Protocol 1A, 37% Protocol 1B, 41%	Protocol 1A, 35% Protocol 1B, 42%
Hepatotoxicity	ALT increased in 36% of the patients Grade 3/4 hyperbilirubinemia in 11% of the patients	ALT increased in 26% of the patients Grade 3/4 hyperbilirubinemia in 10% of the patients
Dyslipidemia	Not reported	Grade 3/4 hypertriglyceridemia in 12% of the patients
Intensification	PEGasparaginase Mean trough level, 899 IU/L	PEGasparaginase Median trough level, 272 IU/L
Pancreatitis (grade ≥ 3)	Incidence, 5%	Cumulative incidence, 8%
Thrombosis (grade ≥ 3)	Incidence, 3%	Cumulative incidence, 2%
Central neurotoxicity (grade ≥ 3)	Incidence 10%	Cumulative incidence, 3%
Infections (No. of patients with at least 1 grade ≥ 2 infection)	Week 1-19, 46% Week 20-35, 53%	Week 1-19, 35% Week 20-35, 38%
Hepatotoxicity	Not reported	ALT increased in 50% of the patients Grade 3/4 hyperbilirubinemia in 3% of the patients
Dyslipidemia	Grade 3/4 hypertriglyceridemia in 47% of the patients	Grade 3/4 hypertriglyceridemia in 37% of the patients

NOTE. The ALL-10 intensification phase included 6 doses of doxorubicin. The ALL-11 intensification phase contained 4 doses or 0 in the case of EVT6/RUNX1-positive patients with leukemia or Down syndrome.

Abbreviation: PEGasparaginase, polyethylene glycol-conjugated *Escherichia coli* asparaginase.

Other toxicity. During induction, the median number of erythrocyte and thrombocyte transfusions administered since the start of asparaginase to 2 weeks after the third dose was 1 (IQR, 1-2 and 0-2 transfusions, respectively) and 0 (IQR, 0-1 and 0-0 transfusions, respectively) during intensification (Data Supplement). Glucose levels were rarely increased throughout the asparaginase treatment (Fig 3), and only 1 patient had grade 3/4 hyperglycemia (Data Supplement).

DISCUSSION

The efficacy and toxicity of individualized asparaginase therapy were analyzed. With our nationwide TDM program, the PEGasparaginase dose was reduced to a median of 450 IU/m², with asparaginase levels > 100 IU/L in 97% of the nonallergic patients. This dose reduction leads to a substantial reduction of the asparaginase-associated costs.²⁷ The starting dose of 1,500 IU/m² proved to be adequate because trough asparaginase levels were > 100 IU/L in 98% of the patients after the first dose and in 95% after the second. In addition, end-induction MRD levels were similar to ALL-10.

Efficacy of the TDM program was evaluated by measurement of asparagine and glutamine. Accurate sample handling is essential to avoid ex vivo asparaginase activity.^{28,29} In our samples, asparagine was measurable,

and glutamine was not decreased, which confirmed that the samples were adequately processed. Asparagine was $< 0.5 \mu\text{M}$ in 96% of the PEGasparaginase samples > 100 IU/L but only in 67% of the Erwinase samples. However, L-asparagine concentrations measured in these samples were very low (73% of the samples $< 0.2 \mu\text{M}$), which showed a considerable influence of the irrelevant D-asparagine on total asparagine concentrations. To our knowledge, this study is the first to report L-asparagine concentrations during asparaginase treatment.

Compared with ALL-10, the pattern of occurrence of a hypersensitivity reaction and the formation of antibodies have changed fundamentally in ALL-11. Patients were treated during induction with native *E coli* asparaginase in ALL-10 and PEGasparaginase in ALL-11, whereas both protocols used PEGasparaginase during intensification. In ALL-10, during induction, 5% had a hypersensitivity reaction to native *E coli* asparaginase.²⁵ In ALL-11, this was 3% for PEGasparaginase. During ALL-10 intensification, 30% had a neutralizing hypersensitivity reaction, which is considerably more than the 7% in ALL-11. In ALL-10, these reactions almost exclusively occurred during the second PEGasparaginase dose, and they were all accompanied by antibodies to *E coli* asparaginase.²⁶ In contrast, in ALL-11, the hypersensitivity reactions occurred during both the first and the second PEGasparaginase dose intensification, and native *E coli* asparaginase antibodies were absent in the

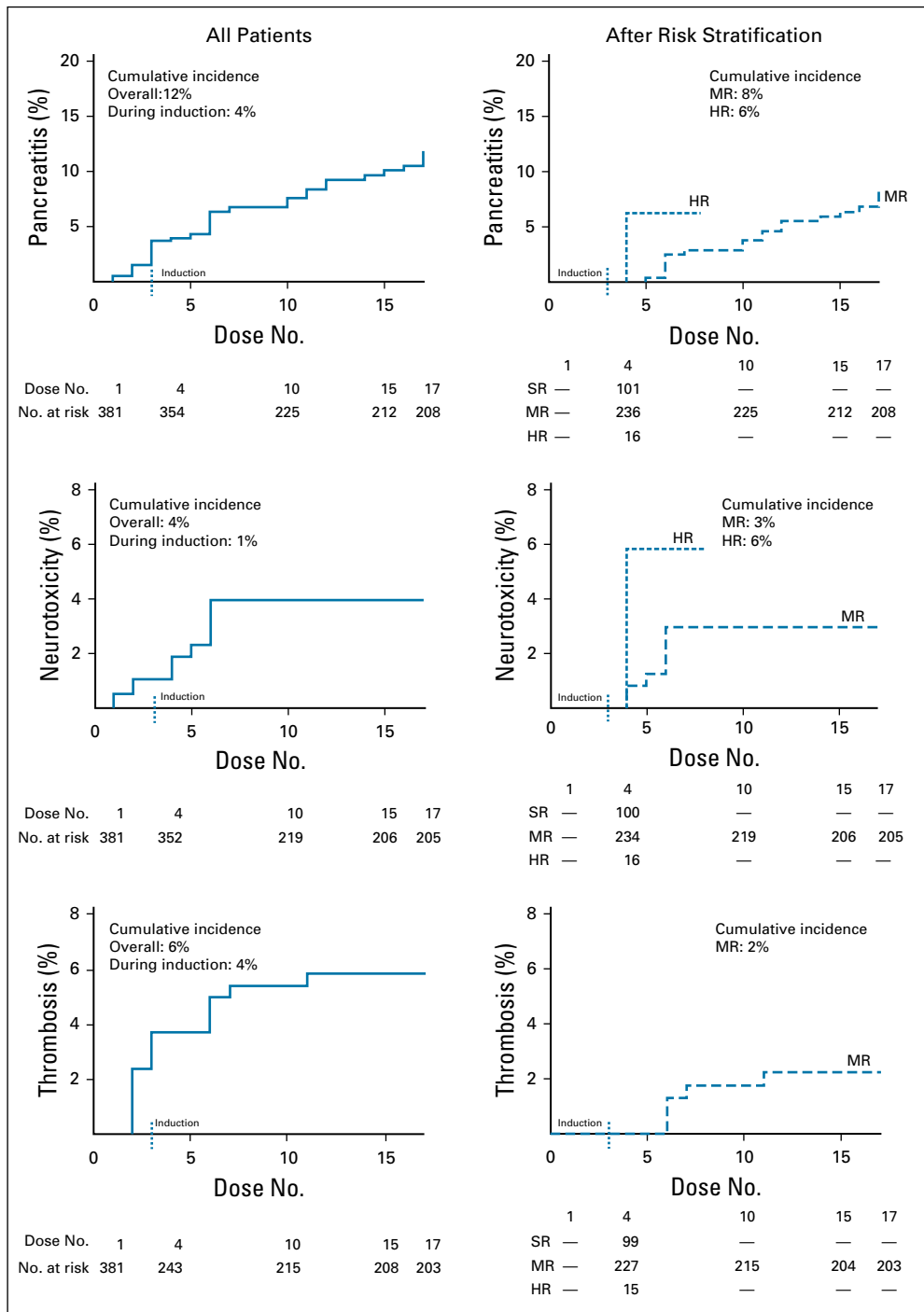


FIG 2. Cumulative incidence of pancreatitis, neurotoxicity, and thrombosis. The cumulative incidence of asparaginase-associated pancreatitis, central neurotoxicity, and thrombosis for all patients (left) and after risk stratification (right) for standard-risk (SR), medium-risk (MR) and high-risk (HR) patients is shown. Of note, only 16 patients were stratified as HR, and the cumulative incidence of 6% for pancreatitis and central neurotoxicity reflects only 1 patient. The end of induction is indicated with a dotted line. The cumulative incidences after risk stratification did not comprise the toxicity that occurred during induction.

majority of the patients, as was the case with the reactions during induction. Only the 3 patients with a reaction during the second PEGasparaginase dose during intensification had, like in ALL-10, antibodies to native *E coli* asparaginase and PEGasparaginase. Thus, by using PEGasparaginase, patients do not necessarily seem to develop reactions to the asparaginase moiety, except for the few with a reaction during the second dose during intensification.

In ALL-11, PEGasparaginase antibodies were also absent in the majority of patients with a neutralizing reaction. More

detailed measurements have shown that by coating the plates with PEGasparaginase in a basic carbonate coating buffer (pH 9.5), PEG hydrolyses from PEGasparaginase (Kloos et al, manuscript submitted for publication). Thus, possible anti-PEG antibodies were not measured with the assay used. As anti-PEG antibodies have been described earlier,³⁰ the majority of the hypersensitivity reactions in ALL-11 were probably against the PEG moiety or even against the linker connecting the PEG to asparaginase (Kloos et al, manuscript submitted for publication).

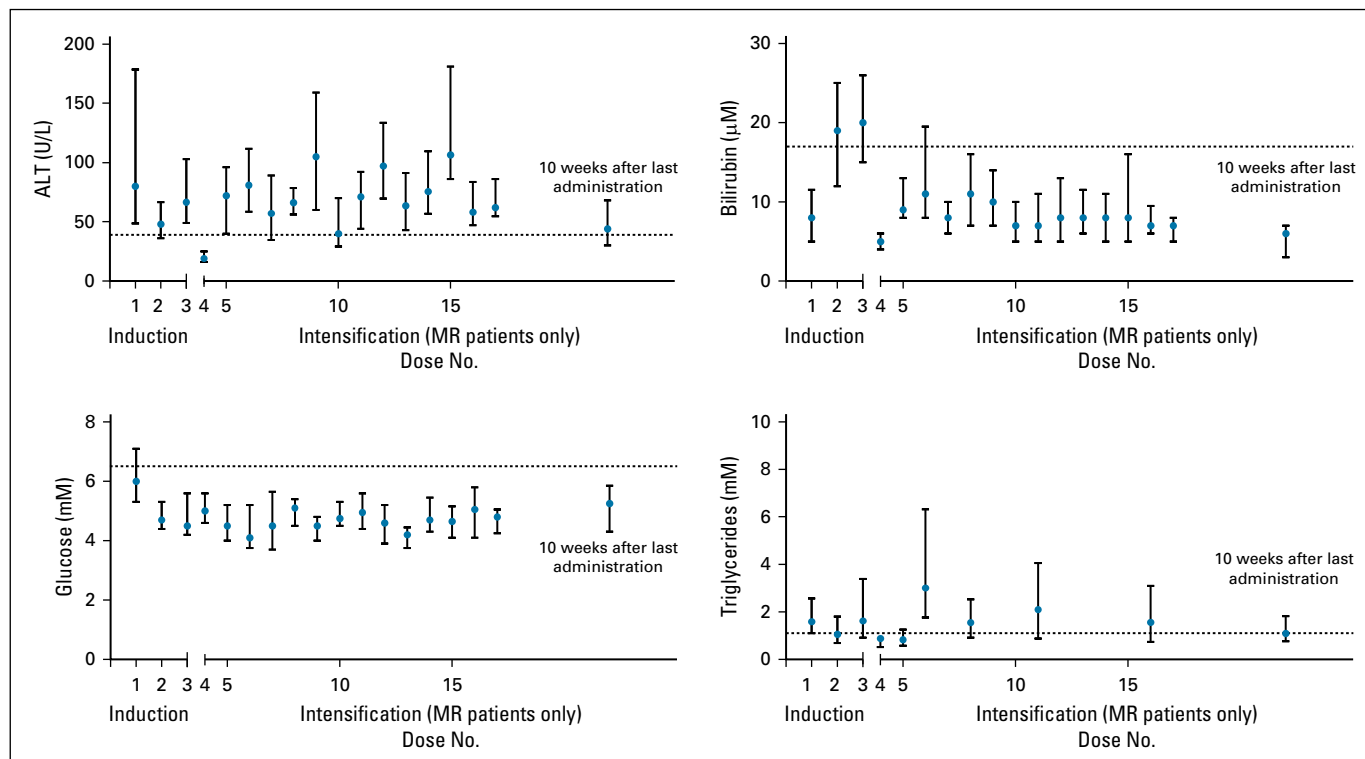


FIG 3. Laboratory measurements. The median and interquartile range of ALT, bilirubin, glucose, and triglyceride levels throughout the asparaginase treatment are shown. The levels during induction include standard-risk, medium-risk (MR), and high-risk patients ($n = 50$); the other levels are measured during MR intensification therapy ($n = 26$). The first measurements were done before the first polyethylene glycol-conjugated *Escherichia coli* asparaginase dose; the measurement at the fourth dose was done before this dose after an asparaginase-free interval of several months. The last measurement was done during week 37, 10 weeks after the last asparaginase dose. The dotted lines indicate the upper limit of normal. Longitudinal analyses showed a correlation between asparaginase activity levels and ALT and bilirubin levels.

To assess whether lowering asparaginase levels by TDM leads to less toxicity, toxicity was correlated with asparaginase levels and compared with ALL-10 (Table 3). In ALL-10, the asparaginase levels were significantly lower during induction but were much higher during intensification compared with ALL-11. During induction, the occurrence of thrombosis and central neurotoxicity was similar between the 2 protocols, and there was no correlation with asparaginase activity levels. Pancreatitis, however, seemed to occur more frequently in ALL-11 (4% v 1% in ALL-10). This might be due to the longer asparaginase exposure during ALL-11 induction (42 v 24 days in ALL-10) because there was no correlation between the PEGasparaginase levels and pancreatitis. The percentage of patients who developed grade 3/4 hyperbilirubinemia did not differ, despite the correlation between asparaginase levels and bilirubin levels, which is, although statistically significant, clinically irrelevant and measured in only a subset of patients.

During the intensification phase, the toxicity was comparable as well. Only central neurotoxicity (10% v 3%) and infection occurred more often in ALL-10 versus ALL-11.⁵ However, the anthracycline therapy was less intensive in

ALL-11, which resulted in less myelosuppression but only during the first 13 weeks of the intensification. Grade 3/4 hypertriglyceridemia occurred less often in ALL-11 (37% v 47% in ALL-10), although a correlation with asparaginase activity levels was not found. This is in contrast to the correlation found in our earlier studies that are possibly explained by less variation in the ALL-11 asparaginase levels as a result of TDM. A comparison of the rough data of ALL-10 triglycerides²⁶ with ALL-11 shows that the median triglycerides are higher during ALL-10 (4.6 mM) than ALL-11 (2.6 mM), with more outliers in ALL-10 (Data Supplement). Thus, the correlation between asparaginase activity levels and triglycerides only holds for higher asparaginase levels.

We conclude that TDM of asparaginase is feasible in a nationwide program and results in a substantial dose reduction without loss of efficacy of treatment, as reflected by asparagine depletion, although survival analyses should be awaited. In addition, TDM leads to detection of SI in 4% of patients and identification of allergic-like reactions in 1%. However, the effect of the PEGasparaginase dose reduction on asparaginase-associated toxicities is limited.

AFFILIATIONS

¹Pediatric Oncology and Hematology, Erasmus MC–Sophia Children's Hospital, Rotterdam, the Netherlands

²Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands

³Dutch Childhood Oncology Group, Utrecht, the Netherlands

⁴Pediatric Oncology and Hematology, Emma Children's Hospital, Amsterdam University Medical Center, Amsterdam, the Netherlands

CORRESPONDING AUTHOR

Inge M. van der Sluis, MD, PhD, Pediatric Oncology and Hematology, Erasmus MC–Sophia Children's Hospital, Wytemaweg 80, Rotterdam 3015, the Netherlands; e-mail: i.m.vandersluis@prinsesmaximacentrum.nl.

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AUTHOR CONTRIBUTIONS

Conception and design: Rob Pieters, Inge M. van der Sluis

Collection and assembly of data: Robin Q.H. Kloos, Florine M.V. Jumelet, Hester A. de Groot-Kruseman, Cor van den Bos, Inge M. van der Sluis

Data analysis and interpretation: Robin Q.H. Kloos, Rob Pieters, Florine M.V. Jumelet, Inge M. van der Sluis

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Individualized Asparaginase Dosing in Childhood Acute Lymphoblastic Leukemia

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Rob Pieters

Honoraria: ERYTECH Pharma, Jazz Pharmaceuticals, Kite Pharma, Novartis

Consulting or Advisory Role: ERYTECH Pharma, Kite Pharma, Jazz Pharmaceuticals, SERVIER

Travel, Accommodations, Expenses: ERYTECH Pharma, Kite Pharma, Jazz Pharmaceuticals, SERVIER

Cor van den Bos

Research Funding: MEDAC (Inst), Sanofi (Inst), Genzyme (Inst), Sanofi (Inst)

Travel, Accommodations, Expenses: Jazz Pharmaceuticals

Inge M. van der Sluis

Consulting or Advisory Role: Jazz Pharmaceuticals

Travel, Accommodations, Expenses: Jazz Pharmaceuticals

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Individualized Asparaginase Dosing in Childhood Acute Lymphoblastic Leukemia

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Supplemental methods

Measurements

Asparaginase activity levels were measured using the L-aspartic β -hydroxamate (AHA) assay as described earlier.(1, 2) Asparaginase inactivation was defined as PEGasparaginase activity <100 IU/L after 7 ± 1 days (week level) and/or <10 IU/L after 14 ± 1 days (trough level).

For asparagine and glutamine measurement, whole blood was directly put on ice and 10% sulfosalicylic acid was added. The samples were stored in -80°C until analysis using liquid chromatography tandem-mass spectrometry (limit of quantification: $0.05\text{ }\mu\text{M}$). Human asparagine measured comprises both L-asparagine and D-asparagine. The latter is also present in humans but not incorporated in proteins, and ranges between $0.017\text{--}0.18\text{ }\mu\text{M}$.(3) Therefore, beside the total asparagine concentration, L-asparagine was measured in 50 patients with varying asparaginase activity levels.

PEGasparaginase and native *E. coli* asparaginase antibodies were measured with Enzyme-Linked Immuno Sorbent Assay (ELISA) as described earlier.(4) The threshold for a positive antibody measurement was calculated using the F-measure.(5)

Statistical analysis

The data was analyzed with the software package SPSS Statistics version 21.0 (IBM Corp, Armonk, New York, USA) and R Sigmaplot Version 3.3.3 (Systat Software Inc, London, UK). Continuous data was presented as mean and standard deviation (SD) or median and interquartile range (IQR). Student t-tests, Mann-Whitney U tests, χ^2 -(trend)tests, and Fisher exact tests were used to compare baseline characteristics. To account for the repeated measurement design to estimate the effect of asparaginase activity levels on laboratory measurements, marginal models were estimated. The cumulative incidence of pancreatitis, central neurotoxicity and thrombosis starting from

asparaginase treatment were estimated by using Kaplan-Meier's methodology. Due to the presence of repeated measurements, a mixed effect model was estimated to study the association between pancreatitis, central neurotoxicity and thrombosis and asparaginase activity levels.

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Supplemental table 1. DCOG ALL-11 treatment protocol

Treatment phase	Therapy
Protocol 1A	
Prednisone	60 mg/m ² /day for 29 days followed by 3x3 days tapering
Vincristine	1.5 mg/m ² /dose at day 8, 15, 22 and 29
Daunorubicin	30 mg/m ² /dose at day 8, 15, 22 and 29 (not in case of Down syndrome)
PEGasparaginase	1,500 IU/m ² at day 12 and 26
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 15 and 33. Only intrathecal methotrexate at day 1.
Protocol 1B	
PEGasparaginase	1,500 IU/m ² at day 40
Cyclophosphamide	1,000 mg/m ² /dose at day 36 and 64
Cytarabine	75 mg/m ² /day at days 38 – 41, 45 – 48, 52 – 55, 59 – 62
6-Mercaptopurine	60 mg/m ² /day at days 36 – 63
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 45 and 59
Protocol M for SR and MR patients	
6-Mercaptopurine	25 mg/m ² /day for 56 days
Methotrexate	5,000 mg/m ² /dose at day 8, 22, 36 and 50
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 8, 22, 36 and 50
Protocol IV for SR patients	
Dexamethasone	10 mg/m ² /day for 15 days followed by 3x3 days tapering
Vincristine	1.5 mg/m ² /dose at day 1 and 8
PEGasparaginase	Individualized dose at day 1
Maintenance for SR patients	
6-Mercaptopurine	50 mg/m ² /day for 81 weeks
Methotrexate	20 mg/m ² /week for 81 weeks
Intensification and maintenance for MR patients	
Dexamethasone	6 mg/m ² /day for 5 days every 3 weeks until week 82
Vincristine	2 mg/m ² /dose every three weeks until week 82
Doxorubicin	30 mg/m ² /dose at week 1, 4, 7 and 10 (not in case of Down syndrome without IKZF1 deletion or TEL/AML1)
PEGasparaginase	Individualized doses biweekly from week 1 – 27
Methotrexate	30 mg/m ² /week from week 13 – 84 (or week 2 – 84 in case of Down syndrome or TEL/AML1), not during intrathecal therapy In case of an IKZF1 deletion, 200 mg/m ² /dose every three weeks from week 85 - 136
6-Mercaptopurine	50 mg/m ² /day from week 1 – 12 in courses of 2 weeks with 1 week interruption (without interruption in case of Down syndrome or TEL/AML1) and from week 13 – 84 daily, without interruption In case of an IKZF1 deletion, 100 mg/m ² /day for 10 days after each methotrexate dose from week 85 - 136

Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at week 1, 19, 37, 55 and 73
High risk blocks for HR patients	
HR block 1	
6-Mercaptopurine	25mg/m ² /day from days 1 - 14
Methotrexate	5,000 mg/m ² at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Cyclophosphamide	1,200 mg/m ² /dose at day 15, 16 and 17
Etoposide	350 mg/m ² /dose at day 15, 16 and 17
PEGasparaginase	1,500 IU/m ² at day 22
Vincristine	1.5 mg/m ² /dose at day 22 and 29
HR block 2	
Methotrexate	5,000 mg/m ² at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Cytarabine	1,500 mg/m ² /dose at day 15, 16, 17, 18 and 19
Mitoxantrone	5.25 mg/m ² /dose at day 15, 16, 17, 18 and 19
PEGasparaginase	1,500 IU/m ² at day 22
Vincristine	1.5 mg/m ² /dose at day 22 and 29
HR block 3	
Methotrexate	5,000 mg/m ² at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Idarubicin	6 mg/m ² /dose at day 15, 16 and 17
Fludarabine	22.5 mg/m ² /dose at day 15, 16, 17, 18 and 19
Cytarabine	1,500 mg/m ² /dose at day 15, 16, 17, 18 and 19
HR block 4*	Equal to HR block 1, but without 6-Mercaptopurine
HR block 5*	Equal to HR block 2
HR block 6*	Equal to HR block 3, but without Idarubicin
Protocol II*	
Dexamethasone	10 mg/m ² /day at days 1 – 21 followed by 3x3 days tapering
Vincristine	1.5 mg/m ² /dose at day 8, 15, 22 and 29
Doxorubicin	30 mg/m ² /dose at day 8, 15, 22 and 29
PEGasparaginase	1,500 IU/m ² at day 8
Cyclophosphamide	1,000 mg/m ² at day 36
6-Thioguanine	60 mg/m ² /day at days 36 – 49
Cytarabine	75 mg/m ² /day at days 36 – 39 and 43 – 46
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 36 and 43
HR maintenance*	
6-Mercaptopurine	50 mg/m ² /day from week 1 – 37
Methotrexate	20 mg/m ² /week from week 1 – 37
* Not if patients are eligible for a stem cell transplantation	

1 Supplemental table 2. Algorithm for dose reductions of PEGasparaginase

PEGasparaginase trough level	Dose adjustment
>600 IU/L	50%
500 – 599 IU/L	60%
400 – 499 IU/L	70%
300 – 399 IU/L	80%
200 – 299 IU/L	100%
100 – 199 IU/L	100%
50 – 99 IU/L	100%
30 – 49 IU/L	150%
10 – 29 IU/L	200%

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7 Supplemental table 3. Transfusions and laboratory measurements during induction and intensification

Maximal toxicity	Induction N=50		Medium risk intensification N=30	
Number of erythrocyte transfusions (median, IQR)	1 (1 – 2)		0 (0 – 1)	
Number of thrombocyte transfusions (median, IQR)	1 (0 – 2)		0 (0 – 0)	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Increased ALT	36 (72%)	13 (26%)	11 (36%)	15 (50%)
Increased bilirubin	34 (68%)	5 (10%)	15 (50%)	1 (3%)
Increased glucose	21 (42%)	1 (2%)	17 (57%)	0
Hypertriglyceridemia	26 (52%)	6 (12%)	13 (43%)	11 (37%)
IQR: interquartile range; ALT: alanine transaminase				

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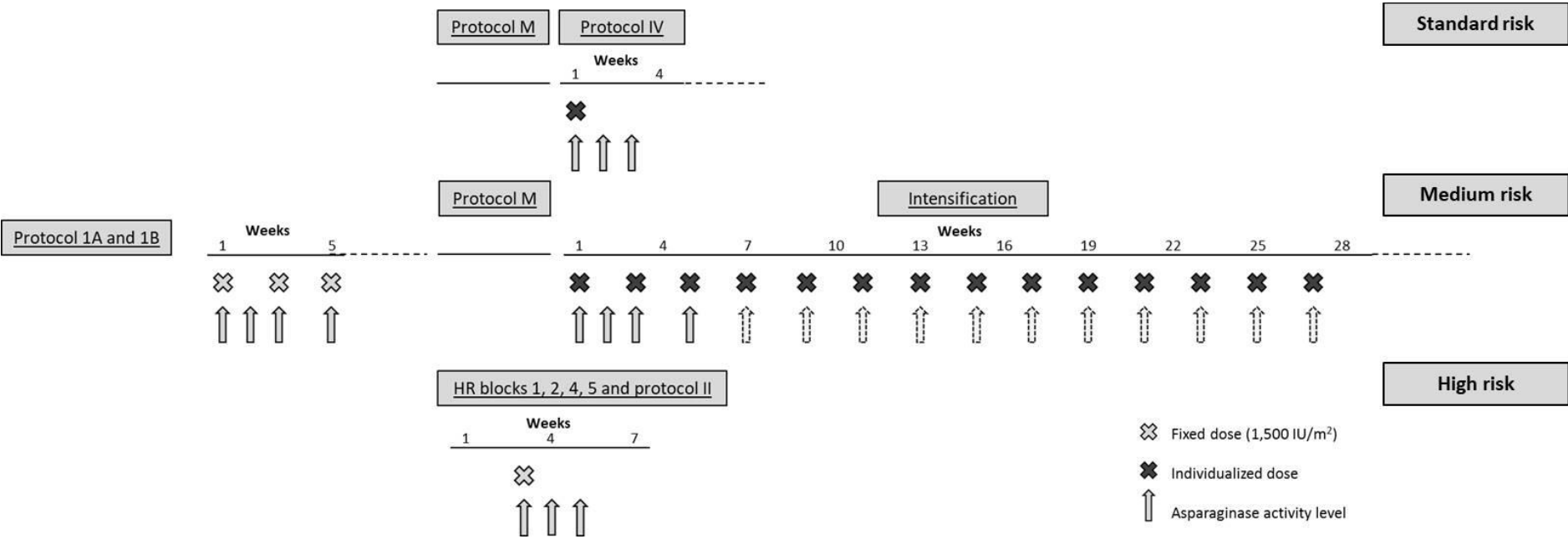
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21 Supplemental figure 1. PEGasparaginase therapeutic drug monitoring



22 *Supplemental figure 1* schematically shows the therapeutic drug monitoring program of the ALL-11 protocol.

23 Patients start with three doses of a fixed dose of PEGasparaginase (1,500 IU/m²) during induction. In standard risk and medium risk patients, the

24 PEGasparaginase doses are individualized after induction. When stable PEGasparaginase levels have been reached in medium risk intensification, trough levels

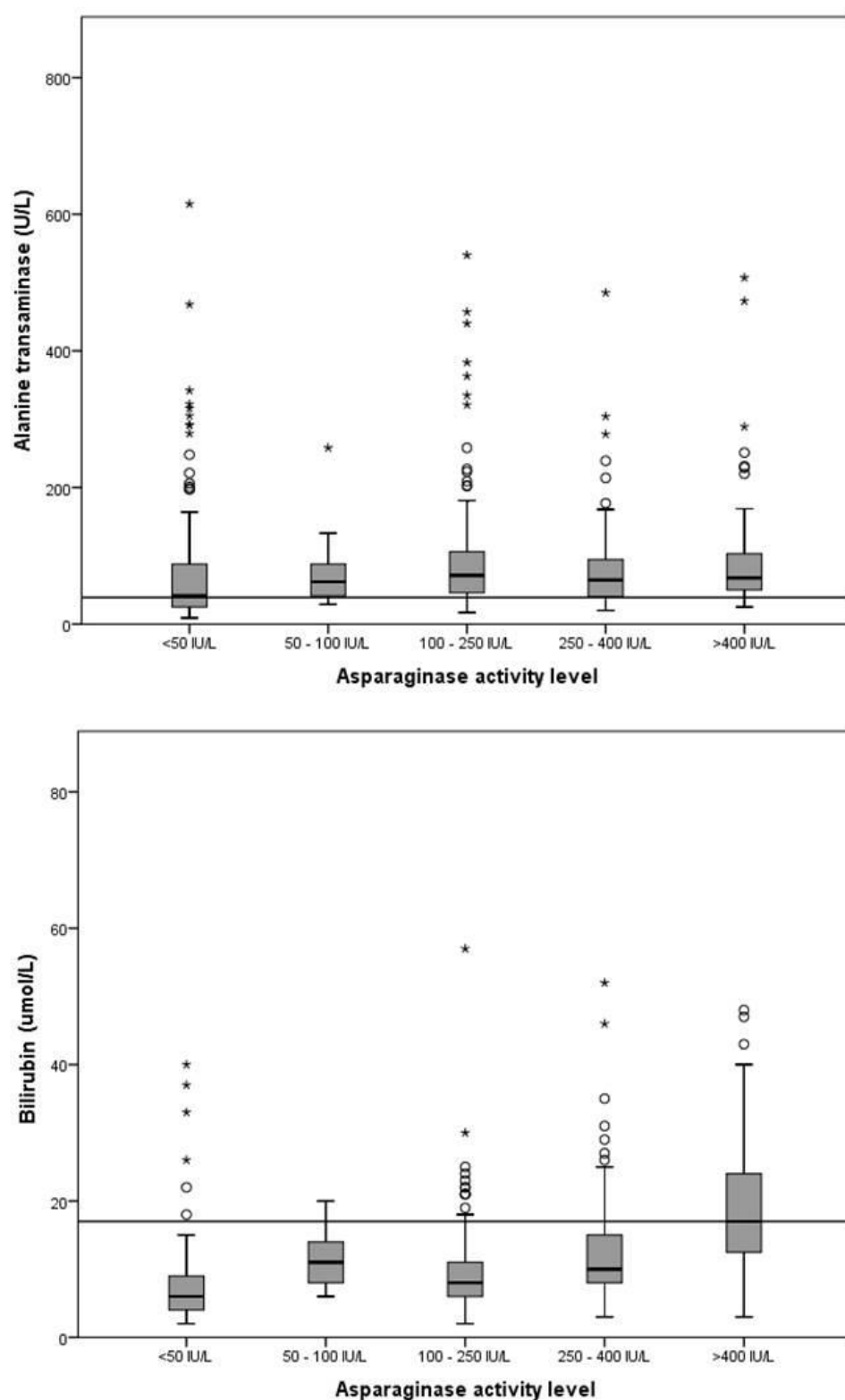
25 are measured only every four weeks (indicated by the dotted arrows). High risk patients are also treated with the fixed dose of induction during the high risk

26 blocks.

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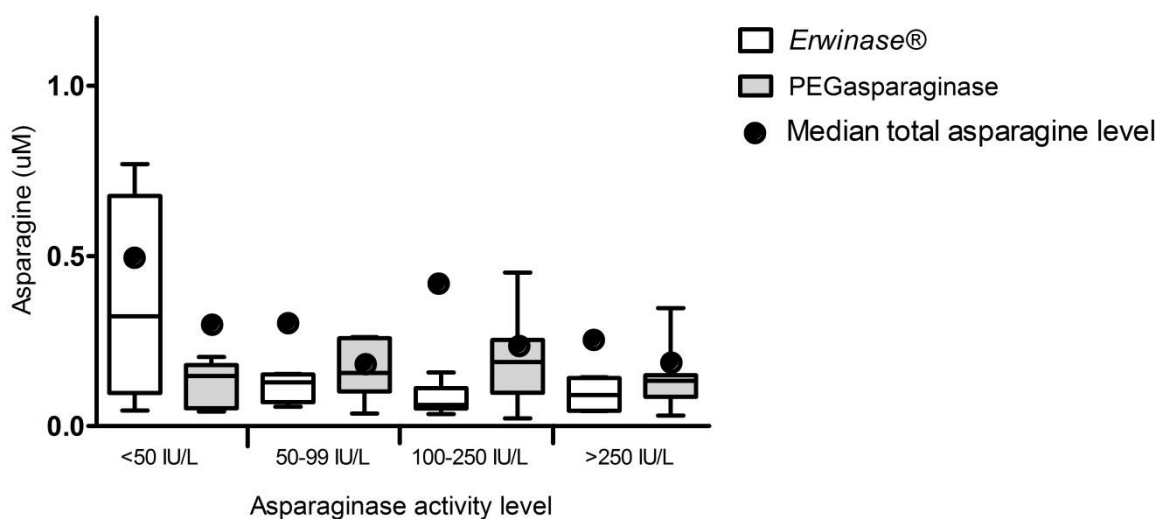
Supplemental figure 2. Correlation asparaginase activity levels and hepatotoxicity



Supplemental figure 2 shows the alanine transaminase (ALT) and bilirubin levels for different asparaginase activity levels. Longitudinal analysis showed that ALT and bilirubin levels were significantly higher with higher asparaginase activity levels ($p=0.004$ and $p<0.001$, respectively). The triglyceride, amylase and glucose levels were not included in this figure as longitudinal analyses did not show a correlation between these parameters and asparaginase activity levels.

The boxplot includes the 25th, 50th and 75th percentile in the boxes, the outliers (o), extreme outliers (*), and the ranges (indicated by the whiskers).

Supplemental figure 3. Asparagine levels

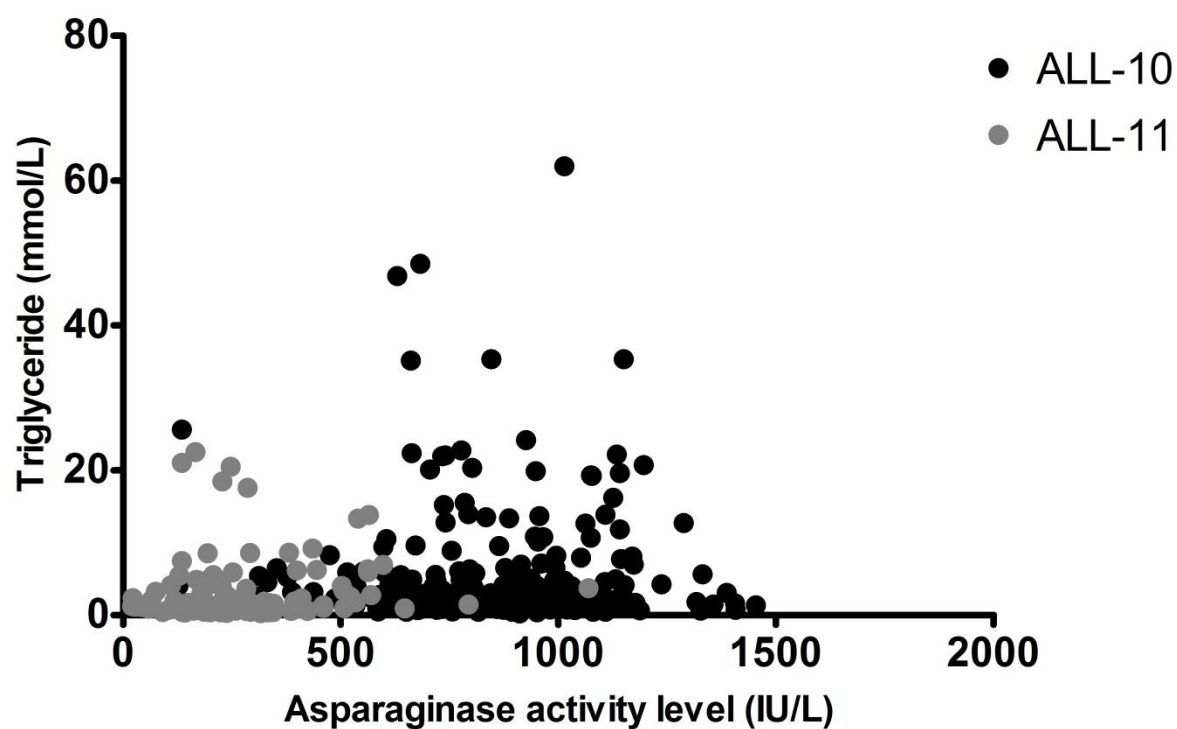


Supplemental figure 3 shows the L-asparagine levels of 50 PEGasparaginase and 20 Erwinase® samples in the box plots. The boxplot includes the 25th, 50th and 75th percentile in the boxes and the ranges (indicated by the whiskers).

For both the PEGasparaginase and Erwinase® samples, there was no association between L-asparagine and asparaginase activity levels ($p=0.587$ and $p=0.267$, respectively).

The median total asparagine levels (L- and D-asparagine) are indicated by the black dots.

Supplemental figure 4. Triglycerides in DCOG ALL-10 and ALL-11



Supplemental figure 4 shows the triglyceride levels, plotted against the asparaginase activity levels, for DCOG ALL-10(1) (black dots) and ALL-11 (grey dots).

Individualized Asparaginase Dosing in Childhood Acute Lymphoblastic Leukemia

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The information provided may not reflect the complete protocol or any previous amendments or modifications. As described in the Author Center (http://jco.ascopubs.org/site/ifc/manuscript-guidelines.xhtml#randomized_phase_one_and_two) only specific elements of the most recent version of the protocol are requested by JCO. The protocol information is not intended to replace good clinical judgment in selecting appropriate therapy and in determining drug doses, schedules, and dose modifications. The treating physician or other health care provider is responsible for determining the best treatment for the patient. ASCO and JCO assume no responsibility for any injury or damage to persons or property arising out of the use of these protocol materials or due to any errors or omissions. Individuals seeking additional information about the protocol are encouraged to consult with the corresponding author directly.

**Research Protocol ‘Individualized
Asparaginase Dosing in Childhood Acute
Lymphoblastic Leukemia’**

1. DCOG ALL-11 treatment protocol

Treatment phase	Therapy
Protocol 1A	
Prednisone	60 mg/m ² /day for 29 days followed by 3x3 days tapering
Vincristine	1.5 mg/m ² /dose at day 8, 15, 22 and 29
Daunorubicin	30 mg/m ² /dose at day 8, 15, 22 and 29 (not in case of Down syndrome)
PEGasparaginase	1,500 IU/m ² at day 12 and 26
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 15 and 33. Only intrathecal methotrexate at day 1.
Protocol 1B	
PEGasparaginase	1,500 IU/m ² at day 40
Cyclophosphamide	1,000 mg/m ² /dose at day 36 and 64
Cytarabine	75 mg/m ² /day at days 38 – 41, 45 – 48, 52 – 55, 59 – 62
6-Mercaptopurine	60 mg/m ² /day at days 36 – 63
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 45 and 59
Protocol M for SR and MR patients	
6-Mercaptopurine	25 mg/m ² /day for 56 days
Methotrexate	5,000 mg/m ² /dose at day 8, 22, 36 and 50
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 8, 22, 36 and 50
Protocol IV for SR patients	
Dexamethasone	10 mg/m ² /day for 15 days followed by 3x3 days tapering
Vincristine	1.5 mg/m ² /dose at day 1 and 8
PEGasparaginase	Individualized dose at day 1
Maintenance for SR patients	
6-Mercaptopurine	50 mg/m ² /day for 81 weeks
Methotrexate	20 mg/m ² /week for 81 weeks
Intensification and maintenance for MR patients	
Dexamethasone	6 mg/m ² /day for 5 days every 3 weeks until week 82
Vincristine	2 mg/m ² /dose every three weeks until week 82
Doxorubicin	30 mg/m ² /dose at week 1, 4, 7 and 10 (not in case of Down syndrome without IKZF1 deletion or TEL/AML1)
PEGasparaginase	Individualized doses biweekly from week 1 – 27
Methotrexate	30 mg/m ² /week from week 13 – 84 (or week 2 – 84 in

	case of Down syndrome or TEL/AML1), not during intrathecal therapy In case of an IKZF1 deletion, 200 mg/m ² /dose every three weeks from week 85 - 136
6-Mercaptopurine	50 mg/m ² /day from week 1 – 12 in courses of 2 weeks with 1 week interruption (without interruption in case of Down syndrome or TEL/AML1) and from week 13 – 84 daily, without interruption In case of an IKZF1 deletion, 100 mg/m ² /day for 10 days after each methotrexate dose from week 85 - 136
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at week 1, 19, 37, 55 and 73
High risk blocks for HR patients	
HR block 1	
6-Mercaptopurine	25mg/m ² /day from days 1 - 14
Methotrexate	5,000 mg/m ² at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Cyclophosphamide	1,200 mg/m ² /dose at day 15, 16 and 17
Etoposide	350 mg/m ² /dose at day 15, 16 and 17
PEGasparaginase	1,500 IU/m ² at day 22
Vincristine	1.5 mg/m ² /dose at day 22 and 29
HR block 2	
Methotrexate	5,000 mg/m ² at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Cytarabine	1,500 mg/m ² /dose at day 15, 16, 17, 18 and 19
Mitoxantrone	5.25 mg/m ² /dose at day 15, 16, 17, 18 and 19
PEGasparaginase	1,500 IU/m ² at day 22
Vincristine	1.5 mg/m ² /dose at day 22 and 29
HR block 3	
Methotrexate	5,000 mg/m ² at day 1
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 1
Idarubicin	6 mg/m ² /dose at day 15, 16 and 17
Fludarabine	22.5 mg/m ² /dose at day 15, 16, 17, 18 and 19
Cytarabine	1,500 mg/m ² /dose at day 15, 16, 17, 18 and 19
HR block 4*	Equal to HR block 1, but without 6-Mercaptopurine
HR block 5*	Equal to HR block 2
HR block 6*	Equal to HR block 3, but without Idarubicin
Protocol II*	

Dexamethasone	10 mg/m ² /day at days 1 – 21 followed by 3x3 days tapering
Vincristine	1.5 mg/m ² /dose at day 8, 15, 22 and 29
Doxorubicin	30 mg/m ² /dose at day 8, 15, 22 and 29
PEGasparaginase	1,500 IU/m ² at day 8
Cyclophosphamide	1,000 mg/m ² at day 36
6-Thioguanine	60 mg/m ² /day at days 36 – 49
Cytarabine	75 mg/m ² /day at days 36 – 39 and 43 – 46
Intrathecal methotrexate, cytarabine and prednisone	8 – 12 mg methotrexate, 20-30 mg cytarabine, 8-12 mg prednisone at day 36 and 43
HR maintenance*	
6-Mercaptopurine	50 mg/m ² /day from week 1 – 37
Methotrexate	20 mg/m ² /week from week 1 – 37
* Not if patients are eligible for a stem cell transplantation	

2. Inclusion and exclusion criteria

2.1 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Patients who are treated according to the DCOG ALL11 protocol:

Inclusion criteria DCOG ALL-11 protocol

1. Newly diagnosed patients with T-lineage or precursor-B lineage ALL (patients with mature B-ALL are not eligible)
2. Age between > 1 and < 19 years
3. Informed consent signed by parents/guardians and patient if 12 years or older
4. Diagnosis ALL confirmed by DCOG laboratory
5. Patient should be treated in a Dutch Childhood Oncology Centre
6. Patient should be >3 months settled in The Netherlands at diagnosis

Exclusion criteria DCOG ALL-11 protocol:

1. Age > 19 years at diagnosis
2. Age < 366 days at diagnosis (infant ALL); these patients are eligible for the Interfant protocol
3. Patients with secondary ALL
4. Patients with mature B-ALL (immunophenotypical or documented presence of karyotype t(8;14), t(2;8), t(8;22) and breakpoint as in B-ALL)
5. Patients with relapsed ALL
6. Pre-existing contra-indications for treatment according to (parts of) protocol ALL11.
7. Essential data missing (in consultation with the protocol chairman)
8. Treatment with systemic corticosteroids and/or cytostatics in a 4-week interval prior to diagnosis. One exception is the use of corticosteroids as emergency treatment.

9. Patients with Ph-positive ALL (documented presence of t(9;22)(q34;q11) and/or of the BCR/ABL fusion transcript). These patients will be transferred to the EsPhALL protocol in induction according to the guidelines of the EsPhALL protocol.

- Informed consent has to be signed by parents/guardians and patient of 12 years or older.

2.2 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Patients who have a contraindication for asparaginase.

3. METHODS

3.1 Study parameters/endpoints

3.1.1 Main study parameter/endpoint

Efficacy of Therapeutic drug monitoring

- Percentage of trough asparaginase activity levels within target range
- Asparagine concentrations: L-asparagine and D-asparagine
- Dose of PEGasparaginase and Erwinase®
- For Erwinase®, dosing interval

Toxicity

- Hypersensitivity (clinical allergies, silent inactivation and allergic-like reactions).
- Hypertriglyceridemia.
- Increased ASAT, ALAT and bilirubin levels.
- Pancreatitis.
- Thromboembolism.
- Central neurotoxicity.

3.2 Randomisation, blinding and treatment allocation

Not applicable.

3.3 Study procedures

Efficacy of Therapeutic drug monitoring

PEGasparaginase is administered during Protocol 1A/1B at day 12, 26 and day 40 with a fixed dose of 1,500 IU/m². Trough levels are measured prior to each dose, complemented with a week level at day 19.

During medium risk intensification, patients are treated with individualized PEGasparaginase doses at week 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27, targeting trough levels of 100-250 IU/L. Trough levels are standardly measured prior to each dose but every four weeks when stable PEGasparaginase levels are reached. At week 2, a week level is measured. Standard risk patients are treated with 1 individualized dose at day 1, followed by

measurement of PEGasparaginase levels 1 and 2 weeks after administration. High risk patients are treated with 2-5 fixed doses of 1,500 IU/m² PEGasparaginase with intervals of approximately 7 weeks.

In case of a neutralizing hypersensitivity reaction, patients were switched to intravenous Erwinase® 20,000 IU/m², starting three times a week for two weeks. After two weeks, the dose and/or dosing schedule was adjusted to ensure Erwinase® activity levels >100 IU/L. Asparagine and glutamine concentrations were measured in serum samples collected in the Erasmus MC. Beside total asparagine levels, L-asparagine and D-asparagine were measured.

Table: PEGasparaginase dose modifications

PEGasparaginase trough level	Dose adjustment
>600 IU/L	50%
500 – 599 IU/L	60%
400 – 499 IU/L	70%
300 – 399 IU/L	80%
200 – 299 IU/L	100%
100 – 199 IU/L	100%
50 – 99 IU/L	100%
30 – 49 IU/L	150%
10 – 29 IU/L	200%

Toxicity

Liver enzymes (ALAT, ASAT, bilirubin), amylase, and glucose are measured before each PEGasparaginase infusion and every 2 weeks in case of Erwinia asparaginase as part of standard care (minor toxicity). Major toxicity including allergy, pancreatitis, neurotoxicity and thrombosis, are monitored by the DCOG ALL11 toxicity registration according to the CTCAE 4.03 criteria, also as part of standard care. Major toxicity parameters will be investigated in all Dutch Oncology Centers.

The minor toxicity parameters, will be investigated in patients who are treated in the Sophia Children's Hospital, Rotterdam or the Academic Medical Centre in Amsterdam. Triglyceride concentration will be added to the standard blood tests at several time points during protocol 1A/1B, the M-protocol and MRG.

Triglyceride concentration will be measured at the following time points prior to the asparaginase dose:

- Protocol 1A/1B: day 12 (baseline measurement), day 26, day 40.
- M-protocol: before the first and third asparaginase dose in the continuous treatment arm and before the first and third HD-MTX dose in the non-continuous arm.
- MRG: week 1, 3, 5, 7, 9, 15, 25 and 37.

Triglyceride levels will be measured at week 37 of MRG in all patients when they are no longer treated with asparaginase, to evaluate if the levels are within normal range again. All triglyceride levels will be measured using the residual blood from the standard blood tests. In week 37 of MRG blood will be obtained during the standard bone marrow puncture. Triglyceride levels will be measured in the hospital where the patient is treated. Asparaginase toxicity will be compared between patients with continuous and non-continuous asparaginase therapy and between patients who are treated according to the fixed dose schedule in the ALL10 protocol and the individualized dosing in ALL11.

3.4 Methods of measurement

Asparaginase activity levels were measured using the L-aspartic β -hydroxamate (AHA) assay as described earlier.(1, 2) Asparaginase inactivation was defined as PEGasparaginase activity <100 IU/L after 7 ± 1 days (week level) and/or <10 IU/L after 14 ± 1 days (trough level).

For asparagine and glutamine measurement, whole blood was directly put on ice and 10% sulfosalicylic acid was added. The samples were stored in -80°C until analysis using liquid chromatography tandem-mass spectrometry (limit of quantification: $0.05\ \mu\text{M}$). Human asparagine measured comprises both L-asparagine and D-asparagine. The latter is also present in humans but not incorporated in proteins, and ranges between $0.017\text{--}0.18\ \mu\text{M}$.(3) Therefore, beside the total asparagine concentration, L-asparagine was measured in 50 patients with varying asparaginase activity levels.

PEGasparaginase and native *E. coli* asparaginase antibodies were measured with Enzyme-Linked Immuno Sorbent Assay (ELISA) as described earlier.(4) The threshold for a positive antibody measurement was calculated using the F-measure.(5)

3.5 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. In case of allergy or silent inactivation, the patient will be withdrawn from the population pharmacokinetic study. These patients, however, will be included in the data-analysis. Furthermore, these patients will then be treated with Erwinia asparaginase and toxicity will still be monitored.

3.5.1 Specific criteria for withdrawal (if applicable)

Not applicable.

3.6 Replacement of individual subjects after withdrawal

There will be no replacement of subjects.

3.7 Follow-up of subjects withdrawn from treatment

There will be no follow-up of patients after withdrawal for this particular studies.

3.8 Premature termination of the study

If there are any alterations in the ALL11 protocol concerning PEGasparaginase treatment, the study have to be prematurely terminated.

4. SAFETY REPORTING

Safety reporting will be performed via the DCOG safety reporting from ALL11. No specific adverse events are expected in the current studies because patients will be treated according standard care and no study medication is used. Therefore this section is considered not applicable.

5. STATISTICAL ANALYSIS

5.1 Primary study parameter(s)

Continuous data was presented as mean and standard deviation (SD) or median and interquartile range (IQR). Student t-tests, Mann-Whitney U tests, χ^2 -(trend)tests, and Fisher exact tests were used to compare baseline characteristics.

Efficacy of Therapeutic drug monitoring

Asparaginase doses and concentrations will be described as median (interquartile range) asparaginase activity levels and doses, and the percentage of concentrations within target range.

Asparagine concentrations will be described as percentage of samples $<0.2 \mu\text{M}$ and $<0.5 \mu\text{M}$. Glutamine concentrations will be described as mean (standard deviation).

Toxicity study

Primary outcomes are hypersensitivity reactions, haemorrhage or thrombosis, pancreatitis, central neural toxicity, hypertriglyceridemia and increased liver enzymes according to CTCAE 4.03 criteria (ordinal data). Continuous toxicity will be analyzed with repeated measurement analyses. Discontinuous toxicity will be analyzed with Kaplan-Meier's methodology.

A two-sided p-value of <0.05 will be considered statistically significant. Intention to treat analysis will be performed.

5.2 Secondary study parameter(s)

Not applicable.

5.3 Other study parameters

Not applicable.

5.4 Interim analysis (if applicable)

Not applicable.

6. ETHICAL CONSIDERATIONS

6.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts.

6.2 Recruitment and consent

All eligible pediatric ALL patients will have the option to be included in this study. The patient and his or her parents/legal surrogates will be informed by their treating physician after the diagnosis. They will receive a patient information letter and be given time to consider the option.

6.3 Objection by minors or incapacitated subjects (if applicable)

All participants in this study will be minors. For this study we will take the following code of conduct into account: Gedragscode bij verzet van minderjarigen die deelnemen aan medisch-wetenschappelijke onderzoek (Code of conduct for dealing with the expression of objection by minors participating in medical research), produced by the Netherlands Association for Paediatric Medicine, Nieuwsbrief NVK (NVK Newsletter) no. 3, June 2001.

6.4 Benefits and risks assessment, group relatedness

There is no direct benefit for the participants. Results of this study will most likely be of importance in future asparaginase treatment for children with cancer. The risk of these sub-studies is negligible. We will not make any changes to standard treatment in these studies.

6.5 Compensation for injury

There is an exemption for insurance requested, see G1: request for exemption, because patients will be exposed to merely negligible risk in these sub-studies.

6.6 Incentives (if applicable)

Not applicable.

7. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

7.1 Handling and storage of data and documents

A database will be established where all data from the study will be documented. Data will be monitored by R.Q.H. Kloos. She will ensure that the clinical trial is conducted, recorded, and reported in accordance with the protocol, ICH-GCP, and the applicable regulatory requirement(s).

Source documents are defined as: patient medical files, letters and laboratory records.

All data of the patients will be coded with a consecutive number. Only the investigator and the study team will have direct access to identifiable patient records together with all parties as defined by national law.

7.2 Monitoring and Quality Assurance

Monitoring will be according to the Erasmus MC Monitoring Plan. In this study there will be negligible risk and therefore 'attachment A' shall apply.

7.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

7.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, other problems, and amendments.

7.5 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the

end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

7.6 Public disclosure and publication policy

The trial will be registered at the 'Nederlands Trial Register' (www.trialregister.nl) and the CCMO. The Nederlands Trial Register is a WHO accredited trial register. The final results of the clinical trial will be published after complete data collection and evaluation. The publication will be prepared by RQH Kloos under supervision of IM van der Sluis. The results of this study will be communicated to colleagues supervised by the coordinating investigator through international publications and presentations at national and international conferences on pediatric oncology/hematology.

8. STRUCTURED RISK ANALYSIS

Not applicable.

8.1 Potential issues of concern

8.2 Synthesis

1. Kloos RQ, Pieters R, Escherich G, van der Sluis IM. Allergic-like reactions to asparaginase: Atypical allergies without asparaginase inactivation. *Pediatr Blood Cancer*. 2016.
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3. Visser WF, Verhoeven-Duif NM, Ophoff R, Bakker S, Klomp LW, Berger R, et al. A sensitive and simple ultra-high-performance-liquid chromatography-tandem mass spectrometry based method for the quantification of D-amino acids in body fluids. *J Chromatogr A*. 2011;1218(40):7130-6.
4. Tong WH, Pieters R, Kaspers GJ, te Loo DM, Bierings MB, van den Bos C, et al. A prospective study on drug monitoring of PEGasparaginase and Erwinia asparaginase and asparaginase antibodies in pediatric acute lymphoblastic leukemia. *Blood*. 2014;123(13):2026-33.
5. Van Rijsbergen CJ. *Information Retrieval*. 2nd edition ed. Butterworth1979.