SUPPLEMENTARY APPENDIX

Individualized dosing guidelines for PEGasparaginase and factors influencing the clearance: a population pharmacokinetic model

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Supplemental methods

Measurements

Asparaginase activity levels were measured using the L-aspartic β-hydroxamate (AHA) assay.[1] Briefly, AHA is added to patient serum containing PEGasparaginase and consequently hydrolyzed to L-aspartic acid and hydroxylamine. Hydroxylamine condenses with 8-hydroxyquinoline and oxidizes to indooxine, which is quantified by photometric detection at 690 nm. The lower limit of quantification (LLQ) was 10 IU/L.

Population PK analysis

The population pharmacokinetic analysis was performed using NONMEM® Version 7.2 (Icon Development Solutions, Ellicott City, Maryland, USA). Other statistical analyses were performed using IBM SPSS Statistics (IBM Corp, Armonk, New York, USA) version 21.0 for Windows. The graphs to evaluate the models were prepared in R and Sigmaplot Version 3.4.1 (Systat Software Inc, London, UK).

In case of missing data of continuous covariates, the last known value or the median was implemented. Missing discontinuous data was excluded from the analysis.

The activity data were logarithmically transformed and the analysis was performed with the First Order Conditional Estimation method with interaction (FOCE+I).

In the development of the structural model, one- and two compartment models were evaluated. Subsequently, models with first- and zero-order elimination, Michaelis-Menten elimination, and time-dependent elimination were explored. The pharmacokinetics were expressed in terms of clearance (CL) and volume of distribution (Vd).

Time profiles of PEGasparaginase activity versus time were adequately described using a one-compartment model. Addition of a second compartment did not improve the model. Models with first- and zero-order elimination did not describe the data adequately. Models with time-dependent CL, previously described by Würthwein et al.[2] described the data better.

Inter-individual variability and inter-occasion variability, with an occasion defined as administration of a new dose, and correlation between Cl and Vd were assessed in the models. Inter-individual and inter-occasion variability in Cl and Vd was characterized with exponential models. For example, the elimination for the i^{th} patient was estimated using the following equation:

$$CLi = \Theta pop \times e^{(\eta i + ki)}$$

Were Θpop is the typical population value for CL. η_i and k_i represent random effects accounting for individual and occasion variation from the typical value. η_i and k_i are assumed to be symmetrically distributed with a mean of 0 and estimated variance of ω^2 and π^2.

Additional and proportional error models were evaluated to account for the residual error. Furthermore, since body surface area (BSA) is known to be an important covariate for PEGasparaginase pharmacokinetics, this was included in the structural model.

$$CLi = \Theta pop\ x\ BSA \times e^{(\eta i + ki)}$$

To allow for asparaginase activity levels below the limit of quantification (LLQ), several methods were applied. Both the M3[3] and M2 method, however, resulted in unstable runs of particularly the time-varying elimination models. Because only 4% of the patients had developed a neutralizing hypersensitivity reaction, this has only little influence on the analysis. Therefore, we have decided to exclude the values <LLQ.

The precision of the parameter estimates, objective function values (OFV's) and goodness of fit plots were used for selection of the models evaluated. A decrease in the OFV of >3.84 points and >10.83 points was considered as a significant improvement of the model with significance of $p<0.05$ and $p<0.001$, respectively.

After obtaining the structural model, several covariates were evaluated as described by the following equations:

Continuous data:

$$\left(\frac{covariate}{median}\right)^{\Theta}$$

Discontinuous data:

$$covariate \times e^{\Theta}$$

For example, the effect of the continuous covariate leukocyte count on Cl was explored by incorporating the leukocyte count divided by the median ($2.4 * 10^9$/L) to the power of Θ in the equations of Cl and Vd. The discontinuous covariate 'infection' was explored by multiplying the Cl and Vd by Θ in case of an infection.

The covariates were first explored with univariate analysis after which the significant covariates (OFV -3.84) were evaluated with stepwise forward inclusion and backward elimination (OFV -10.83).

A bootstrap analysis with 1000 bootstrap replicates was used to assess the robustness of the model. Visual predictive check (VPC) plots were used for internal validation of the model. An independent validation dataset, obtained by randomly selecting 25% of the total population, was used to validate the final model externally. The VPCs were prediction corrected to correct for the dose adjustments of PEGasparaginase.

To develop dosing guidelines, Monte Carlo simulations were performed. Starting doses were calculated targeting trough asparaginase activity levels >100 IU/L, >250 IU/L and >350 IU/L, taking into account the significant covariates. By stepwise increasing the dose in simulations, it was evaluated which loading and maintenance dose provides adequate trough levels in 95% of the patients.

Dosing guidelines were developed targeting at a trough asparaginase activity level of 100-250 IU/L or 250-400 IU/L based on week- or trough levels. For adjustment of the PEGasparaginase dose based on week levels, trough levels were predicted based on individual simulated time profiles of PEGasparaginase activity.

Supplemental results

PK analysis

First, the asparaginase activity levels were log transformed. To account for residual error, an additive and proportional model were evaluated. As a combined model of proportional and additive error was superior, this was further used in development of the model. Linear models and models with time-constant elimination did not adequately describe the data. This analysis, however, showed that a one-compartment model was sufficient and adding body surface area (BSA) as a covariate did significantly improved the model (OFV -22.7). Also inter-individual variability (IIV) on Cl and Vd, inter-occasion variability (IOV) on Cl, and correlation between Cl and Vd significantly improved the model. Next, the models with time-varying clearance as described by Würthwein et al.[2] were tested. These models comprised several exponential elimination equations with initial and induced clearance. However, these models did not adequately describe the data as well. Würthwein et al.[2] have concluded that a split point model best describes the PEGasparaginase pharmacokinetics by exploring transit models. They concluded that the Cl was constant at first but increased after approximately 10 days. Therefore, we next have evaluated a transit model, estimating after how many days the clearance increases. This model most adequately described the data, estimating the split point at 12.9 days after administration. Hence, the final structural model was as follows:

$$Cl\ (first\ 13\ days) = \Theta_1 * e^{\eta + \eta IOV} * BSA$$

$$Cl\ (after\ 13\ days) = \Theta_1 * e^{\eta + \eta IOV} * BSA * Cl_{ind}$$

$$Cl_{ind} = 1 + \Theta_2 * (TAD - split\ point)$$

$$Vd = \Theta_3 * e^{\Theta_4 * \eta} * BSA$$

Where TAD is time after dose and the Cl_{ind} increases with Θ_2 per day. In the equation of Vd, Θ_4 represents the correlation between Cl and Vd. Table 2 shows the parameter estimates of the final model with a Cl of 0.075 L/day/m^2 , increasing with 0.079 L/day/m^2 after 12.9 days, and a Vd of 0.92 L/m^2. IIV on Vd could not be estimated as this completely correlated with the correlation between Cl and Vd.

After obtaining the structural model, the covariates were evaluated one by one. Univariate analysis resulted in 16 significant covariates influencing the clearance (Table 3). However, the anti-asparaginase antibodies, creatinine and leukocytes had large relative standard errors and the 95% confidence interval included 0. Infection, treatment phase and intensive care unit (ICU) admission resulted in the largest decrease of OFV and were therefore first evaluated during the multivariate analysis. Multivariate analysis with treatment phase and the presence of an infection significantly improved the model (OFV -21.6) compared to the structural model. Further addition of ICU admission did not improve the model (OFV -2.6) and was, therefore, excluded. Similar results were found for anti-asparaginase antibodies, creatinine and leukocyte levels. As explained in the main article, only methotrexate and doxorubicin significantly improved the model on top of treatment phase and infection (OFV -10.3, mean effect (RSE): 0.88 (5%) and OFV -6.0, mean effect (RSE): 1.24 (6%), respectively). Adding both drugs in the analysis did not improve the model (OFV -0.04) and both drugs were not significant during backward elimination. Finally, treatment phase and infection were included in the final model.

Simulations

Using the final population model, Monte Carlo simulations were performed for 2000 virtual patients with BSA ranging from 0.52 to 2.3 m^2. All patients received bi-weekly steady-state doses of PEG-asparaginase with doses ascending from 100 IU/m^2 to 3000 IU/m^2 in 100 IU/m^2 steps. Trough levels and levels one week after administration were evaluated. Target trough levels of 100 – 250 IU/ml corresponded to levels of 200 – 450 IU/ml at one week after administration. Similarly, target trough levels of 250-400 IU/ml corresponded to levels of 450-750 IU/ml at one week after administration. When simulated levels were outside the target range it was evaluated to what extent the dose had to be increased or decreased to obtain adequate levels.

References

1. Lanvers C, Vieira Pinheiro JP, Hempel G, Wuerthwein G, Boos J. Analytical validation of a microplate reader-based method for the therapeutic drug monitoring of L-asparaginase in human serum. *Anal Biochem* 2002 Oct 1; **309**(1)**:** 117-126.

2. Wurthwein G, Lanvers-Kaminsky C, Hempel G, Gastine S, Moricke A, Schrappe M*, et al.* Population Pharmacokinetics to Model the Time-Varying Clearance of the PEGylated Asparaginase Oncaspar(R) in Children with Acute Lymphoblastic Leukemia. *Eur J Drug Metab Pharmacokinet* 2017 Mar 27.

3. Beal SL. Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn* 2001 Oct; **28**(5)**:** 481-504.

Supplemental Table 1: 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, 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
PEGasparaginase	
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 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	

Supplemental Table 2. Patient characteristics

	Index dataset n = 92			**Validation dataset n = 28**		
Sex Male	51 (55%)			18 (64%)		
Female	41 (45%)			10 (36%)		
Age, years (median, IQR)	4.8 (3.3 – 8.2)			7.7 (3.3 – 12.5)		
Weight, kg (median, IQR)*	19.2 (14.9 – 29.3)			28.0 (16.5 – 47.9)		
BSA, m^2 (median, IQR)*	0.76 (0.65 – 1.05)			1.04 (0.68 – 1.44)		
Type of ALL						
Pro-B cell	0			0		
Common B-cell	38 (41%)			14 (50%)		
Pre-B cell	11 (12%)			7 (25%)		
Common T-cell	5 (6%)			3 (11%)		
Unknown[%]	38 (41%)			4 (14%)		
Genetics of ALL						
TEL/AML1	19 (21%)			6 (21%)		
t(1;19)	0			1 (4%)		
MLL-rearrangements	0			0		
Hyperdiploid	15 (16%)			5 (18%)		
Other B cell	15 (16%)			9 (32%)		
Other T cell	5 (6%)			3 (11%)		
IKZF1-deletion	5 (6%)			0		
Unknown[%]	38 (41%)			4 (14%)		
Risk group						
Standard risk	13 (14%)			3 (11%)		
Medium risk (%)	76 (83%)			23 (82%		
Continuous	*27*			*4*		
Discontinuous	*49*			*20*		
High risk	2 (2%)			-		
Not stratified	1 (1%)			2 (7%)		
Asparaginase related toxicity	**No**	**Yes**	**Unknown[%]**	**No**	**Yes**	**Unknown[%]**
Allergy	90 (98%)	2 (2%)	0	29 (100%)	0	0
Silent inactivation	90 (98%)	2 (2%)	0	29 (100%)	0	0
Central neurotoxicity[#]	54 (58%)	1 (PRES)	38 (41%)	23 (79%)	1 (4%)	4 (14%)
Thrombosis[#]	54 (58%)	1 (1%)	38 (41%)	23 (79%)	1 (4%)	4 (14%)
Pancreatitis[#]	88 (96%)	4 (4%)	0	22 (75%)	2 (8%)	4 (14%)
Number of infections[$]	19			12		
Unknown, number of patients (%)[%]	38 (41%)			4 (14%)		
Number of ICU admissions	3			2		
Unknown, number of patients (%)[%]	38 (41%)			4 (14%)		
Leukocytes, * 10^9/L (median, IQR)	2.4 (1.5 – 4.0)			2.4 (1.6 – 3.4)		
Measurements missing (%)[%]	100 (8%)			20 (5%)		
AST, U/L (median, IQR)	44 (30 – 65)			46 (33 – 66)		
Measurements missing (%)[%]	364 (45%)			155 (38%)		
ALT, U/L (median, IQR)	65 (40 – 95)			67 (45 – 97)		
Measurements missing (%)[%]	364 (45%)			155 (38%)		
Creatinine, µmol/L (median, IQR)	27 (22 – 33)			27 (21 – 38)		
Measurements missing (%)[%]	508 (62%)			237 (59%)		
Albumin, g/L (median, IQR)	33 (29 – 40)			32 (27 – 39)		
Measurements missing (%)[%]	721 (88%)			370 (91%)		
Native *E. coli* asp AB, OD (median, IQR)	0.018 (0.010 – 0.030)			0.008 (0.006 – 0.018)		

Measurements missing (%)[%]	333 (41%)	228 (56%)
PEGasp AB, OD (median, IQR) Measurements missing (%)[%]	0.019 (0.010 – 0.034) 333 (41%)	0.009 (0.006 – 0.017) 228 (56%)

IQR: interquartile range; BSA: body surface area; PRES: posterior reversible encephalopathy syndrome; ICU: intensive care unit; AB: antibodies; AST: aspartate transaminase ; ALT: alanine transaminase; OD: optical density; AB: antibodies asp: asparaginase; PEGasp: PEGasparaginase. Laboratory measurements were done during asparaginase activity level measurement.

[%] Clinical data of the patients not treated in the Sophia Children's Hospital was missing.

* Weight and BSA measured at start PEGasparaginase therapy.

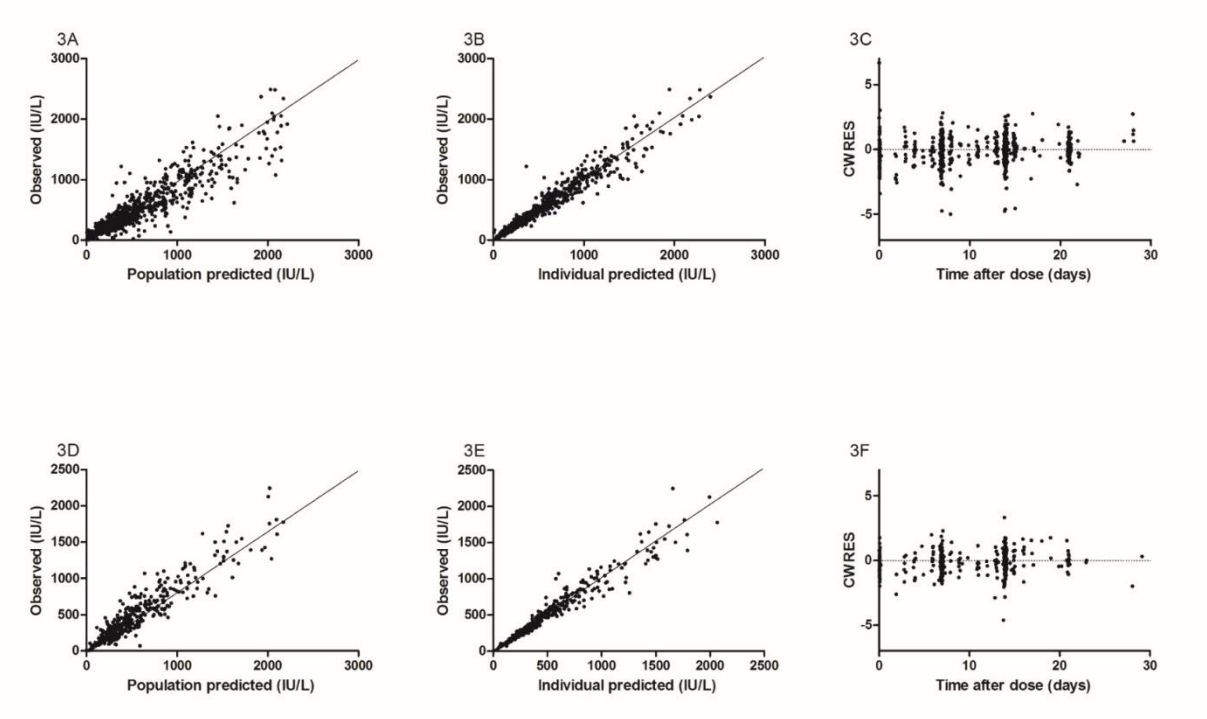
[#] Only Common Terminology Criteria for Adverse events 4.03 grade 3 and 4.

[$] Infections were defined as fever (>38° Celsius) and hospital admission or prescription of antibiotics.

Supplemental table 3. 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	125%
30 – 49 IU/L	150%
10 – 29 IU/L	200%

Supplemental figure 1. Goodness of fit plots



Supplemental Figure 1. Figure 2A and 2D show the observed asparaginase activity levels plotted against the population predicted values for the main and external database, respectively. In these figures, the dots are evenly distributed around the line of unity.
Figure 2B and 2E show the observed values plotted against the individual predicted values. Also in this figure, the dots are evenly distributed around the line of unity.
Figure 2C and 2F show the conditional weighted residuals (CWRES) plotted against the time after dose. Here, most dots are between -2 and 2, and show no trend.