



# Impact of Antibodies Against Polyethylene Glycol on the Pharmacokinetics of PEGylated Asparaginase in Children with Acute Lymphoblastic Leukaemia: A Population Pharmacokinetic Approach

Christian Siebel<sup>1</sup> · Claudia Lanvers-Kaminsky<sup>1</sup> · Julia Alten<sup>2</sup> · Petr Smisek<sup>3</sup> · Christa E. Nath<sup>4,5</sup> · Carmelo Rizzari<sup>6</sup> · Joachim Boos<sup>1</sup> · Gudrun Würthwein<sup>1</sup>

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## Abstract

**Background and Objectives** Besides allergic reactions, antibodies against polyethylene glycol (PEG) have been associated with reduced PEG-asparaginase (PEG-ASNase) activity. Population pharmacokinetics (popPK) allow for an in-depth investigation of the influence of anti-PEG antibodies on PEG-ASNase pharmacokinetics.

**Methods** PEG-ASNase activity (6261 samples) and anti-PEG antibodies (2082/6412 samples prior to/post administration) in 1444 children with acute lymphoblastic leukaemia treated in the AIEOP-BFM ALL 2009 trial were evaluated. Patients received two doses of PEG-ASNase during induction (2500 U/m<sup>2</sup>, intravenous, biweekly) and a third dose during reinduction treatment. Anti-PEG IgG and IgM measured prior to and post administration were explored for their influence on the initial clearance of PEG-ASNase using a previously established popPK model. Categorical and continuous antibody data, including each isotype individually as well as in combination, were assessed.

**Results** High pre-existing levels of anti-PEG antibodies increase the initial drug clearance. Analysed separately, both anti-PEG IgG<sub>prior</sub> and IgM<sub>prior</sub> were significant covariates; the stronger effect was observed for anti-PEG IgM<sub>prior</sub>. Hockey stick models best described the data. For anti-PEG IgM<sub>prior</sub>, each additional log unit above the estimated cut point was related to a 41.4% increase in initial clearance after the first dose in induction. Antibody levels below the cut point were not associated with an effect on clearance. The combination of both isotypes did not provide additional information compared to anti-PEG IgM<sub>prior</sub> alone. Antibody levels post administration were not associated with an effect on clearance.

**Conclusion** Pre-existing antibodies against PEG-ASNase significantly increased the initial clearance in a subgroup of patients showing high antibody levels. (Trial registration: EU clinical trials register; EudraCT No: 2007-004270-43; first registered 23 October 2009.)

## 1 Introduction

Polyethylene glycol (PEG)-conjugated asparaginase (PEG-ASNase, Oncaspar<sup>®</sup>) is used in frontline treatment of children with acute lymphoblastic leukaemia (ALL) in a large number of contemporary treatment protocols [1–5]. PEGylation prolongs the circulation time of the enzyme and reduces its immunogenicity [6]. Though initially assumed to be biologically inert, PEG has been found to be immunogenic itself. Antibodies against PEG have been detected in patients upon treatment with PEG-ASNase, but also with other drugs

### Key Points

High pre-existing levels of anti-PEG IgG and IgM antibodies significantly increase PEG-ASNase clearance during ALL induction treatment and thus affect the individually experienced treatment intensity.

This effect is especially pronounced for pre-existing anti-PEG IgM. In contrast, no effect was observed for antibodies identified in monitoring samples after PEG-ASNase administration.

It is recommended that pre-existing antibodies are taken into account when adjusting the PEG-ASNase dose to predefined activity ranges.

✉ Gudrun Würthwein  
wurthwg@uni-muenster.de

Extended author information available on the last page of the article

such as PEGylated uricase or PEGylated recombinant phenylalanine ammonia lyase [7–11]. Furthermore, an increasing prevalence of antibodies against PEG has been observed in the general population (ranging from 0.2 to 72%), which is attributed to the widespread use of PEG in cosmetics or food products (summarised in [12]).

In the AIEOP-BFM ALL 2009 trial, PEG-ASNase was applied in frontline therapy of children with ALL (EudraCT number: 2007-004270-43). As part of the trial, extensive monitoring of PEG-ASNase serum activity as well as anti-PEG antibody levels was conducted. In a previous analysis of part of the study data performed by our working group, a high prevalence of anti-PEG IgG (13.9%) and IgM (29.1%) prior to the first PEG-ASNase dose was observed [12]. Remarkably, these pre-existing anti-PEG IgG and IgM were associated with a reduction in PEG-ASNase activity following the first drug administration in induction treatment. One plausible explanation for the observed decrease in PEG-ASNase activity is accelerated elimination of antigen–antibody complexes.

Apart from pre-existing anti-PEG IgG and IgM, therapy-induced antibodies may also affect the distribution and elimination of PEG-ASNase. Hence, the aim of the present analysis was to provide a more detailed analysis of the potential influence of anti-PEG antibodies on PEG-ASNase pharmacokinetics in children with ALL. Specifically, the focus was on the role of anti-PEG antibodies in patients not affected by hypersensitivity reactions (clinically allergic reactions accompanied by a loss of ASNase activity) or clear silent inactivation (insufficient ASNase activity without allergy symptoms) [13]. Hypersensitivity reactions and silent inactivation constitute well-defined clinical categories associated with the development of distinct antibodies [7, 8, 14, 15]. However, even beyond these clear-cut phenomena, anti-PEG antibodies may have a quantifiable impact on the pharmacokinetics of PEG-ASNase and may in part explain the observed variability of PEG-ASNase pharmacokinetics in paediatric ALL patients [16].

Population pharmacokinetic (popPK) modelling was applied as a tool. A popPK model for PEG-ASNase during induction and reinduction treatment within the AIEOP-BFM ALL 2009 trial was recently published by our working group and served as a starting point to analyse the impact of anti-PEG antibodies on the pharmacokinetics of PEG-ASNase [16, 17].

## 2 Methods

### 2.1 Study Design

Patients receiving induction and reinduction treatment according to the AIEOP-BFM ALL 2009 protocol (EudraCT

number: 2007-004270-43) were included in the analysis. During induction treatment (protocol IA), patients received two doses of PEG-ASNase on days 12 and 26 (all patients). A third dose was given during reinduction treatment (protocol II) on day 8 (non-high-risk patients only). PEG-ASNase was applied as a 2 h infusion at a dose of 2500 U/m<sup>2</sup>/day (max. 3750 U/day). The analysis refers to samples from patients enrolled in the German and Czech part of the multinational trial. The trial was approved by the appropriate national and local review boards and conducted in accordance with the Declaration of Helsinki, the ICH Guideline for Good Clinical Practice and applicable national legislation. Informed consent was obtained from the parents or legal representatives of the children. As the analysis described here extended the original informed consent, an additional ethics committee vote was obtained (Ethics Committee of the Westfalen-Lippe Medical Association and the University of Münster; reference number 2021-071-f-S).

### 2.2 Data

Pharmacokinetic sampling was scheduled prior to the first PEG-ASNase administration in induction and reinduction treatment as well as 7 and 14 days after each PEG-ASNase administration. Samples were analysed for PEG-ASNase activity as well as anti-PEG IgG and anti-PEG IgM, as described in Section 1 of the Electronic Supplementary Material (ESM). Samples with PEG-ASNase activities below the lower limit of quantification (LLOQ) of the bio-analytical method were not included in the data set due to the small number of samples < LLOQ (3.1% of all samples). Both anti-PEG IgG and IgM were analysed. Results of antibody measurements were expressed as the mean fluorescence intensity (MFI). Outliers were not defined for anti-PEG antibody levels.

Criteria for the exclusion of patients and samples from the analysis are summarised in Fig. 1. As the analysis focused on the impact of anti-PEG antibodies on the standard pharmacokinetic processes of PEG-ASNase, samples obtained at or after the development of a hypersensitivity reaction or silent inactivation (defined as PEG-ASNase activity < 100 U/L within 8 days and/or PEG-ASNase activity < LLOQ within 15 days after administration) [13] were excluded.

Antibody levels obtained prior to the first PEG-ASNase administration per treatment phase and after each administration were included in the analysis as both continuous and categorical variables:

- Log of the absolute value
- Log of the ratio of antibody levels after administration vs. prior to administration
- Categorical into  $\leq$  cut point vs.  $>$  cut point

To categorise antibody levels, cut points previously defined for a PEG-naïve reference population were used (see [12] for details). Antibody levels were categorized as positive or negative for anti-PEG IgG (MFI > 8) or IgM (MFI > 2).

## 2.3 Pharmacokinetic Analysis

### 2.3.1 Population Pharmacokinetic Model

The popPK model for PEG-ASNase previously published by our working group served as a starting point (described in detail in [16, 17]). The model consists of a chain of 14 transit compartments to describe the increase in elimination over time (see Fig. S1 in the ESM). The initial clearance ( $CL_{initial}$ ) and volume of distribution ( $V$ ) were found to be dependent on the administration (difference between the first and second doses in induction) and treatment phase (difference between induction and reinduction). Demographic covariates (age, sex) were also found to affect  $CL_{initial}$  [16].

### 2.3.2 Anti-PEG Antibody Covariate Model Building

Anti-PEG IgG and IgM measured prior to and after the administration of PEG-ASNase were evaluated as potential influential covariates affecting  $CL_{initial}$ . In a first step, the

influences of anti-PEG IgG and IgM on  $CL_{initial}$  were tested separately with regard to the high collinearity between both potential covariates. In a second step, models that combine both antibody isotypes were evaluated. In both steps, antibodies were included in the models as either categorical or continuous (log-transformed) variables (see Section 2 in the ESM for details).

In the case of categorical covariates, distinct models for

- Anti-PEG IgG or IgM prior to PEG-ASNase administration (“anti-PEG IgG<sub>prior</sub>”/“anti-PEG IgM<sub>prior</sub>”)
- Anti-PEG IgG or IgM after PEG-ASNase administration (“anti-PEG IgG<sub>after</sub>”/“anti-PEG IgM<sub>after</sub>”)

were assessed.

Categorised antibody data were evaluated using the following proportional shift model:

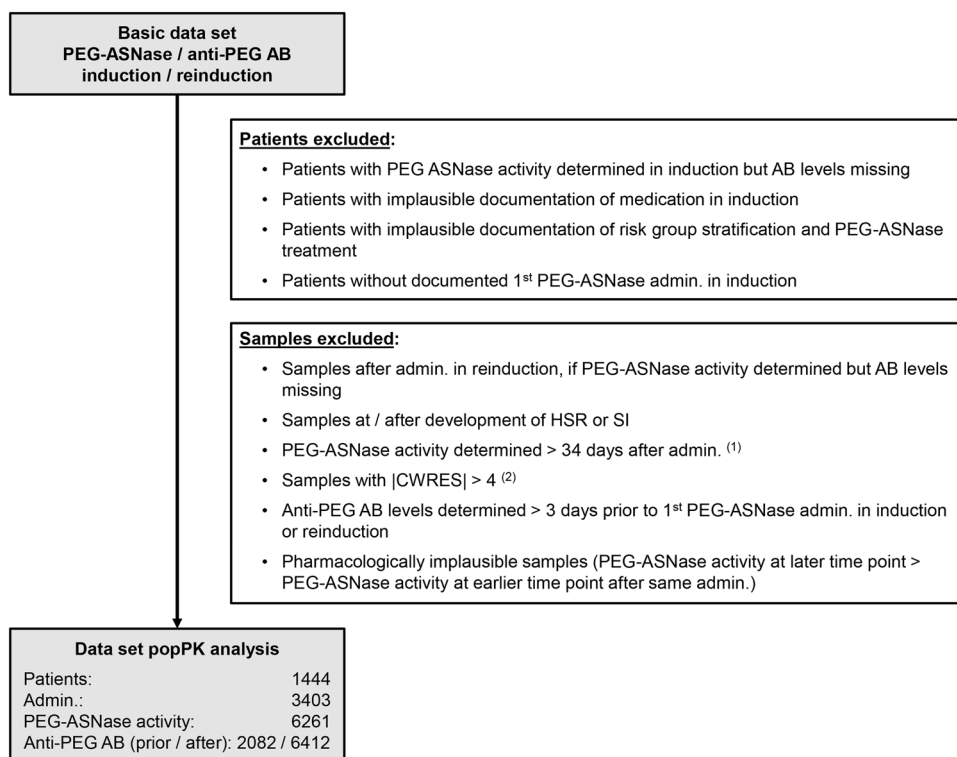
$$CL_{initial} = \theta_{pop} \times (1 + \theta_{AB}) \quad (1)$$

Here,  $\theta_{pop}$  is the  $CL_{initial}$  value when antibody levels were below the respective cut point. In this case,  $\theta_{AB}$  is fixed at 0.  $\theta_{AB}$  is the fractional change in  $CL_{initial}$  when antibody levels were above the cut point.

In the case of continuous covariates, different models evaluating

- Anti-PEG IgG<sub>prior</sub> or IgM<sub>prior</sub>

**Fig. 1** Overview of the data set used to analyse the impact of anti-PEG antibodies on the pharmacokinetics of PEG-ASNase. <sup>(1)</sup>Anti-PEG AB levels determined > 34 days after administration were included in the data set (flagged with EVID = 2). <sup>(2)</sup>Outliers identified during previous modelling steps (26 samples overall) [16]. *AB* antibody, *admin.* administration, *CWRES* conditional weighted residual, *HSR* hypersensitivity reaction, *popPK* population pharmacokinetic, *SI* silent inactivation



- Anti-PEG IgG<sub>after</sub> or IgM<sub>after</sub>
- The ratio of antibody levels after administration vs. prior to administration

were assessed.

Antibody data were log-transformed due to the large range of antibody levels. Log-transformed variables were included in the model using linear covariate–parameter relationships, as described in

$$CL_{\text{initial}} = \theta_{\text{pop}} \times (1 + \theta_{\text{AB}} \times (\text{AB} - \text{AB}_{\text{median}})) \quad (2)$$

Here,  $\theta_{\text{pop}}$  is the  $CL_{\text{initial}}$  value in individuals with antibody levels (AB) equal to the median antibody level.  $\theta_{\text{AB}}$  is the fractional change in  $CL_{\text{initial}}$  with each log unit difference in the antibody level from the median antibody level in the population. When assessing the ratio of antibody levels after vs. prior to PEG-ASNase administration, centring of the effect of the covariate on the median was not performed. Thus,  $\theta_{\text{pop}}$  reflected the  $CL_{\text{initial}}$  value when the antibody level after administration was not different from that prior to administration.

As the anti-PEG antibodies represent time-varying covariates, two extended approaches described by Wählby et al. [18] were additionally tested:

- In the first approach, the covariate effect was split into a baseline covariate effect of pre-existing antibodies ( $\theta_{\text{BCOV}}$ ) and a change from baseline covariate effect expressed as the difference between the (log-transformed) antibodies after administration and the (log-transformed) antibodies prior to administration ( $\theta_{\text{DCOV}}$ ); i.e. different relationships were estimated for between- and within-individual variation in antibody levels:

$$CL_{\text{initial}} = \theta_{\text{pop}} \times (1 + \theta_{\text{BCOV}} \times (\text{AB}_{\text{prior}} - \text{AB}_{\text{prior median}}) + \theta_{\text{DCOV}} \times (\text{AB}_{\text{after}} - \text{AB}_{\text{prior}})) \quad (3)$$

- In the second approach, an additional inter-individual variability (IIV) in the covariate effect ( $\eta_{\text{AB,CLinitial}}$ ) was included, i.e. the model allowed the magnitude of the antibody effect to differ between individuals with otherwise identical antibody levels. A possible interpretation of such a model could be the occurrence of antibodies with different affinities. This model required a logit transformation of the covariate–parameter relationship to ensure that individual  $CL_{\text{initial}}$  values remained positive (Eqs. 4, 5). As described above, centring of the covariate effect on the median was not performed when evaluating the change from baseline antibody levels.

$$\text{Logit} = \theta_{\text{AB}} \times (\text{AB} - \text{AB}_{\text{median}}) + \eta_{\text{AB,CLinitial}} \quad (4)$$

$$CL_{\text{initial}} = \theta_{\text{pop}} \times (0.5 + \exp(\text{Logit}) / (1 + \exp(\text{Logit}))) \quad (5)$$

## 2.4 Model Selection Criteria

Anti-PEG antibodies were tested for their significance in a univariate analysis. The best model from each modelling step was selected based on the likelihood ratio test ( $\alpha = 0.01$ ) (nested models), the Bayesian information criterion (BIC) (non-nested models), relative standard error estimates (%RSE) of the model parameters, and graphical criteria (goodness-of-fit (GOF) plots). Once the final antibody covariate model was established, the demographic covariates age and sex were successively excluded from the model to test whether these covariate effects remained significant after the inclusion of the anti-PEG antibody effect. For key model development steps, prediction-corrected visual predictive checks (pcVPCs;  $n = 1000$ ) were performed. A bootstrap analysis ( $n = 1000$ ) of the final antibody covariate model was conducted to calculate nonparametric confidence intervals for the model parameters.

## 2.5 Impact of Anti-PEG Antibodies on PEG-ASNase Activity

Monte Carlo simulations were performed to visualize the impact of anti-PEG antibodies on PEG-ASNase activity. Different antibody levels were selected for comparison. Other covariates were fixed to the median (BSA, age) or the most frequent value (sex). The standard dosing regimen of the AIEOP-BFM ALL 2009 trial was applied (2500 U/m<sup>2</sup>; 2 h infusion). Further, a PEG-ASNase dose of 1500 U/m<sup>2</sup> (1 h infusion), as used in other treatment protocols, was also evaluated [4]. Overall, 1000 patients were simulated for each anti-PEG antibody level and dosing regimen.

## 2.6 Software

NONMEM (7.4.4, ICON, Dublin, Ireland), R (R-4.1.0, <https://www.r-project.org/>), R-Studio (1.4.1106, <https://www.rstudio.com/>), PSN (4.9.0, <https://uupharmacometrics.github.io/PsN/>) and Pirana (3.0.0, <https://www.certara.com/software/pirana-modeling-workbench/>) were used for popPK analysis and model diagnostics [19–23]. The first-order conditional estimation method with interaction (FOCE+I) was applied for model estimation. Data preparation was performed with SAS (9.4, TS1M4; SAS Institute GmbH, Heidelberg, Germany). Statistical and graphical analyses were performed with SAS and R.

### 3 Results

#### 3.1 Data

Overall, 1444 patients who received 3403 PEG-ASNase administrations during induction and reinduction treatment were included in the analysis (Table 1). 6261 measurements of PEG-ASNase activity were available from these patients (Table S1 in the ESM). Antibody levels were available for 2082 samples obtained prior to PEG-ASNase administration and 6412 samples obtained after administration, respectively. Table 2 gives an overview of the distribution of anti-PEG IgG and IgM levels. The time course of antibody levels during induction and reinduction treatment is presented in Fig. 2.

#### 3.2 Population Pharmacokinetic Analysis

##### 3.2.1 Anti-PEG Antibody Covariate Model Building

- Modelling antibody isotypes separately

**Table 1** Summary of patient characteristics

Variable	Number or median (range)
Sex (male/female)	842/602
Age (years)	5.13 (1.06–18.32)
Body surface area (m <sup>2</sup> )	0.78 (0.41–2.58)
PEG-ASNase dose (U)	1950 (720–5300)
PEG-ASNase dose (U/m <sup>2</sup> )	2500 (985–4464)

**Table 2** Overview of anti-PEG IgG and IgM antibodies

Parameter	Time point	Induction	Reinduction
		Number or median (range)	
Total number of samples	Prior to 1st admin. <sup>a</sup>	1444	638
	After admin. <sup>b</sup>	5213	1199
Number of samples per patient		5 (1–8)	3 (1–5)
Anti-PEG IgG (MFI)	Prior to 1st admin.	4.18 (0.73–70.3)	3.67 (0.48–30.1)
	After 1st admin.	3.30 (0.29–51.6)	2.94 (0.36–30.7)
	After 2nd admin.	3.12 (0.39–110)	–
Anti-PEG IgM (MFI)	Prior to 1st admin.	1.37 (0.19–18.1)	0.96 (0.13–13.5)
	After 1st admin.	0.83 (0.06–22.9)	0.68 (0.07–8.28)
	After 2nd admin.	0.76 (0.07–18.0)	–

*Admin.* administration, *ASNase* asparaginase, *LLOQ* lower limit of quantification, *MFI* mean fluorescence activity, *PEG* polyethylene glycol

<sup>a</sup>Samples taken prior to the second dose in induction were not available, as the measurement coincided with the day 14 sample after the first PEG-ASNase administration

<sup>b</sup>Data set includes 151 samples with information on antibody levels but without PEG-ASNase activity data or with PEG-ASNase activity < LLOQ

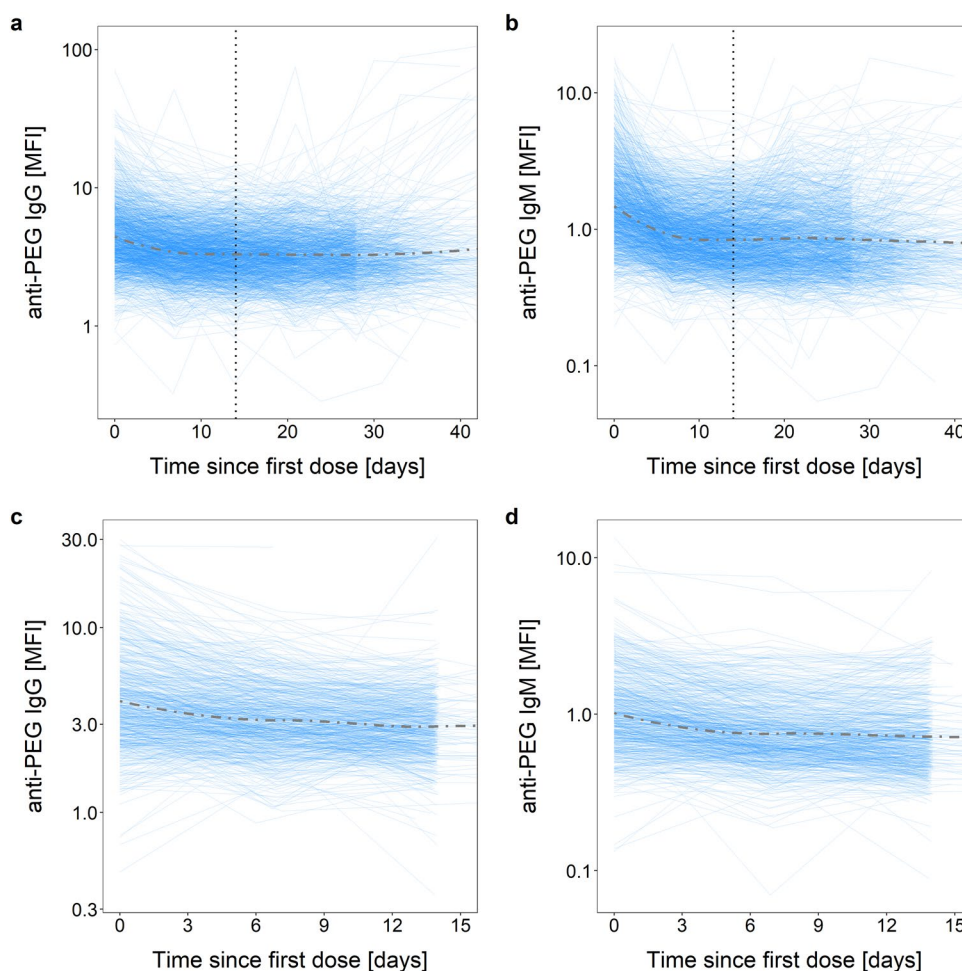
Different models for continuous and categorical anti-PEG IgG or IgM data were evaluated. For a detailed description of covariate model development, see Section 2 of the ESM. Overall, models including continuous antibody data were more informative than models including categorical data. Antibody levels obtained prior to PEG-ASNase administration were generally a better predictor of individual  $CL_{initial}$  compared to antibody levels obtained after drug administration or the ratio of antibody levels after vs. prior to administration. Modelling anti-PEG IgG<sub>prior</sub> or IgM<sub>prior</sub> together with an additional covariate effect for the difference between the antibody level after administration and the antibody level prior to administration (Eq. 3) did not lead to a better description of the variability in  $CL_{initial}$  compared to a model that only included prior antibody levels (anti-PEG IgG<sub>prior</sub>:  $\Delta OFV = -0.617$ , degrees of freedom (*df*) = 1; anti-PEG IgM<sub>prior</sub>:  $\Delta OFV = -0.450$ , *df* = 1). Likewise, adding an additional IIV in the covariate effect (Eqs. 4, 5) of anti-PEG IgG<sub>prior</sub> or IgM<sub>prior</sub>, respectively, did not improve the model (increase in BIC > 8.8).

Next, the linear relationship between anti-PEG IgG<sub>prior</sub> or IgM<sub>prior</sub> and  $CL_{initial}$  was assessed for each of the three PEG-ASNase administrations separately. However, the covariate effect could only be estimated with sufficient precision following the first drug administration in induction, not following any of the other two PEG-ASNase administrations (here, the relative standard error of parameter estimates was > 50%). These models were superior to models with the same covariate effect on all three administrations (anti-PEG IgG<sub>prior</sub>:  $\Delta OFV = -4.810$ , *df* = 0; anti-PEG IgM<sub>prior</sub>:  $\Delta OFV = -6.248$ , *df* = 0).

Khalil et al. defined cut points to distinguish between antibody-positive and antibody-negative samples (cut point

**Fig. 2** Changes in the anti-PEG IgG (a, c) and IgM (b, d) levels over time after the first dose.

*Upper panel:* antibody levels during induction; *lower panel:* antibody levels during reinduction. Each *blue line* depicts the course of the antibody level over time after the first PEG-ASNase administration for an individual patient. The *dashed grey line* is a LOESS (smoothed) curve through the data. The nominal time point of the second dose in induction treatment is indicated as a *dotted black line* (14 days after the first administration). ASNase asparaginase, MFI mean fluorescence intensity, PEG polyethylene glycol



anti-PEG IgG<sub>prior</sub>: MFI = 8; cut point anti-PEG IgM<sub>prior</sub>: MFI = 2 (linear scale) [12]. These cut points were taken into account by applying hockey stick models in place of the standard linear models. In these hockey stick models, antibody levels below the cut point showed no effect on PEG-ASNase pharmacokinetics, whereas (log-transformed) antibody levels above the cut point were linearly related to  $CL_{initial}$ . In a first step, the cut points originally defined by Khalil et al. were employed; in a second step, these cut points were additionally re-estimated within NONMEM. For anti-PEG IgG<sub>prior</sub>, a hockey stick model using the cut point defined by Khalil et al. was slightly superior to the standard linear model ( $\Delta OFV = -3.055$ ,  $df = 0$ ), and re-estimation of the cut point did not further improve the model. In contrast, taking into account the cut point defined by Khalil et al. for anti-PEG IgM<sub>prior</sub> led to a more pronounced drop in the OFV ( $\Delta OFV = -17.045$ ,  $df = 0$ ), and re-estimation of the cut point (MFI = 3.67 instead of 2; linear scale) within NONMEM improved the model even further ( $\Delta OFV = -11.546$ ,  $df = 1$ ). The hockey stick model including anti-PEG IgM<sub>prior</sub> led to a greater reduction in the OFV from the

reference model than the model including anti-PEG IgG<sub>prior</sub> ( $\Delta OFV = -49.184$  vs.  $\Delta OFV = -22.899$ ).

- Combination of both antibody isotypes

Combination of the hockey stick models of both antibody isotypes did not provide additional information when compared to anti-PEG IgM<sub>prior</sub> alone ( $\Delta OFV = -4.509$ ,  $df = 1$ ). Though both antibodies were informative for predicting individual  $CL_{initial}$ , the results indicate that anti-PEG IgM<sub>prior</sub> is a better predictor of  $CL_{initial}$ .

Taken together, the hockey stick model with a linear relationship between (log-transformed) anti-PEG IgM<sub>prior</sub> above the cut point of 3.67 (linear scale, expressed as MFI) and  $CL_{initial}$  was the best predictor of individual  $CL_{initial}$ . On the basis of the data set, an effect of anti-PEG IgM<sub>prior</sub> on  $CL_{initial}$  could only be estimated following the first dose in induction. Compared to the reference model (model without antibody covariates), inclusion of the covariate anti-PEG IgM<sub>prior</sub> led to a drop in OFV of  $-49.184$  ( $df = 2$ ) (Table 3). IIV in  $CL_{initial}$  marginally decreased

from 25.8 to 25.4% and inter-occasion variability (IOV) decreased from 23.5 to 22.8%.

### 3.2.2 Model Evaluation

Following the identification of the best anti-PEG antibody covariate model, an examination of whether the covariates age and sex remain significant after including the effect of anti-PEG IgM<sub>prior</sub> on CL<sub>initial</sub> was performed. Elimination of the relationship between CL<sub>initial</sub> and either age ( $\Delta\text{OFV} = 7.260$ ,  $df = 1$ ) or sex ( $\Delta\text{OFV} = 17.199$ ,  $df = 1$ ) significantly impaired the model.

The pcVPC demonstrates that the anti-PEG IgM<sub>prior</sub> covariate model adequately predicts the data (Fig. 3). Just as for the published model (see [16]), day 14 samples after

the second dose in induction were slightly underpredicted. GOF plots of the final anti-PEG IgM<sub>prior</sub> covariate model are presented in the ESM (Fig. S2). The trend observable when plotting individual  $\eta_{\text{CLinitial}}$  and  $\kappa_{\text{CLinitial}}$  values against anti-PEG IgM<sub>prior</sub> was largely compensated for after the inclusion of the covariate effect, with the exception of the administration in reinduction (Fig. S3 in the ESM). Here, the remaining trend indicates an influence of high anti-PEG IgM<sub>prior</sub> levels on CL<sub>initial</sub>, but this relationship was removed from the model due to the high parameter imprecision.

Table 3 summarises the results of the bootstrap analysis for the final covariate model. The median bootstrap estimates were in close agreement with the estimates for the final covariate model, indicating high model accuracy. Further, none of the 95% confidence intervals included the value

**Table 3** Parameter estimates for the reference model and final covariate model, along with results of the bootstrap analysis

Parameter	Typical values (%RSE)		1000 Bootstrap replicates (97.8% successful)	
	Reference model	Final covariate model	Estimate	95% CI
OFV	73,391.518	73,342.334		
$V$ (L/m <sup>2</sup> )	1.71 (1.4%)	1.71 (1.4%)	1.71	1.65 to 1.78
CL <sub>initial</sub> (L/day/m <sup>2</sup> )	0.130 (1.4%)	0.128 (1.4%)	0.128	0.123 to 0.133
$Q_{tr}$ (L/day/m <sup>2</sup> )	0.965 (2.5%)	0.954 (2.4%)	0.957	0.901 to 1.01
$F_{\text{BSA}}^a$ on $V$	1.60 (2.1%)	1.60 (2.1%)	1.60	1.54 to 1.67
$F_{\text{BSA}}^a$ on CL <sub>initial</sub> + $Q_{tr}$	1.50 (2.1%)	1.49 (2.1%)	1.49	1.43 to 1.55
Proportional error (%)	19.1 (2.9%)	0.191 (2.8%)	0.191	0.180 to 0.202
Additive error (U/L)	5.37 (47.3%)	5.36 (45.1%)	5.16	2.23 to 11.5
$F(V_{\text{Induction 2.admin}})^b$	-0.155 (6.8%)	-0.158 (6.7%)	-0.157	-0.179 to -0.136
$F(V_{\text{Reinduction}})^b$	-0.284 (3.8%)	-0.288 (3.7%)	-0.287	-0.306 to -0.265
$F(\text{CL}_{\text{Initial induction 2.admin}})^b$	-0.125 (10.6%)	-0.107 (12.8%)	-0.108	-0.135 to -0.078
$F(\text{CL}_{\text{Initial reinduction}})^b$	-0.451 (3.3%)	-0.438 (3.4%)	-0.439	-0.469 to -0.407
$F(\text{age} > 8 \text{ years})^c$	0.010 (42.7%)	0.011 (38.8%)	0.011	0.003 to 0.021
$F(\text{sex})^d$	-0.069 (24.1%)	-0.070 (23.4%)	-0.070	-0.101 to -0.036
$F(\text{IgM}_{\text{Prior induction 1.admin}} > \text{CP})^e$	-	0.414 (18.0%)	0.416	0.237 to 0.831
CP <sup>f</sup>	-	1.30 (2.5%)	1.30	0.984 to 1.68
IIV CL <sub>initial</sub> (%)	25.8 (4.5%)	25.4 (4.5%)	25.4	22.9 to 27.7
IOV $V$ (%)	11.9 (9.7%)	11.9 (9.6%)	11.8	9.6 to 14.5
IOV CL <sub>initial</sub> (%)	23.5 (5.1%)	22.8 (4.9%)	22.9	20.7 to 25.2

Typical values for  $V$ , CL<sub>initial</sub> and  $Q_{tr}$  are reported for a child with BSA = 1 m<sup>2</sup> for better comparison  
*admin* administration, *ASNase* asparaginase, *BSA* body surface area, *95% CI* 95% confidence interval, *CL<sub>initial</sub>* initial clearance of PEG-ASNase, *CP* cut point, *IgM<sub>prior</sub>* anti-PEG IgM level prior to first PEG-ASNase administration in induction or reinduction, *IIV* inter-individual variability, *IOV* inter-occasion variability, *OFV* objective function value, *PEG* polyethylene glycol, *Q<sub>tr</sub>* intercompartmental clearance, *%RSE* percent relative standard error, *V* volume of distribution

<sup>a</sup>Linear increase in  $V/\text{CL}_{\text{initial}}/Q_{tr}$  with BSA (centred on the median)

<sup>b</sup>Fractional change in  $V/\text{CL}_{\text{initial}}$  compared to the first administration in induction

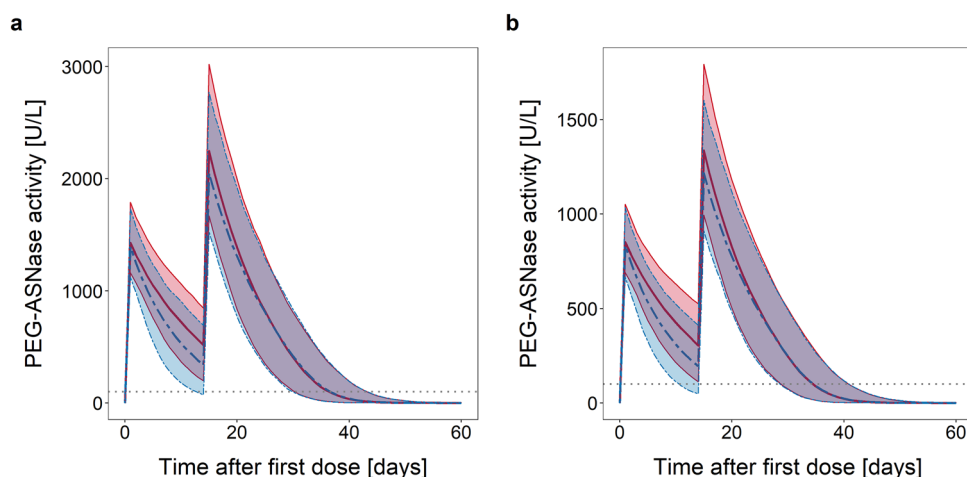
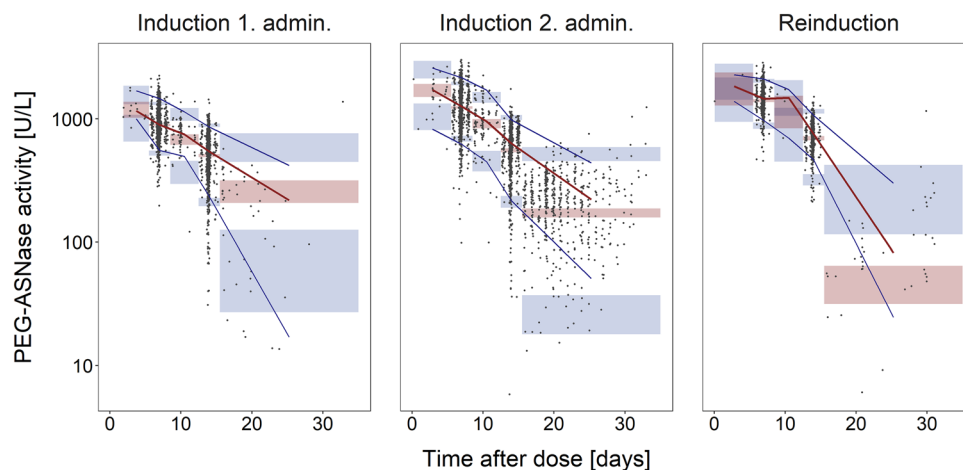
<sup>c</sup>Linear increase in CL<sub>initial</sub> for patients older than 8 years

<sup>d</sup>Fractional change in CL<sub>initial</sub> for females

<sup>e</sup>Linear increase in CL<sub>initial</sub> per log unit of anti-PEG IgM<sub>prior</sub> above the cut point following the first administration in induction

<sup>f</sup>Cut point on log scale

**Fig. 3** Prediction-corrected visual predictive check for the final anti-PEG IgM<sub>prior</sub> covariate model stratified by PEG-ASNase administration. Red line is the median of the observations; blue lines are the 2.5 and 97.5 percentiles of the observations; shaded areas are the 95% confidence intervals for simulated data (1000 simulated datasets) for the corresponding percentiles; dots are observed PEG-ASNase activity levels. ASNase asparaginase, PEG polyethylene glycol



**Fig. 4** Simulated PEG-ASNase activity over time in induction treatment. Dosing regimens applying PEG-ASNase at 2500 U/m<sup>2</sup> over 2 h (a) and 1500 U/m<sup>2</sup> over 1 h (b) were evaluated. Patients were simulated with anti-PEG IgM<sub>prior</sub> levels equal to the cut point (MFI = 1.30 on the log scale; 3.67 on the linear scale) or one log unit above the cut point (MFI = 2.30 on the log scale; 9.97 on the linear scale). For

each antibody level and dosing regimen, 1000 patients were simulated. The solid red lines and dashed blue lines indicate the median PEG-ASNase activity at each antibody level. The shaded red and blue areas depict the respective 95% prediction intervals. The dotted grey lines indicate the threshold PEG-ASNase activity of 100 U/L. ASNase asparaginase, PEG polyethylene glycol

zero, thus proving the significance of the covariate effects in the model.

### 3.3 Impact of Anti-PEG Antibodies

According to the final covariate model, each additional log unit of anti-PEG IgM<sub>prior</sub> above the estimated cut point (i.e. an increase in anti-PEG IgM<sub>prior</sub> from 1.30 to 2.30 on the log scale or, equivalently, from 3.67 to 9.97 on the linear scale) was related to an increase in CL<sub>initial</sub> of 41.4%. Figure 4 presents the PEG-ASNase activity simulated for hypothetical patients with anti-PEG IgM<sub>prior</sub> levels equal to the cut point or one log unit above the cut point. For a dose of 2500 U/m<sup>2</sup> median simulated PEG-ASNase, trough levels at day 14 after the first administration in induction

were 517 U/L (interquartile range (IQR) 391–632 U/L, anti-PEG IgM<sub>prior</sub> level of 3.67) and 339 U/L (IQR 222–457 U/L, anti-PEG IgM<sub>prior</sub> level of 9.97). Overall, 0.5% of simulated patients with an anti-PEG IgM<sub>prior</sub> level of 3.67 fell below the threshold of 100 U/L, which is considered to be required for complete asparagine depletion [13], compared to 4.9% of simulated patients with an anti-PEG IgM<sub>prior</sub> level of 9.97. For a dose of 1500 U/m<sup>2</sup> median PEG-ASNase, trough levels 14 days after the first administration were 305 U/L (IQR 241–367 U/L) and 194 U/L (IQR 135–262 U/L); 1.7% and 14.9% of the patients were below 100 U/m<sup>2</sup>.

Simulations are consistent with data observed in the AIEOP-BFM ALL 2009 trial: overall, 0.86% of patients (10/1157) with anti-PEG IgM<sub>prior</sub> below the cut point of 3.67 and 3.85% of patients (5/130) with anti-PEG IgM<sub>prior</sub> above



the cut point of 3.67 had PEG-ASNase activities  $< 100$  U/L at day 13–15 after the first administration in induction (odds ratio = 4.59; 95% confidence interval: 1.54–13.6; see Table S5 in the ESM).

## 4 Discussion

Paediatric ALL patients considerably benefit from intensive PEG-ASNase therapy associated with prolonged asparagine depletion [24–27]. Maintenance of adequate PEG-ASNase treatment intensity is therefore essential and requires knowledge of factors that interfere with PEG-ASNase pharmacokinetics, which may necessitate adjustment of dosages. On the other hand, individualisation of PEG-ASNase treatment has been proposed in order to reduce PEG-ASNase doses and such treatment-related toxicities, but also to decrease the costs of PEG-ASNase therapy [4, 28]. Adjustment of individual PEG-ASNase doses on the basis of therapeutic drug monitoring has recently been clinically evaluated within the Dutch Childhood Oncology Group ALL-11 protocol [4]. Moreover, Kloos et al. developed dosing guidelines on the basis of a popPK model [29]. Obviously, such dosing guidelines need to take into account all factors that significantly affect PEG-ASNase pharmacokinetics to reduce the risk of underdosing.

Anti-PEG antibodies have been associated with the development of clinically overt hypersensitivity reactions against PEG-ASNase as well as with a lack of enzyme activity (so-called silent inactivation) [7, 8]. Apart from hypersensitivity reactions and silent inactivation, the observation of reduced PEG-ASNase activities in patients with pre-existing anti-PEG antibodies indicates that antibodies against the PEG moiety also exert a more general effect on the pharmacokinetics [12]. Using popPK modelling, we were able to investigate in detail the relationship between anti-PEG antibodies and PEG-ASNase elimination and to quantify their impact on individual treatment intensity.

Different functional forms for both antibody levels obtained prior to as well as after PEG-ASNase administration were explored to characterise their impact on  $CL_{\text{initial}}$ . Consistently, for both the IgG and the IgM isotypes, the strongest relationship was between  $CL_{\text{initial}}$  and the antibody levels obtained prior to drug administration when considered as continuous data. However, on the basis of the data, the relationship between  $CL_{\text{initial}}$  and anti-PEG IgG<sub>prior</sub> or IgM<sub>prior</sub> could only be precisely estimated following the first dose in induction, not following any of the other two drug administrations. With regard to the second dose in induction, the missing predictive power of antibody levels prior to first dosing is plausible, as the time lag between antibody determination ( $\leq 3$  days prior to the first dose) and second dosing was about 2 weeks, and the antibody concentration

can vary substantially during this time as a consequence of the administration of the first PEG-ASNase dose. However, regarding the administration in reinduction, one could expect that anti-PEG IgG<sub>prior</sub> or IgM<sub>prior</sub> (determined  $\leq 3$  days prior to dosing in reinduction) exert a similar influence on  $CL_{\text{initial}}$  to their effects following the first dose in induction (median 150 days apart). Presumably, the small number of samples with high antibody levels in reinduction impedes precise estimation of the covariate–parameter relationship. Thus, only 89 samples prior to administration in reinduction showed anti-PEG IgM levels above the cut point defined by Khalil et al., compared to 434 samples prior to the first dose in induction [12]. Nevertheless, it has to be taken into account that the treatment context (comedication, disease stage, physical condition) strongly differs between treatment phases, which might influence the relationship between anti-PEG antibodies and  $CL_{\text{initial}}$  as well as the antibody level itself. Of note, an association between the treatment context and PEG-ASNase activity has also been observed, with increasing PEG-ASNase activity seen in reinduction compared to the PEG-ASNase activity seen following the first dose in induction [16, 29].

Though the relationship between  $CL_{\text{initial}}$  and anti-PEG antibodies exists for both isotypes, anti-PEG IgM<sub>prior</sub> was a better predictor of individual  $CL_{\text{initial}}$ . When both antibodies were combined, anti-PEG IgG<sub>prior</sub> did not add any further information compared to anti-PEG IgM<sub>prior</sub> alone (see Section 2 in the ESM). According to the final covariate model, anti-PEG IgM<sub>prior</sub> levels below the cut point of 3.67 do not exert an influence on  $CL_{\text{initial}}$ , whereas  $CL_{\text{initial}}$  increases for anti-PEG IgM<sub>prior</sub> above the cut point. Thus, an anti-PEG IgM<sub>prior</sub> level of 9.97 (an increase of one log unit) is associated with an increase in  $CL_{\text{initial}}$  of 41.4% compared to the  $CL_{\text{initial}}$  associated with an antibody level of 3.67 or below. Kloos et al. explored the influence of antibodies against PEG-ASNase on drug CL during popPK model building [29]. In contrast to our findings, the relationship between antibodies and CL turned out to be nonsignificant. However, in their study, antibodies were only determined post dosing, and pre-existing antibodies were not assessed. Furthermore, the exact moiety recognised by the antibodies (i.e. PEG or the asparaginase itself) has not been characterised, precluding any comparison between the two studies. Poppenborg and colleagues explored the impact of anti-PEG IgM on PEG-ASNase activity in mice pre-sensitized with 40 kD PEG-diol [30]. An accelerated decrease in PEG-ASNase activity in sera from successfully sensitised mice could not be observed, indicating that pre-existing anti-PEG IgM did not affect the pharmacokinetics of PEG-ASNase. Whether the experimental setup is appropriate to reflect the situation in humans is, however, uncertain. Pre-sensitisation with PEG-diol did not induce a constant response to PEG, and the amount of induced anti-PEG IgM might have been too low

to affect PEG-ASNase activity. As suggested by our model, antibody levels need to exceed a certain threshold to exert an influence on  $CL_{\text{initial}}$ .

During the modelling process, pre-existing antibody levels as well as the entire time course of antibody levels subsequent to PEG-ASNase administration were evaluated as covariates. Neither anti-PEG IgG nor IgM as a time-varying covariate post dosing were predictive for  $CL_{\text{initial}}$ . For the example of infliximab, Edlund et al. have shown that the total anti-drug antibody concentration was a more appropriate descriptor of infliximab CL, whereas measurement of the unbound antibody concentration underestimated the effect on CL [31]. A similar explanation might apply here, as only the free antibody fraction was determined by the analytical assay, not the fraction of antibodies bound to PEG-ASNase. Moreover, an induction of antibody levels following PEG-ASNase administration might not be detectable by measurement of the unbound antibody level, thus masking the true relationship between antibodies and  $CL_{\text{initial}}$ .

The pharmacokinetics of PEG-ASNase have been found to be dependent on the administration and treatment phase [16, 29, 32]. Various factors such as differences in the comedication have been discussed as possible underlying causes of the variations in PEG-ASNase pharmacokinetics. On the basis of our analysis, anti-PEG antibodies explain only a small part of the observed alterations in PEG-ASNase pharmacokinetics. Thus, inclusion of anti-PEG IgM<sub>prior</sub> in the popPK model led to a reduction in the factor describing the change in  $CL_{\text{initial}}$  between the first and second PEG-ASNase administrations in induction from  $-0.125$  to  $-0.107$ .

It should be emphasized that the increase in  $CL_{\text{initial}}$  associated with anti-PEG antibodies only affects a minority of patients who show very high pre-existing antibody levels. For the data set used for popPK analysis, only 146/1444 patients in induction and 9/638 patients in reinduction showed anti-PEG IgM<sub>prior</sub> levels above the finally estimated cut point. This is reflected in the very small decrease in the random variability of  $CL_{\text{initial}}$  following the inclusion of the covariate effect in the popPK model. Compared to the reference model, the inclusion of the effect of anti-PEG IgM<sub>prior</sub> on  $CL_{\text{initial}}$  led to a decrease in IIV from 25.8 to 25.4% and a reduction in IOV from 23.5 to 22.8% (Table 3). Though the absolute number of affected patients is rather small, our analysis nevertheless indicates that these patients show an increased risk of inadequate PEG-ASNase therapy (Fig. 4). In particular, application of low PEG-ASNase doses such as 1500 U/m<sup>2</sup> harbour the risk that an increased proportion of patients will exhibit insufficient PEG-ASNase activity, again highlighting the value of PEG-ASNase therapeutic drug monitoring. Adjustment of the very first PEG-ASNase dose on the basis of pre-existing anti-PEG antibody levels might be an additional option to further optimise PEG-ASNase

therapy, but this is hampered by the lack of widespread assays for anti-PEG antibody measurement.

## 5 Conclusion

In conclusion, popPK modelling showed that pre-existing anti-PEG IgG and IgM both influence the pharmacokinetics of PEG-ASNase, with the IgM isotype being the better predictor for individual  $CL_{\text{initial}}$  values. Treatment intensity is considerably affected in a subgroup of patients that show very high levels of pre-existing antibodies. In these patients, the final covariate model allows the activity–time course of PEG-ASNase to be more precisely predicted and highlights the value of monitoring anti-PEG antibody levels, at least prior to the very first PEG-ASNase administration.

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## Declarations

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**Conflicts of interest** CLK held invited talks for Sigma Tau, Erytech, Jazz Pharmaceuticals and Servier and received honoraria for consultancy from Erytech. CR received honoraria for consultancy and fees for invited lectures from Jazz Pharmaceuticals and Servier. JB serves as a consultant for Servier, held invited lectures and received a research grant—always in the form of an institutional grant in the context of ASNase drug monitoring. CS, JA, PS, CN and GW declare no conflicts of interest.

**Ethics approval** The AIEOP-BFM ALL 2009 trial (EudraCT No: 2007-004270-43) was approved by each national and local review board and was conducted in accordance with the Declaration of Helsinki and applicable national legislation. Informed consent was obtained from the parents or guardians of each patient included in the study, as required by ethical standards and national guidelines. For the pharmacokinetic analysis described here, an additional vote of the Ethics Committee of

the Westfalen-Lippe Medical Association and the University of Münster was obtained (reference number 2021-071-f-S).

**Consent to participate** Informed consent was obtained from the parents or legal representative of each patient included in the study, as required by ethical standards and national guidelines.

**Consent for publication** Not applicable.

**Availability of data and material** The datasets generated and/or analysed during the current study are not publicly available due to data protection rules and the fact that the complexity of the data does not allow for full anonymization.

**Code availability** The NONMEM code for the final anti-PEG antibody covariate model is provided in the ESM.

**Author's contribution** JB, CLK, CS and GW designed the research. JA and PS collected the data. CS and GW performed the pharmacokinetic analysis. Results were discussed and interpreted by all authors. CS and GW wrote the draft manuscript; all authors reviewed the manuscript and approved the final version of the manuscript.

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## Authors and Affiliations

Christian Siebel<sup>1</sup>  · Claudia Lanvers-Kaminsky<sup>1</sup>  · Julia Alten<sup>2</sup> · Petr Smisek<sup>3</sup>  · Christa E. Nath<sup>4,5</sup>  · Carmelo Rizzari<sup>6</sup>  · Joachim Boos<sup>1</sup>  · Gudrun Würthwein<sup>1</sup> 

<sup>1</sup> Department of Paediatric Haematology and Oncology, University Children's Hospital Muenster, Albert-Schweitzer-Campus 1, A1, 48149 Muenster, Germany

<sup>2</sup> Department of Paediatrics, Christian-Albrechts-University Kiel and University Medical Centre Schleswig-Holstein, Kiel, Germany

<sup>3</sup> Department of Paediatric Haematology and Oncology, Charles University and University Hospital, Motol, Prague, Czech Republic

<sup>4</sup> Biochemistry Department and Cancer Centre for Children, The Children's Hospital at Westmead, Westmead, NSW, Australia

<sup>5</sup> Faculty of Health and Medicine, University of Sydney, Sydney, NSW, Australia

<sup>6</sup> Paediatric Haematology-Oncology Unit, Department of Paediatrics, University of Milano-Bicocca, MBBM Foundation, ASST-Monza, Monza, Italy