ORIGINAL RESEARCH ARTICLE

Population Pharmacokinetics of PEGylated Asparaginase in Children with Acute Lymphoblastic Leukemia: Treatment Phase Dependency and Predictivity in Case of Missing Data

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Abstract

Background and Objectives The pharmacokinetics of polyethylene glycol-conjugated asparaginase (PEG-ASNase) are characterized by an increase in elimination over time, a marked increase in ASNase activity levels from induction to reinduction, and high inter- and intraindividual variability. A population pharmacokinetic (PopPK) model is required to estimate individual dose intensity, despite gaps in monitoring compliance.

Methods In the AIEOP-BFM ALL 2009 trial, two PEG-ASNase administrations (2500 U/m^2 intravenously) during induction (14-day interval) and one administration during reinduction were administered in children with acute lymphoblastic leukemia. ASNase activity levels were monitored weekly. A PopPK model was used for covariate modeling and external validation. The predictivity of the model in case of missing data was tested for observations, as well as for the derived parameters of the area under the concentration time curve (AUC$_{0-\infty}$) and time above different thresholds.

Results Compared to the first administration in induction (1374 patients, 6069 samples), the initial clearance and volume of distribution decreased by 11.0% and 15.9%, respectively, during the second administration during induction and by 41.2% and 28.4% during reinduction. Furthermore, the initial clearance linearly increased for children aged > 8 years and was 7.1% lower for females. The model was successfully externally validated (1253 patients, 5523 samples). In case of missing data, > 52% of the predictions for observations and > 82% for derived parameters were within ± 20% of the nominal value.

Conclusion A PopPK model that describes the complex pharmacokinetics of PEG-ASNase was successfully externally validated. AUC$_{0-\infty}$ or time above different thresholds, which are parameters describing dose intensity, can be estimated with high predictivity, despite missing data. (www.clinicaltrials.gov, NCT01117441, first submitted date: May 3, 2010).

1 Introduction

For more than 40 years, L-asparaginase (ASNase) has been an important component in the treatment of children with acute lymphoblastic leukemia (ALL). The polyethylene glycol-conjugated ASNase (PEG-ASNase) shows a longer terminal half-life with lower immunogenicity than native *Escherichia coli* ASNase, and has started to replace its unmodified form in the first-line treatment of ALL [1–6].

The antileukemic effect of ASNase has been explained by depletion of circulating asparagine and selective killing of leukemic cells [7]. Tumor cells depend on the extracellular supply of asparagine for protein synthesis, and starve to death if extracellular asparagine depletion continues. Therapeutic drug monitoring of ASNase activity levels is recommended to identify patients with levels that are insufficient to cause asparagine depletion (silent inactivation), and has been used to adapt treatment schedules [3, 4, 8, 9].

In the AIEOP-BFM ALL 2009 trial, PEG-ASNase was used as first-line treatment for children with ALL. The correlation of PEG-ASNase activity levels with clinical parameters, such as treatment response or toxic events, was one of the objectives of the trial. ASNase activity levels at predefined time points might serve as a first attempt for these pharmacokinetic–pharmacodynamic correlations. However, due to clinical practice, some samples could not be collected

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Key Points

The pharmacokinetics of polyethylene glycol-conjugated asparaginase are characterized by an increase in clearance over time and by a high influence of the treatment context.

A population pharmacokinetic model allows for the estimation of individual drug exposure parameters, despite missing values, and thus reliably fills monitoring gaps, which are not fully avoidable in drug monitoring programs in children.

This significantly expands the options to (1) include more patients and (2) provide meaningful individual pharmacokinetic estimates for the highly complex analyses of clinical trial results.

at the scheduled time points or samples could be missing. On the other hand, as shown in the literature, high inter- and intraindividual variability of ASNase activity levels, together with the observed increase in clearance over time after dose (TAD), questions the use of single ASNase activity levels as a basis for clinical decisions [8–12].

Population pharmacokinetics (PopPK) take the pharmacokinetic variability of the drug into account. This model-dependent approach serves to predict missing data and to calculate individual dose intensity parameters, despite missing data (e.g., the area under the concentration time curve ($AUC_{0-\infty}$) or the time above predefined ASNase activity thresholds in the respective treatment phase). We recently published an empirical transit model for PEG-ASNase within the AIEOP-BFM ALL 2009 trial (Fig. 1) [10]. This model might be interpreted in terms of mimicking the de-PEGylation of PEG-ASNase as a multistep process through a chain of compartments, together with an increase in elimination of the partly de-PEGylated molecules [10]. In an alternative approach, Kloos et al. [9] implemented a one-compartment model with a constant clearance (CL) for 12.7 days, which increases thereafter to describe this time-dependent CL. Furthermore, the authors observed a higher CL during induction treatment than during intensification and maintenance.

The aim of the present project was (1) to investigate the impact of the treatment phases and demographic covariates on PEG-ASNase pharmacokinetics, (2) to externally validate the model, and (3) to evaluate the predictivity of the model for ASNase activity levels and dose intensity in case of missing data.

2 Methods

2.1 Study Design

In the international AIEOP-BFM ALL 2009 trial protocol (https://www.clinicaltrials.gov/NCT01117441), children aged ≥ 1 year and < 18 years with newly diagnosed ALL were treated with multiple antileukemic agents, including PEG-ASNase as the first-line preparation. The treatment outline of the standard protocol, together with the PEG-ASNase administrations (2 h infusion, 2500 U/m²/day, maximal absolute dose 3750 U), included in the present analyses are presented in the Electronic Supplementary Material (ESM) Fig. S1. During induction, two administrations were given, one on day 12 and one on day 26. For the non-high risk (non-HR) group, a third dose was administered during reinduction (scheduled 18 weeks after the administration of the last PEG-ASNase dose during induction). Drug monitoring was scheduled 7 days and 14 days after administration of each dose of PEG-ASNase. The analysis refers to samples from patients enrolled in the German and Czech part of the multinational trial. Body weight and height were recorded before each dose.

The trial protocol (EudraCT Number 2007-004270-43) was approved by the appropriate national and local review boards and was conducted in accordance with the Declaration of Helsinki and national laws. Informed consent was obtained from the parents or guardians of each patient included in the study, as required by ethical standards and national guidelines. This trial was registered at www.clinicaltrials.gov with the identifier NCT01117441.

2.2 Determination of ASNase Activity Levels

ASNase activity levels were centrally analyzed as previously described [13] (for details see section 1 of the ESM). The lower limit of quantification (LLOQ) was 5 U/L.
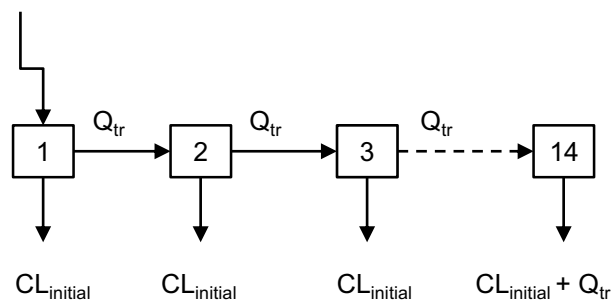

Fig. 1 Schematic view of the transit compartment model. The transit model published by Würthwein et al. [10] was simplified by replacing the additional $CL_{induced}$ in the last compartment by Q_{tr}. $CL_{initial}$ initial clearance value, Q_{tr} intercompartmental clearance

2.3 Datasets

A detailed overview of the datasets is provided in Fig. S2 of the ESM.

- Based on the Structural Model Dataset with date of sample before or on December 31, 2013, the Structural Model [10] was built and further reviewed in this study.
- Based on the Model Building Dataset with date of diagnosis before or on December 31, 2015, we built the Covariate Model.
- Based on the Final Dataset with date of diagnosis before or on December 31, 2016, including all patients enrolled in the trial, we built the Final Pharmacokinetic Model. In this dataset, patients with major protocol deviations or insufficient documentation were excluded from pharmacokinetic analysis. Missing information for time of administration or time of sampling was replaced (see Table S1 of the ESM). The subgroup of patients not included in the Model Building Dataset served for external validation (Testing Dataset).

In order to describe the standard elimination processes of PEG-ASNase, we excluded samples in all pharmacokinetic datasets (1) after a hypersensitivity reaction, (2) indicating silent inactivation, defined as ASNase < 100 U/L within 8 days and/or undetectable ASNase within 15 days without hypersensitivity reaction [14], and (3) being pharmacologically implausible, with higher ASNase activity levels at later time points, compared to earlier time points, within the same administration.

2.4 Population Pharmacokinetic Analysis

NONMEM 7.3.0 and 7.4.4, R (R-3.6.1, http://r-project.org/), PSN (4.9.0, http://psn.sourceforge.net/), and Pirana (2.9.9, https://www.certara.com/pirana-software) were used for the PopPK analysis and model diagnostics [15–18]. Data preparation and statistical analysis were performed with SAS (9.4, TS1M4; SAS, Heidelberg, Germany).

The published structural transit model [10] was used as a starting point for further pharmacokinetic modeling [19]. Body surface area (BSA) was calculated according to the Mosteller formula [20]. Data < LLOQ (Final Dataset: 1.9%; 221 of 11,707 samples) were omitted, and the first-order conditional estimation method with interaction was employed.

2.5 Investigation of Covariate Influence

Age (categorical or continuous change), sex, and body weight were evaluated as covariates on the initial clearance ($CL_{initial}$). In addition, treatment phase and differences between the first and second administrations during induction were evaluated on $CL_{initial}$ and volume of distribution (V). BSA was included as a covariate in the basic structural model. Covariate selection was performed by forward inclusion ($\alpha = 0.05$) and backward exclusion ($\alpha = 0.01$). For the highly correlated covariates, i.e., body weight and BSA ($r = 0.988$), the covariate with the best improvement in objective function value (OFV) was retained in the model. Further details and equations are given in section 2 of the ESM.

2.6 Model Selection and Diagnostics

Model selection was based on the likelihood ratio test ($\alpha = 0.01$) for nested models, the Bayesian information criterion for non-nested models and the goodness-of-fit plots. For key steps in model development, prediction-corrected visual predictive checks (pcVPCs; $n = 1000$) were performed.

Explored demographic covariates were age, body weight, BSA, and sex.

2.6.1 Internal Model Validation

For the Final Pharmacokinetic Model, a bootstrap analysis with 1000 runs was performed. The simulation properties were evaluated by pcVPCs.

2.6.2 External Model Validation

- Using the Covariate Model,
  (i) individual predictions (IPRED) and
  (ii) Bayesian maximum a posteriori estimates of the $CL_{initial}$ ($CL_{initial, Bayesian}$) and V ($V_{Bayesian}$)
  were calculated for each patient in the Testing Dataset ($ESTIMATION with MAXEVAL = 0)
- The prediction error (PE) was calculated according to Eqs. 1 and 2 as:
  (i) for observed data (DV):

$$PE\ [\%] = (IPRED - DV)/DV \times 100 \qquad (1)$$

  (ii) for a priori predictions (taking V as an example):

$$PE\ [\%] = \left(V_{pop} - V_{Bayesian}\right)/V_{Bayesian} \times 100 \qquad (2)$$

  with V_{pop} = a priori prediction using the covariates and $V_{Bayesian}$ = Bayesian maximum a posteriori estimate.
- Bias (median PE) and precision (median absolute PE) were derived.

2.7 Predictivity in Case of Missing Data

The predictivity of the Final Pharmacokinetic Model in case of missing data was assessed using the Final Dataset. To achieve this, patients with complete drug monitoring data (full dataset) were selected, and samples of these patients were deliberately deleted at defined time points by setting their DV values to missing and categorizing them with EVID = 2. Thus, we generated reduced datasets with artificial monitoring gaps (detailed description of patient selection criteria, test scenarios, example datasets and calculations are presented in section 3 of the ESM). The remaining samples and patient covariates were used for Bayesian forecasting of ASNase activity levels of the excluded samples (the individual predictions of the missing DV values were obtained) and the derived pharmacokinetic parameters. These data were compared with the nominal values obtained for the full dataset as follows:

- For reduced datasets,
  (i) IPRED for excluded samples and
  (ii) derived parameters $AUC_{0-\infty}$ and time above 100, 250, 500, and 1000 U/L for induction and reinduction were evaluated using the Final Pharmacokinetic Model ($ESTIMATION with MAXEVAL = 0).
- The PE was calculated according to Eqs. 3 and 4 as:
  (i) for excluded samples:

$$PE\ [\%] = (IPRED - DV)/DV \times 100 \quad (3)$$

  with DV = observed (nominal) value in the full dataset
  (ii) for derived parameters (taking $AUC_{0-\infty}$ for induction as an example):

$$
\begin{aligned}
PE\ [\%] = (&AUC_{0-\infty,\ \text{reduced dataset, induction}} \\
&- AUC_{0-\infty,\ \text{full dataset, induction}})/ \\
&AUC_{0-\infty,\ \text{full dataset, induction}} \times 100
\end{aligned}
\quad (4)
$$

  with $AUC_{0-\infty\ \text{full dataset, induction}}$ = nominal $AUC_{0-\infty}$ in the full dataset evaluated for induction.
- The percentage of predictions within ± 10% and ± 20% of the nominal value and bias and precision were calculated.

For analyses, samples in the time windows day 7 ± 1 and day 14 ± 1 were included. In text, tables, and figures, time points were reported as 'day 7' and 'day 14'.

Broeker et al. [21] used a comparable approach to test the predictive performance of published PopPK models for Bayesian forecasting of vancomycin concentrations and AUC values.

3 Results

3.1 Dataset

Descriptive statistics of patient characteristics and PEG-ASNase doses in the Model Building Dataset (1374 patients, 6069 samples ≥ LLOQ, 115 samples < LLOQ) and the Testing Dataset (1253 patients, 5523 samples ≥ LLOQ, 107 samples < LLOQ) are provided in Table 1. There was no difference in terms of covariate distribution between the two datasets.

As a consequence of the monitoring strategy, 45% of the samples were collected within a time window of day 7 ± 1 and 41.9% within day 14 ± 1 after PEG-ASNase administration (Final Dataset, Fig. S1 of the ESM). Dose-normalized ASNase activity levels versus TAD, stratified by PEG-ASNase administration, are presented in Fig. 2 and Table 2.

3.2 Population Pharmacokinetic Analysis

3.2.1 Development of the Covariate Model

A detailed description of the PopPK modeling is provided in section 2 of the ESM. The basic structural model [10] was simplified by setting $CL_{induced}$ (the additional clearance term in the last transit compartment) equal to the intercompartmental clearance Q_{tr} (Model Building Dataset, Fig. 1, Table 3). After inclusion of interoccasion variability on CL_{inital} and V (decrease in OFV (dOFV)= − 651), interindividual variability on Q_{tr} or V could be neglected.

For non-HR patients, a marked increase in ASNase activity levels from induction to reinduction was observed (Final Dataset: median dose-normalized ASNase activity levels of 901 U/L on day 7 and 547 U/L on day 14 after the first

Table 1 Summary of demographics and PEG-ASNase dose

Variable		Model building dataset (*n* = 1374)		Testing dataset (*n* = 1253)	
Sex (male/ female)		779/595		741/512	
Age	[years]	5.13	(1.03–17.9)	5.06	(1.03–18.0)
Height	[cm]	113	(71–195)	112	(71–199)
Body weight	[kg]	19.55	(8.5–128)	19	(7.7–118)
Body surface area	[m²]	0.78	(0.41–2.42)	0.77	(0.39–2.44)
PEG-ASNase dose	[U]	1940ᵃ	(710–4850)	1900	(620–5300)
	[U/m²]	2494ᵃ	(902–4559)	2492	(984–4988)

Data are presented as median (range) or number

ᵃThree administrations were excluded due to corrections after transfer of data for the Model Building Dataset

PEG-ASNase dose during induction and 1437 U/L on day 7 and 751 U/L on day 14 after reinduction). These differences were modeled by lower $CL_{initial}$ and V (dOFV = − 1351) during reinduction in comparison with the first administration during induction, which was used as a reference. A slight accumulation of PEG-ASNase activities during induction was described by lower $CL_{initial}$ and V (dOFV = − 286) after the second administration compared to the first administration during induction.

Further testing identified age (constant $CL_{initial}$ up to 8 years and a linear increase for older children) and sex (7.1% lower $CL_{initial}$ for females compared to males) as covariates on $CL_{initial}$.

3.2.2 Development of the Final Pharmacokinetic Model

The Model Building Dataset included about 50% of the patients compared to the Final Dataset. Therefore, we repeated and confirmed the covariate selection. The NON-MEM control file of the Final Pharmacokinetic Model is provided in section 4 of the ESM.

3.2.3 Internal Model Validation

Goodness-of-fit plots of the Final Pharmacokinetic Model showed almost evenly distributed conditional weighted residuals over the population predictions and time ranges, respectively (Fig. S3 of the ESM). However, a slight underprediction of high ASNase activity levels might be observed, which is possibly explained by the lack of peak levels (only 0.9% of the samples were collected in the time intervals day 0–5 after PEG-ASNase administration). Therefore, there is little information in the dataset for the first days after administration with observed high ASNase activity levels. The pcVPC indicated good simulation properties (Fig. 3). Only day 14 levels after the second PEG-ASNase administration during induction were slightly underpredicted when sampling was sparse compared to the other scheduled time points. Bootstrap analysis of 1000 runs showed that the median and 95% confidence intervals were in line with the model estimations (Table 3), indicating high accuracy of the Final Pharmacokinetic Model.

Fig. 2 Asparaginase activity levels normalized to PEG-ASNase dose of 2500 U/m^2 versus time after dose, stratified by PEG-ASNase administration. Levels below the lower limit of quantification were set to 2.5 U/L. *Admin* administration
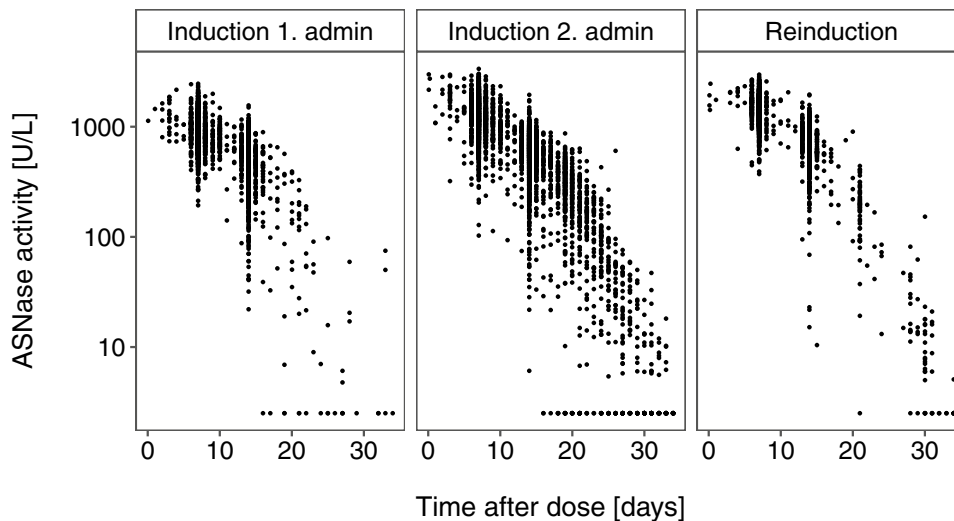
Table 2 Asparaginase activity levels (in U/L) at day 7 ± 1 and day 14 ± 1 after the respective PEG-ASNase administration

PEG-ASNase administration	N	Min	25th percentile	Median	75th percentile	Max
Day 7 ± 1 after the respective administration						
Induction 1st admin	1958	193	737	896	1076	2458
Induction 2nd admin	1765	103	991	1259	1623	3353
Reinduction	1431	371	1205	1437	1722	2970
Day 14 ± 1 after the respective administration						
Induction 1st admin	2193	22	428	543	662	1556
Induction 2nd admin	1288	6	473	629	782	1939
Reinduction	1324	10	618	751	883	1958

These data of the Final Dataset are normalized to a PEG-ASNase dose of 2500 U/m^2

Admin administration, *N* number of samples

Table 3 Population pharmacokinetic parameter estimates for the structural [10], covariate, and final pharmacokinetic Models, with nonparametric bootstrap analysis for the final pharmacokinetic model

Parameter	Typical values (%RSE)			1000 Bootstrap replicates (92.9% successful)	
	Structural model	Covariate model	Final PK model	Estimate	2.5–97.5% confidence interval
V [L/m^2]	1.27 (1.6)	1.69 (1.5)	1.69 (1.1)	1.68	1.63–1.73
$CL_{initial}$ [L/day/m^2]	0.107 (0.9)	0.130 (1.4)	0.126 (1.0)	0.126	0.123–0.130
$CL_{induced}$ [a] [L/day/m^2]	0.740 (10.9)				
Q_{tr} [L/day/m^2]	0.848 (2.4)	0.913 (3.1)	0.918 (1.9)	0.918	0.870–0.960
F[b] $_{BSA}$ on all PK parameters	1.52 (1.8)				
F[b] $_{BSA}$ on V		1.57 (2.1)	1.56 (1.5)	1.56	1.51–1.61
F[b] $_{BSA}$ on $CL_{initial}$ + Q_{tr}		1.43 (2.3)	1.44 (1.6)	1.44	1.40–1.49
Proportional error [%]	32.5 (1.5)	19.0 (3.1)	18.9 (2.2)	18.9	18.0–19.7
Additive error [U/L]	4.59 (37.3)	13.2 (41.7)	8.74 (32.2)	8.64	3.96–14.6
F[c] (V Induction 2. admin)		−0.160 (7.5)	−0.159 (5.1)	−0.159	−0.174 to −0.142
F[c] (V Reinduction)		−0.292 (4.1)	−0.284 (2.9)	−0.283	−0.300 to −0.266
F[c] ($CL_{initial}$ Induction 2. admin)		−0.113 (13.1)	−0.110 (9.5)	−0.110	−0.131 to −0.087
F[c] ($CL_{initial}$ Reinduction)		−0.433 (3.3)	−0.412 (2.4)	−0.411	−0.430 to −0.391
F[d] (Age> 8 years)		0.021 (22.2)	0.018 (19.3)	0.018	0.011–0.025
F[e] Sex		−0.077 (20.3)	−0.071 (16.7)	−0.071	−0.095 to −0.046
IIV $CL_{initial}$ [%]	19.8 (4.8)	22.7 (5.3)	24.0 (3.4)	23.9	22.4–25.5
IIV Q_{tr} [%]	12.1 (10.7)				
IOV V [%]		13.5 (8.7)	13.3 (6.7)	13.4	11.3–15.1
IOV $CL_{initial}$ [%]		23.1 (4.9)	22.4 (3.4)	22.4	20.9–24.0

During NONMEM estimation, values for $CL_{initial}$, $CL_{induced}$, Q_{tr} and V are reported for BSA=0.79 m^2; values were converted from L/day/0.79m^2 or L/0.79m^2 to L/day/m^2 or L/m^2 for better comparison [10]

BSA body surface area, $CL_{initial}$ and $CL_{induced}$ clearance values, *IIV* interindividual variability, Q_{tr} intercompartmental clearance, *IOV* interoccasion variability, *V* volume of distribution, *%RSE* percent relative standard error

[a] In the Covariate Model and the Final Pharmacokinetic Model: $CL_{induced} = Q_{tr}$

[b] $F_i = F(1 + F_{BSA} \cdot (BSA_i - 0.79))$

[c] Fractional change of V or $CL_{initial}$, respectively, compared to induction 1. administration

[d] Linear increase in $CL_{initial}$ for patients older than 8 years: $F_i = F(1 + F_{age>8years}(age_i - 8))$

[e] Fractional change in $CL_{initial}$ for females

Fig. 3 Prediction-corrected visual predictive check stratified by PEG-ASNase administration. Red line, median of the observations; blue lines, 2.5 and 97.5 percentiles of the observations; shaded areas, 95% confidence intervals for simulated data (1000 simulated datasets) for the corresponding percentiles; dots, observed asparaginase activity levels (during pharmacokinetic modeling, levels below the lower limit of quantification were excluded)
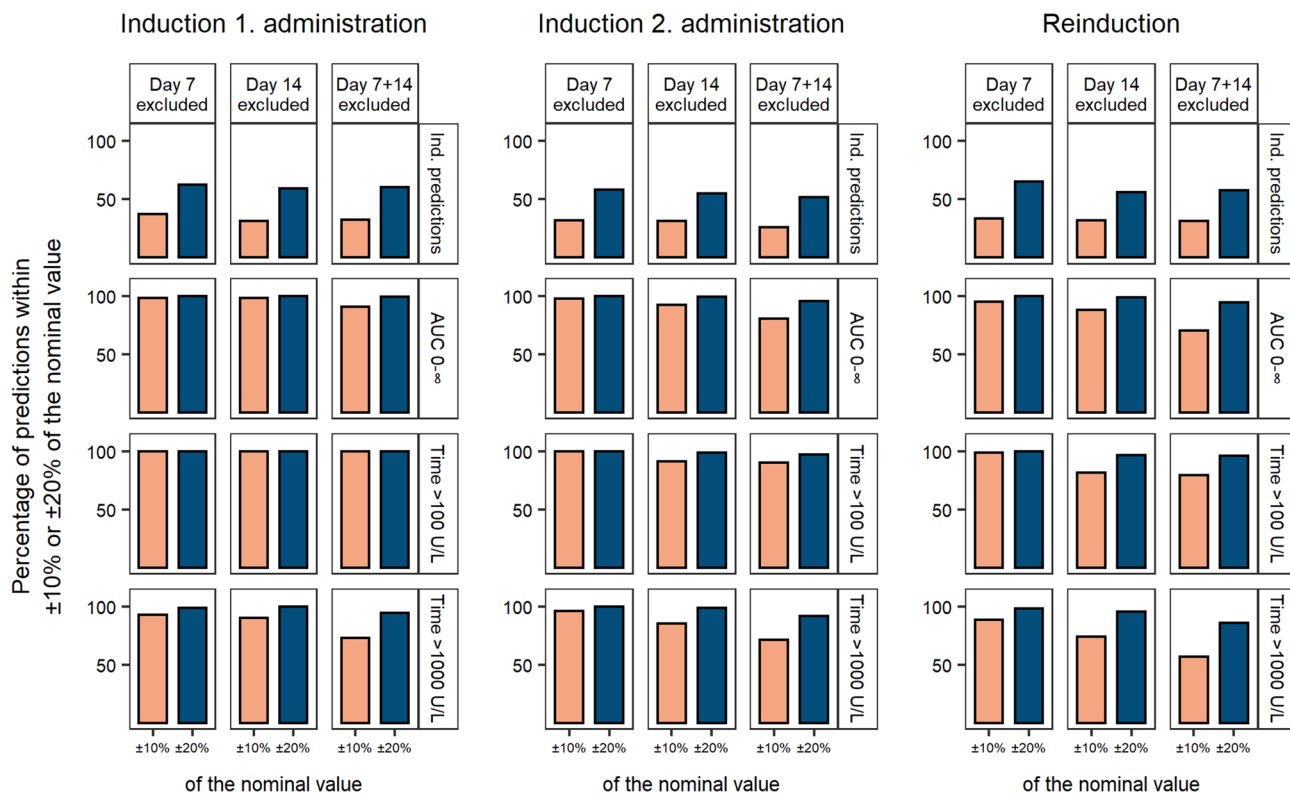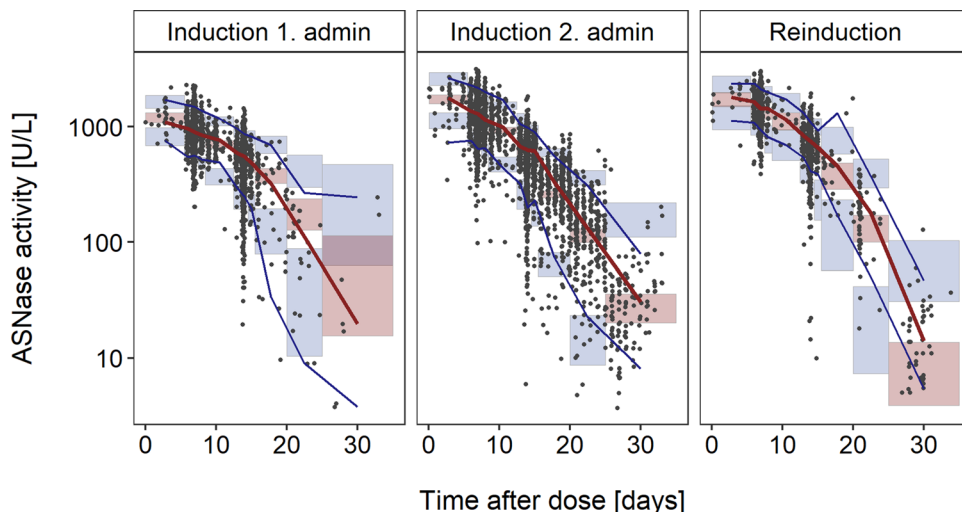

Fig. 4 Predictivity of the final pharmacokinetic model. For patients with all scheduled monitoring samples available, defined samples after the same administration were excluded. The population pharmacokinetic model was used for Bayesian forecasting of (1) individual predictions for these excluded samples, (2) area under the concentration time curve $AUC_{0-\infty}$, (3) time > 100 U/L, and (4) time > 1000 U/L. Percentage of predictions within ± 10% (light red bar) or within ± 20% (blue bar) of the nominal value were calculated (nominal values were defined for: (1) ASNase activity levels: observed value and (2–4) $AUC_{0-\infty}$ and time above different thresholds: parameters evaluated based on the final dataset)

3.2.4 External Model Validation

The Covariate Model accurately predicted ASNase activity levels in the Testing Dataset not used for model building, with low bias (− 3.6%) and high precision (8.6%). A priori individual parameter estimates were unbiased ($CL_{initial}$: bias + 2.5%; V: bias: − 1.0%), with high precision for both $CL_{initial}$ (17.9%) and V (3.5%). Subgroup analyses showed only a slightly higher bias of − 5.5% for day 14 after PEG-ASNase administration during induction compared to the other time points (bias: − 2.7 to − 0.2%).

3.3 Predictivity in Case of Missing Data

A Bayesian forecasting approach was employed to test the predictivity of the Final Pharmacokinetic Model in case of samples being excluded (Final Dataset, see section 2.6). Results for the percentage of predictions within ± 10% or ± 20% of the nominal value are given in Fig. 4 (scenarios with samples after the same administration being excluded), Tables S2 (all scenarios, results stratified by treatment phase), and S3 (all scenarios, results for excluded samples stratified by time point) of the ESM. Percentiles of the PEs are provided in Fig. S4 of the ESM.

For the different scenarios tested, 26–37% of the individual predictions for excluded samples were within ± 10%, and 52–65% were within ± 20% of the observed (nominal) value. Results were irrespective of the time point or number of excluded samples. The bias was in the range of − 7.6 to 0.4%. Subgroup analyses stratified by time point indicated slight underpredictions for day 14 samples during induction (first PEG-ASNase administration: − 8.0 to − 2.6%; second PEG-ASNase administration: − 9.2 to − 7.1%), whereas no bias was observed for the other time points (− 3.0 to 2.1%). Irrespective of the number of excluded samples, good precision (14.8–19.2%) was obtained.

For the derived parameter $AUC_{0-\infty}$, the percentage of predictions within ± 10% of the nominal value slightly decreased with the number of excluded samples (one sample: > 88%, two samples during induction: > 81%, two samples during reinduction: > 70%, and three samples during induction: > 67%). For all scenarios > 91% of the predictions were within ± 20% of the nominal value.

For time > 500 U/L and time > 1000 U/L (the pharmacokinetic parameters with pronounced influence on $AUC_{0-\infty}$ calculation) deviations of predictions from the parameter evaluated based on the full dataset (nominal value) were slightly higher compared to the results observed for $AUC_{0-\infty}$ (e.g., for time > 1000 U/L with three samples during induction being excluded, > 82% of the predictions were within ± 20% of the nominal value).

Predictions for $AUC_{0-\infty}$, time > 500 U/L, and time > 1000 U/L were almost unbiased (− 3.2 to 1.8%) and showed high precision (0.2–9.4%). Bias and imprecision only slightly increased with the number of excluded samples.

For time > 100 U/L and time > 250 U/L, > 86% of predictions were within ± 10% of the nominal value. Results were irrespective of the number of excluded samples. Only scenarios with samples on day 14 or day 7 + 14 during reinduction being excluded showed slightly lower percentages (> 78%). For all scenarios, > 95% of the predictions were within ± 20% of the nominal value. Predictions were unbiased (− 0.5 to 1.5%) with high precision (0.0–4.9%).

4 Discussion

The pharmacokinetics of PEG-ASNase are characterized by an increase in elimination over TAD, which might be explained by in vivo hydrolysis of the unstable ester bond between PEG and the enzyme combined with increased elimination of the partly de-PEGylated enzyme [10]. ASNase activity levels at predefined time points might serve for pharmacokinetic–pharmacodynamic correlations within the AIEOP-BFM ALL 2009 trial. However, due to clinical practice, monitoring compliance decreased from 86% on day 14 after the first PEG-ASNase administration to 56% at day 14 after the second PEG-ASNase administration during induction. Missing data, together with the observed high inter- and intraindividual variability of the drug, show the need to better understand the pharmacokinetics. Based on the recently published empirical transit PopPK model for PEG-ASNase [10] (Fig. 1), we built up the Covariate Model, and evaluated the predictivity of the model in case of missing data. Such evaluations are important preconditions for subsequent analyses of potential pharmacokinetic–pharmacodynamic correlations. The nonlinear mixed-effects modeling approach used in our PopPK analysis is especially appropriate in children, because it allows for sparse data situations with flexible sampling time points, where the traditional two-stage approach is not applicable [22, 23].

4.1 Population Pharmacokinetic Analysis

The covariate selection was built up based on the Model Building Dataset (1374 patients, 6069 samples), and confirmed based on the Final Dataset (2545 patients, 11,486 samples). For non-HR patients, marked differences in drug exposure between induction and reinduction were observed with about 60% higher median dose-normalized ASNase activity levels (about 1450 U/L after reinduction compared to about 900 U/L on day 7 ± 1 after the first PEG-ASNase administration during induction) (see section 2 of the ESM). This huge difference was modeled by lower $CL_{initial}$ (41.2%) and V (28.4%) during reinduction in comparison with the first administration during induction, which was used as a

reference. Furthermore, $CL_{initial}$ (11.0%) and V (15.9%) were lower during the second administration in comparison with the first administration during induction. Covariate analyses showed a linear increase in $CL_{initial}$ for children aged > 8 years, and lower $CL_{initial}$ for females. Our model showed good predictive performance for the Testing Dataset (1253 patients).

The observed differences in the pharmacokinetics of PEG-ASNase between administrations and treatment phases can hardly be explained by changes in chemotherapy (induction: daunorubicin; reinduction: doxorubicin), corticosteroids (induction: prednisone or dexamethasone; reinduction: dexamethasone) or immunomodulators. Moreover, changes in the demographic and biologic characteristics might be responsible for the observed differences. However, particularly with regard to V, changes between induction and reinduction are first based on statistical terms to describe the observed huge increase in ASNase activity levels. For PEG-ASNase (a large molecule with molecular weight of about 513 kDa showing no protein binding [7]), V might reflect the plasma volume of the patient, which is about 50 mL/kg and almost constant in children [24]. Of note, only 0.9% of the samples were collected within days 0–5 after administration; these samples would provide the most information on the change of V between treatment phases. However, the V in reinduction (median: 44.4 mL/kg for males and females) still seems pharmacologically plausible, as 88% of the data for males and 96% for females were above the lower confidence limit reported for plasma volume in children [24].

In a patient cohort of 110 pediatric patients, Kloos et al. [9] implemented a PopPK model with time-dependent CL for PEG-ASNase. In their evaluations, the V was estimated to be 0.94 L/m^2, which was equivalent to about 30 mL/kg, and thus significantly lower than the plasma volume. However, the authors also observed differences in CL between treatment phases that were less pronounced. CL appeared 11% lower during medium risk (MR) intensification and 19% lower during MR maintenance compared to that during treatment phase 1A. Administration of specific chemotherapeutic agents, the physical condition of the patients, and changes in the mononuclear phagocyte system are discussed as possible causes [9]. Further work is required to explore this, which up to now is not fully understood treatment phase-dependent pharmacokinetic behavior. Our general PopPK model, which is based on demographic covariates, now allows direct comparisons of the pharmacokinetics of PEG-ASNase between different clinical trials. The treatment phase-dependent pharmacokinetics concern the question of dosing guidelines, as suggested by Kloos et al. [9]. It also explains that the model reported by Hempel et al. [11], which is based on mixed monitoring data from induction and/or relapse treatment of different trials, was not predictive for our cohort.

4.2 Predictivity in Case of Missing Data

We evaluated the predictivity of the Final Pharmacokinetic Model in case of missing data. For patients with all scheduled monitoring samples being available, some samples were excluded, and the PopPK model was used to forecast (1) the individual predictions for these excluded samples and (2) the derived pharmacokinetic parameters $AUC_{0-\infty}$ and time above different thresholds for induction and reinduction.

Only > 52% of the predictions for excluded samples, our 'artificial monitoring gaps', were within ± 20% of the observed values. However, this has to be interpreted in the context of the EMA guideline for the analysis of drug concentrations in biological matrices [25]. For at least 75% of the calibration standards and for 67% of the quality control samples, the back calculated concentrations should be within ± 15% of the nominal values. Additionally, for at least 67% of the reanalyzed samples, the percent difference should not be > 20% of their mean. This adds four aspects to our precision modeling: (1) we have to accept that even the 'true' analyzed monitoring activity levels do have an internal uncertainty as described in the guidelines, (2) the rather high residual error (18.9%) is in line with the analytical uncertainty, (3) high intraindividual variability is observed for PEG-ASNase activities [12], and (4) we can take this frame of acceptable uncertainty as justification for our PopPK model. The model describes, despite missing values, the clinically relevant dose intensity parameters, with an accuracy being within the ranges defined for single monitoring analysis. For the derived pharmacokinetic parameters, > 67% of the predictions were within ± 10%, and > 91% were within ± 20% of the nominal values, irrespective of the number of samples being excluded. The best predictions were observed for time > 100 U/L (levels considered to be sufficient for complete asparagine depletion [14]), with > 80% of predictions within ± 10% of the nominal value. If three samples during induction or both samples during reinduction were excluded, only for time > 1000 U/L, > 53% of the predictions were within ± 10% and > 82% were within ± 20% of the nominal values.

In the scenarios tested, we observed a slight underprediction for excluded samples, which was more pronounced for day 14 samples during induction (bias − 9.2 to − 2.6%) compared to the other time points (bias − 3.0 to 2.1%). The pcVPC and the external validation also indicated a slight underprediction of day 14 samples during induction. However, derived pharmacokinetic parameters were unbiased (− 3.2 to 1.8%). Furthermore, individual predictions (14.8–19.2%) and pharmacokinetic parameters (0–9.4%) were evaluated with high precision. However, the model cannot predict peak levels of ASNase activity with absolute certainty. High ASNase activity levels may have been slightly underpredicted, which might result in slight underprediction

of $AUC_{0-\infty}$ values or times above high ASNase thresholds (e.g., 1000 U/L). This shortcoming of the model does not affect nonparametric, distribution-free interindividual comparisons of $AUC_{0-\infty}$ values or times above threshold estimates for 100 U/L or 400 U/L. The methodological and pharmacological issues presented here need an unbiased scientific discussion. The dose intensity parameters can be used for a wide range of analyses to be prospectively planned once the whole spectrum of clinical data becomes available.

5 Conclusion

A PopPK model for PEG-ASNase was successfully established and externally validated to account for the time-dependent CL, the marked differences in drug exposure between induction and reinduction, and the slight accumulation during induction within the AIEOP-BFM ALL 2009 trial. For patients treated with PEG-ASNase according to this protocol, the PopPK model allows for the calculations of $AUC_{0-\infty}$ or times above different thresholds with sufficient precision, even in case of missing data. Such parameters describe the individual pharmacokinetics with significantly more information obtained from the patient, as well as from the population, than simple trough levels can do. In addition, the surrogates for drug exposure can be obtained from significantly more patients compared to fully realized monitoring protocols. The PopPK approach permits high tolerance for variation in the practical schedule procedures and allows for flexibility in patient needs in pediatric clinical trials. Even gaps in monitoring due to non-compliance or the practical needs of the wards can be overcome.

Declarations

Conflict of Interest M. Schrappe and the ALL-BFM Study Center received funding from medac, Shire, Sigma-Tau, Servier, and Jazz Pharmaceuticals for research, and for functions on advisory boards. C. Rizzari received institutional grants for PEG-asparaginase pharmacological studies aimed at the therapeutic drug monitoring of asparaginase within the AIEOP-BFM ALL 2009 study, fees for participation to advisory boards, and invited lectures for medac, Sigma-Tau, Baxalta, Shire, and Servier who were and currently are the owners of the product that is the object of this pharmacological study. C. Lanvers-Kaminsky held invited talks for Sigma-Tau, Erytech, and Jazz Pharmaceuticals, received honoraria for consultancy from Erytech, and received a travel grant from Servier. A. Möricke received honoraria from Baxter. J. Boos served personally as a consultant and participated in advisory as well as in safety boards for medac GmbH. He received support for travel from Eusa Pharma, Jazz Pharmaceuticals, Baxalta, Shire, and Servier. For medac GmbH, Eusa Pharma, Jazz Pharmaceuticals, Baxalta, Servier, Shire, and Sigma-Tau, he held invited lectures. In addition, institutional grants in the context of ASNase drug monitoring from more or less all ASNase providers contributed to the therapeutic drug monitoring program, including medac GmbH, Eusa Pharma, Jazz Pharmaceuticals, Baxalta, Servier, Shire, and Sigma-Tau (all representing the varying marketing authorization holders of *E. coli* ASNase, PEG-ASNase, and Erwinase). No other authors declare a conflict of interest.

Authors' contributions JB, GW and CL-K designed the research; AM, MZi, JS, PS and MS collected the data; GW and JG performed the statistical analyses; GW, CL-K, CS, GH, SGW, and JB performed and discussed the pharmacokinetic analysis; JB, GW, CL-K, JG, AM, MZi, JS, PS, MS, CR, and MZu discussed and interpreted the results during scientific meetings of the Asparaginase Working Party. GW, CL-K, CS, and JB wrote the manuscript; all authors reviewed the manuscript.

Ethics approval The study (EudraCT Number 2007-004270-43) was approved by each national and local review board and was conducted in accordance with the Declaration of Helsinki and national laws.

Consent to participate Informed consent was obtained from the parents or guardians 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 analyzed 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 of the final pharmacokinetic model together with an example NONMEM dataset are provided in the supplement.

References

1. Asselin BL, Whitin JC, Coppola DJ, Rupp IP, Sallan SE, Cohen HJ. Comparative pharmacokinetic studies of three asparaginase preparations. J Clin Oncol. 1993;11(9):1780–6.

2. Müller HJ, Löning L, Horn A, et al. Pegylated asparaginase (Oncaspar) in children with ALL: drug monitoring in reinduction according to the ALL/NHL-BFM 95 protocols. Br J Haematol. 2000;110(2):379–84.

3. EU Clinical Trials Register. Dutch Childhood Oncology Group: Protocol ALL-11: Treatment study protocol of the Dutch Childhood Oncology Group for children and adolescents (1-19 year) with newly diagnosed acute lymphoblastic leukemia [online]. 2020. https://www.clinicaltrialsregister.eu/ctr-search/trial/2012-000067-25/NL. Accessed 25 Sep 2020.

4. ClinicalTrials.gov. Dana-Farber Cancer Institute. Treatment of Newly Diagnosed Acute Lymphoblastic Leukemia in Children and Adolescents [online]. 2020. https://clinicaltrials.gov/ct2/show/record/NCT03020030?cond=ALL%2C+Childhood&view=record. Accessed 25 Sep 2020.

5. EU Clinical Trials Register. AIEOP-BFM ALL 2009: International collaborative treatment protocol for children and adolescents with acute lymphoblastic leukemia [online]. 2020. https://www.clinicaltrialsregister.eu/ctr-search/trial/2007-004270-43/DE. Accessed 25 Sep 2020.

6. Lanvers-Kaminsky C. Asparaginase pharmacology: challenges still to be faced. Cancer Chemother Pharmacol. 2017;79(3):439–50.

7. Food and Drug Administration. Oncaspar (pegaspargase) injection label. Revised 01/2019 [online]. 2020. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/193411s5196lbl.pdf. Accessed 25 Sep 2020.

8. Kloos RQH, Pieters R, Jumelet FMV, de Groot-Kruseman HA, van den Bos C, van der Sluis IM. Individualized asparaginase dosing in childhood acute lymphoblastic leukemia. J Clin Oncol. 2020;38(7):715–24.

9. Kloos RQH, Mathôt R, Pieters R, van der Sluis IM. Individualized dosing guidelines for PEGasparaginase and factors influencing the clearance: a population pharmacokinetic model. Haematologica. 2020. https://doi.org/10.3324/haematol.2019.242289.

10. Würthwein G, Lanvers-Kaminsky C, Hempel G, et al. Population pharmacokinetics to model the time-varying clearance of the PEGylated Asparaginase Oncaspar® in children with acute lymphoblastic leukemia. Eur J Drug Metab Pharmacokinet. 2017;42(6):955–63.

11. Hempel G, Müller HJ, Lanvers-Kaminsky C, Würthwein G, Hoppe A, Boos J. A population pharmacokinetic model for pegylated-asparaginase in children. Br J Haematol. 2010;148(1):119–25.

12. Würthwein G, Lanvers-Kaminsky C, Gerss J, et al. Therapeutic drug monitoring of asparaginase: intra-individual variability and predictivity in children with acute lymphoblastic leukemia treated with PEG-asparaginase in the AIEOP-BFM Acute Lymphoblastic Leukemia 2009 Study. Ther Drug Monit. 2020;42(3):435–44.

13. 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;309(1):117–26.

14. van der Sluis D, Vrooman LM, Pieters R, et al. Consensus expert recommendations for identification and management of asparaginase hypersensitivity and silent inactivation. Haematologica. 2016;101(3):279–85.

15. R Core Team. R: A language and environment for statistical computing: a language and environment for statistical computing: R Foundation for Statistical Computing, Vienna, Austria; 2018.

16. Lindbom L, Pihlgren P, Jonsson EN. PsN-Toolkit—a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Comput Methods Programs Biomed. 2005;79(3):241–57.

17. Keizer RJ, Karlsson MO, Hooker A. Modeling and Simulation Workbench for NONMEM: Tutorial on Pirana, PsN, and Xpose. CPT Pharmacometrics Syst Pharmacol. 2013;2:e50.

18. Icon Development Solutions. NONMEM Users's Guides: Ellicott City, Maryland; 2009.

19. Byon W, Smith MK, Chan P, et al. Establishing best practices and guidance in population modeling: an experience with an internal population pharmacokinetic analysis guidance. CPT Pharmacometrics Syst Pharmacol. 2013;2:e51.

20. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med. 1987;317(17):1098.

21. Broeker A, Nardecchia M, Klinker KP, et al. Towards precision dosing of vancomycin: a systematic evaluation of pharmacometric models for Bayesian forecasting. Clin Microbiol Infect. 2019;25(10):1286.e1-1286.e7.

22. Food and Drug Administration. Guidance for Industry. Draft Guidance 07/2019 [online]. 2020. https://www.fda.gov/media/128793/download. Accessed 4 Jan 2021.

23. Sun H, Fadiran EO, Jones CD, et al. Population pharmacokinetics. A regulatory perspective. Clin Pharmacokin. 1999;37(1):41–58.

24. Raes A, van Aken S, Craen M, Donckerwolcke R, Vande WJ. A reference frame for blood volume in children and adolescents. BMC Pediatr. 2006;6:3.

25. European Medicines Agency. Guideline on bioanalytical method validation [online]. 2020. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Accessed 25 Sep 2020.

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