Development of A Physiological-Based Pharmacokinetic Model to Predict Bendamustine Exposure in Paediatric Patients

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Abstract

Background the study aimed to predict bendamustine pharmacokinetics in paediatric patients by employing physiological-based pharmacokinetic (PBPK) model that considers age-dependent physiological maturity.

Methods Using PK-Sim, a paediatric PBPK model was constructed based on a validated PBPK adult model using the recommended paediatric dose at 120 mg/m² bendamustine intravenous (IV) infusion for 60 minutes and age-dependent mechanistic scaling. Parameter optimisation was done based on model fitting to the observed *in vivo* population pharmacokinetic (PK) data using the visual predictive check. The final paediatric PBPK model was extrapolated to various paediatric age categories (term newborn, infant, toddler, preschool, school-age, adolescent) for PK estimate and comparisons. The PBPK paediatric model was compared with the adult model, and PK comparisons were made for different age categories with the reference paediatric model.

Results The model estimated a bendamustine fraction unbound of 4% in a paediatric patient. Renal clearance, biliary clearance and total hepatic clearance in a typical child were 1.38 mL/min/kg, 0.02 mL/min/kg and 13.76 mL/min/kg respectively. The paediatric model was comparable to the adult model. Compared to a typical child, a term newborn has the highest estimated maximum plasma concentration (C_{max}) and systemic exposure (AUC_{0-24h}), which were 1.24-fold respectively. Clearance in term newborn and infant were 1.4-1.5-fold higher than a typical child.

Conclusions We developed a bendamustine PBPK model for a paediatric patient with relapsed/refractory Acute Lymphocytic Leukaemia (ALL) and Acute Myeloid Leukaemia (AML). The current model may be useful to estimate systemic exposure in paediatric for various age categories in different settings. More *in vivo* studies are needed in paediatric patients to challenge the robustness of this model.

Keywords Anticancer, bendamustine, paediatric, physiological-based pharmacokinetic model (PBPK) modelling, simulation

Background

Acute Lymphocytic Leukaemia (ALL) and Acute Myeloid Leukaemia (AML) are the two commonest childhood leukaemia subtypes of all diagnosed leukaemia among children and teens [1]. ALL and AML are commonly treated with intensive polychemotherapy [2, 3]. Despite the reported high survival rate [4], 10-15% of patients who entered complete remission still experience relapse and are refractory to induction of chemotherapy [5, 6].

Bendamustine is among the alkylating agents being evaluated in clinical trials among children for its use in relapsed/refractory AML and ALL [7, 8]. Structurally, bendamustine has 3 active moieties- mechlorethamine alkylating group, purine-analogue benzimidazole ring, and a butyric side chain (Fig 1a) [9]. It is approved by the United States Food and Drug Safety Agency (USFDA) for the front-line treatment of Chronic Lymphocytic Leukaemia (CLL) and rituximab-refractory indolent B-cell non-Hodgkin Lymphoma (NHL) [10]. The indication has also been studied for multiple myeloma (MM) [11, 12], T-cell lymphoma [13], mantle cell lymphoma (MCL) [14], and other solid tumours [15, 16]. Bendamustine either used alone or in combination with other agents has shown superior activity in indolent NHLs, MCL, and CLL [17].

The exact mode of action of bendamustine is not known, but studies have shown bendamustine confers a unique mode of action compared to other conventional nitrogen mustards with a similar structure such as cyclophosphamide, chlorambucil, and melphalan [9]. Distinct to other alkylators, bendamustine forms more durable and extensive interstrand crosslinks with DNA that distort DNA double helix [18]. Additional proposed mechanisms of action include the activation of intrinsic apoptosis, inefficient cell repair mechanism via base incision repair pathway and inhibition of mitotic checkpoint control that induces a downstream 'mitotic catastrophe' [9, 18]. *In vitro*, bendamustine also demonstrated a lack of cross-resistance with other alkylators [19]. These combined abilities of bendamustine mechanistically show that it is a more potent agent suitable for patients with relapsed leukaemia refractory to other alkylators [9].

In plasma, approximately 94-96% of the drug is bound to protein, mainly with human serum albumin [10, 20] and the binding is not affected by age (>70 years), low serum albumin (<31 g/L), or presence of advance tumours [10]. Bendamustine is extensively metabolised mainly via non-enzymatic hydrolytic degradation to produce inactive metabolites monohydroxy bendamustine (HP1) and dihydroxy bendamustine (HP2), and minorly via enzymatic cytochrome P450 (CYP) 1A2 in the liver to form cytotoxic metabolites γ -OH-bendamustine (M3) and N-desmethyl-bendamustine (M4) (Fig 1b and 1c) [21, 22]. The relative potency of M3 is approximate to the parent compound, whilst M4 is 1/5 to 1/10 of bendamustine [21]. The cytotoxic activities of these active metabolites are

minimal, owing to the low concentrations relative to their parent compound [23]. Bendamustine also undergoes phase II metabolism via conjugation with glutathione [24]. The phase II metabolites were characterised as cysteine-S-conjugates with up to 96% secreted in bile [24, 25], thus phase II conjugation may be a major route of bendamustine metabolism [24]. In a human mass balance study with intravenous (IV) infusion of [¹⁴C]bendamustine, 76% of the administered dose was recovered, of which 45-50% in urine, 25% in faeces and 5.2% in bile 168-hour post-infusion [22].

In vitro, bendamustine showed cytotoxic activities against paediatric ALL T-cell (CCRF-CEM), B-cell (CCRF-SB) lines, and in the AML HL-60 cell line warranted further research on its potential use for paediatrics [26]. In combination with other antimetabolites (cladribine, cytarabine fludarabine, clofarabine), bendamustine has shown synergistic inhibition *in vitro* against refractory/relapse paediatric B cell precursor acute lymphoblastic leukaemia (BCP-ALL) cell lines [27]. Only one recommended phase II dose (R2PD) open-label clinical trial has been conducted to date, which involves bendamustine monotherapy at 120 mg/m² IV infusion daily for 2 days of each 21-day cycle in children with ALL and AML [7]. The results demonstrated limited efficacy against childhood relapsed/refractory ALL with no activity against AML despite a satisfactory safety profile [7].

With limited clinical trials and current unlicensed use in paediatrics patients [10], assessment of bendamustine pharmacokinetic (PK) in paediatric is warranted. It is well known that children display uniqueness in absorption, distribution metabolism, and excretion that may be significantly different from adults that subsequently affect drug disposition. Progression in paediatric malignancy is also different from the adult equivalent which may alter drug PK and responses in children [28]. From this perspective, it could be argued that the developmental changes should be considered as an additional variability that may affect PK prediction in children.

In recent years, drug PK in the understudied population can be estimated using physiology-based pharmacokinetic (PBPK) modelling and simulation. PBPK uses a computational and mechanistic approach to account for the physical and anatomical characteristics of paediatrics and the physicochemical property of the tested compound [29]. At present, a bendamustine PBPK model has been developed in adult patients, but not in paediatrics. Hence, the current study aimed to develop a paediatric PBPK model and to describe the PK parameters of bendamustine in individuals from various paediatric age groups.

Methods

Simulation of a paediatric PBPK model was done based on the workflow proposed by Maharaj et al. [30]. The starting point of adult to paediatric bridging is typically an IV adult model, then using the physiological information about growth and maturation of relevant process to simulate a paediatric model.

A previous study (unpublished manuscript by HPT) has simulated a base PBPK adult model using pharmacokinetic data of 6 patients from Dubbelman et al. [22]. The data represented the PK profile of patients over age 18 (mean age 66 years old) with histologically and cytologically verified relapsed/refractory NHL, which were unresponsive to established care after receiving an IV infusion of bendamustine 120 mg/m². We reconstructed this base model using the extracted information from Table 1. Table 1 shows the input parameters for a typical European male, the physiochemical input values for bendamustine from Drugbank and the clearance parameters for the adult model. The adult model has been validated with visual predictive checks, and percentage error was calculated to assess model precision. The results indicated a good predictive performance of the base model and serve as a basis for paediatric scaling.

Table 1 Patient characteristics, physiochemical and the clearance parameters used in the simulation of adult PBPK model

Patient characteristics	Values [median (range)]	Source
Age (years)	66 (48-75)	[22]
Weight (kg)	72.7 (59-94)	[22]
Height (cm)	173.2 (155-181)	[22]
Physiochemical parameters		
Log P (Log Units)	1.66	[31]
Fraction unbound (%)	6	[31]
Molecular weight (g/mol)	394.7	[31]
Effective molecular weight (g/mol)	328.7	PK-Sim estimated
Solubility (mg/ml) at pH 4.1	1.1	
Clearance parameters		
Hepatic plasma clearance (ml/min/kg)	4.41 (3.4 - 14.25)*	[22]
Renal plasma clearance (ml/min/kg)	0.112 (0.087 - 0.461)*	[22]

Biliary plasma clearance (ml/min/kg)	0.0014 (0 - 0.0034)*	[21, 22]
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Table 1 legend: * All the parameters have been adjusted based on the weight [mean (range)] in Dubbelman et al. [22]

Scaling of paediatric PBPK model parameters

a) Anatomy and physiology

We followed a physiological-based mechanistic scaling previously proposed by Edginton et al. [32]. For anatomy and physiology scaling, PK-Sim default values were used for the age-dependent parameters such as body weight, height, organ weight, organ volume, organ blood flow, binding protein concentration, hematocrit, and cardiac output. Integrated values were based on the anthropometric distribution database of the International Commission on Radiological Protection (ICRP, 2002) for European Caucasian males [33] and Edginton et al. for organ composition [34].

b) Fraction unbound

The built-in albumin ontogeny function in PK-Sim was used to estimate the age-dependent unbound fraction of bendamustine [35]. The non-linear ontogeny was based on McNamara and Alcorn equation for human serum albumin [36] and unbound fraction of bendamustine in adult at 6%. We assumed similar binding affinity of bendamustine to albumin in adults and children.

c) Clearance parameters

Prediction of clearance in children that uses a combination of physiological-based mechanistic approach and enzyme ontogeny approach has been previously described [32]. For scaling of renal clearance, the pre-defined age-dependent value for glomerular filtration rate in PK-Sim was used. We assume similar renal elimination in adults and children based on equation 1:

$$CLGFR, \text{child} = \frac{GFR, \text{child}}{GFR, \text{adult}} \times \frac{fu, \text{child}}{fu, \text{adult}} \times CLGFR, \text{adult}$$

(1)

Where CLGFR, child and CLGFR, adult are the clearance of compound due to glomerular filtration in paediatric and adult; fu, child and fu, adult are the fraction unbound in paediatric and adult; GFR, child and GFR, adult are the estimated glomerular filtration rate (GFR) of the paediatric and adult (mL/min).

For scaling of biliary clearance, the PK-Sim default function was used, which incorporates an $\frac{3}{4}$ allometric liver model based on liver weight and fraction unbound in children. Equation 2 was used for scaling of biliary clearance:

$$CL_{\text{biliary, child}} = CL_{\text{biliary, adult}} \left(\frac{LW_{\text{child}}}{LW_{\text{adult}}} \right)^{0.75} \times \frac{f_{u, \text{child}}}{f_{u, \text{adult}}}$$

(2)

Where $CL_{\text{biliary, child}}$ and $CL_{\text{biliary, adult}}$ are biliary clearance in child and adult; LW_{child} and LW_{adult} are the liver weight of the child and adult respectively taken from PK-Sim; $f_{u, \text{child}}$ and $f_{u, \text{adult}}$ are the fraction unbound in child and adult respectively.

For hepatic clearance scaling, the PK-Sim database for CYP 1A2 enzyme expression was used. The database considers age-dependent enzyme ontogenies and distribution in various organs [35]. The process of hepatic scaling of clearance assumes that [37]:

- a) clearance pathway in children is the same as in adults
- b) enzyme metabolism follows the first-order kinetic where there is no enzyme saturation
- c) hepatic uptake of the compound is limited by perfusion rate within a ‘well-stirred’ model.

The scaled paediatric intrinsic clearance for metabolic enzyme CYP 1A2 was calculated based on equation 3:

$$CL_{\text{int, child}} = (CL_{\text{int, adult}}) \times OSF$$

(3)

Where $CL_{\text{int, child}}$ is the scaled intrinsic clearance for CYP 1A2 per gram of liver; $CL_{\text{int, adult}}$ is the intrinsic clearance for CYP 1A2 per gram of liver in adults; OSF is the ontogeny scaling factor for enzyme CYP 1A2.

Since bendamustine undergoes renal, hepatic, and biliary clearance pathway, the total hepatic clearance is the sum of its individual scaled pathway and can be represented by equation 4:

$$\text{Total hepatic clearance} = CL_{\text{GFR, child}} + CL_{\text{int, child}} + CL_{\text{biliary, child}} \quad (4)$$

Where $CL_{\text{GFR, the child}}$ is the compound clearance attributed by glomerular filtration in paediatric; $CL_{\text{int, the child}}$ is the scaled intrinsic clearance in paediatric, and $CL_{\text{biliary, the child}}$ is the biliary clearance in paediatric.

Clinical data

Using databases in Pubmed and Medline, keywords such as ‘bendamustine’, ‘pharmacokinetics’, and ‘paediatrics’ were used to search for relevant literature. Only studies that have concentration-time data following administration of IV infusion until the age of 18 were included. Two relevant studies were found [7, 8]. The population PK data was based on an open-label, single-arm recommended phase II dose (RP2D) study among children with histologically proven ALL and AML. The subjects received 120 mg/m² bendamustine IV infusion for 60 minutes on day 1 and 2 of each 21-day cycle for relapsed/refractory acute leukaemia, whereby blood samples were collected shortly prior and following the infusion at 3, 6, 10 (±2) and 24 hours [7]. The PK data from 38 patients of age 1 to 19 years old (median age 7 years old) were extracted, and the plasma concentration plot was converted to data points using WebPlotDigitiser Version 4.4 [38] (see Additional file 1). Table 2 summarises the demographic of the paediatric population.

Table 2 Demographics of the paediatric patient population

Demographics	Values, N=38
Age (years) [median (range)]	7 (1-19)
Sex (%)	
Male	26 (68%)
Female	12 (32%)
Body surface area (m ²) [mean (SD)]	1.10 (0.41)

Table 2 legend: SD, standard deviation

Model simulation, parameter optimisation, model evaluation and sensitivity analysis

The PBPK model was constructed using PK-Sim version 9.1 (OSP Suite, Bayer Technology Service, Leverkusen, Germany) [39]. All simulation works are available as Additional file 2.

Based on the adult characteristics in Table 1, a virtual individual representing a typical European child was created using the scaling method previously described. The median age of the subject population in Table 2 was used to create the individual. The administration protocol used was a single 120 mg/m² bendamustine IV infusion for 60 minutes, which is the paediatric dose derived from a dose-escalating phase I/II trial [7]. During the simulation, Rodger and Rowland's calculations [40] were used to estimate the partition coefficient and Schmitt's charge-dependent calculation [41] for cellular permeability. Based on these building blocks, a paediatric PBPK model was created.

The simulated paediatric PBPK model was then compared with the observed mean PK data using visual predictive checks for model fitting. Visual check ensures the appropriateness of line shape. Using the Monte-Carlo algorithm, the chosen parameters (renal, hepatic, biliary clearance, fraction unbound, and lipophilicity) were then optimised to reduce the error between the observed and predicted data points. All observed data and simulated data below the lower limit of quantitation (LLOQ) were set to LLOQ and were reduced to single observations. We also performed a sensitivity analysis to estimate the impact of parameter changes on the output metrics [area under the curve (AUC_{0-24h}) and maximum concentration (C_{max})] to inform the reliability and significance of simulated results. Clearance related parameters, albumin ontogeny factor, lipophilicity and fraction unbound were chosen based on value uncertainties and the biological potential in influencing the exposure and peak concentrations. Based on this model fitting and parameter adjustment, the final paediatric PBPK model was generated.

To visually compare the disposition of bendamustine across different paediatric age groups, one individual was created from each paediatric population of term newborn (0 days to 28 days of life), infants (1 month to 12 months), toddler (1 year to 2 years), preschool (2 years to 6 years), school-age (6 years to 12 years) and adolescent (12 years to 18 years), using the scaling method previously described. These age ranges are similar to FDA classification [42] with an increased age bracket. A total of 6 virtual individuals were created:

- 0 day- term newborn (post-menstrual age= 40 weeks)
- 1 month- infant
- 1-year-old- toddler
- 2 years old- preschool
- 7 years old- school-age
- 16 years old- adolescent

Administration protocol and simulation conditions remain unchanged for all age groups. Comparisons of PK parameters were then made with the reference paediatric PBPK model and the adult model using visual predictive check and ratio calculations for PK parameters.

Results

Superimposing the predicted concentration-time curve of a typical European child with the observed data resulted in optimisation of compound log P value to 1.11 log unit and fraction unbound to 4%. Renal clearance, biliary clearance and total hepatic clearance were optimised to 1.38 mL/min/kg, 0.02 mL/min/kg and 13.76 mL/min/kg

respectively. Fig 2 shows the PBPK model of a typical paediatric patient. The experimental data was described with sufficient accuracy after adjustment with these values for model fitting. Table 3 shows the predicted PK parameter of a typical European paediatric patient.

Table 3 Model predicted pharmacokinetic parameters in a typical European child and observed paediatric population

Pharmacokinetic parameters	Model predicted values	Observed mean values
$C_{\max}$ (ng/mL)	6927.64	6806.00
AUC _{0-24h} (ng* h/mL)	7656.04	3233.85
$t_{\max}$ (h)	1.00	1.10
MRT (h)	0.38	0.38
Plasma Vd/F (mL/kg)	6179.03	-
Plasma Vss/F (mL/kg)	224.18	-
Half-life (h)	7.35	Infinity
Total plasma clearance (L/h/kg)	0.58	-

Table 3 legend: $C_{\max}$, maximum concentration; AUC_{0-24h} area under the curve; $t_{\max}$, time to reach $C_{\max}$; MRT, mean residence time; Vd/F, apparent volume of distribution; Vss/F apparent volume of distribution at steady-state

Based on the virtual individual of various paediatric age categories, each simulation was performed and compared with the concentration profile of a typical child (Fig 2). Fig 3a-3e shows the split view and Fig 3f shows the relative profiles. The adult PK profile was included for comparison with the predicted paediatric model. Table 4 summarises the differences of PK parameters in various paediatric age categories with our predicted paediatric PK as reference.

Table 4 Predicted PK parameters in individual child of different age categories and adult patient

Age category	Age	$C_{\max}$ (ng/mL)	Ratio of $C_{\max}^{**}$	AUC _{0-24h} (ng* h/mL)	Ratio of AUC _{0-24h} **	Total plasma clearance (L/h/kg)	Ratio total plasma clearance **
Newborn (term)	0 day	8574.00	1.24	9463.73	1.24	0.81	1.4

Infant	1 month	7714.01	1.11	8333.67	1.09	0.87	1.5
Toddler	1 year	7524.94	1.09	8264.22	1.08	0.67	1.2
Preschool	2 years	7232.94	1.04	7931.31	1.04	0.66	1.1
School-age*	7 years	6927.64	-	7656.04	-	0.58	-
Adolescent	16 years	7310.92	1.06	8478.26	1.11	0.40	0.7
Adult	66 years	5276.74	1.31***	6495.33	1.18***	0.48	1.20***

Table 4 legend: * School-age child represents the reference paediatric model ** Ratio of child: reference paediatric values ***Ratio of reference paediatric: adult values. C_{max} , maximum concentration; AUC_{0-24h} area under the curve; t_{max} , time to reach C_{max}

Comparing the PK parameters, a child have slightly higher yet comparable C_{max} , AUC_{0-24h} and total plasma clearance compared to an adult, as reflected in the model predicted paediatric: adult ratio of 1.31, 1.18 and 1.20 respectively (Table 4). Values close to 1 represent closeness between child and adult PKs.

Based on the parameter ratio of child: reference paediatric values, C_{max} and AUC_{0-24h} for a term newborn is 1.24-fold higher than the reference paediatric model, which is the greatest difference among all the paediatric age categories. The differences in peak and exposure then reduce with age in a non-linear manner. For total plasma clearance, we observed a greater difference in the first 1 month of life, where a term newborn and infant have 1.4-1.5-fold higher plasma clearance than a typical child. The difference in plasma clearance also reduces non-linearly with age until adolescent years.

Sensitivity analysis

In a typical paediatric patient, the most important parameters that influence both the exposure prediction (AUC_{0-24h}) and peak plasma concentration (C_{max}) was lipophilicity, followed by fraction unbound, albumin ontogeny factor and total hepatic clearance.

Discussion

Bendamustine is widely studied in adult patients but not in paediatrics. Using PBPK simulation, the goal is to predict drug disposition of different age groups in paediatrics under various physiological and pathological conditions. Arguably, PK estimation may be performed using other methods such as the classical pharmacokinetic models and Bayesian estimate. However, the classical model lacks the physiological and biological basis for PK parametrisation [43], whereas using Bayesian estimate to develop population PK model are largely empirical [44].

On the other hand, the PBPK model consists of multiple organ compartments that represent corresponding organs with their actual volume and perfusion rate, and each compartment is connected by blood flow [29]. Hence, PBPK modelling provides a more mechanistic estimation with a pharmacological and physiological basis. We developed a paediatric PBPK model using the ‘bottom-up’ approach driven by the understanding of physicochemical properties of tested compound and children physiology. The previously developed whole-body adult IV PBPK model provides a good starting point for paediatric extrapolation as it circumvents most of the preliminary absorption barriers. Our study extrapolated this model to a paediatric PBPK model using the recommended paediatric dose at 120 mg/m² IV infusion for 60 minutes, and age-dependent anatomical and physiological mechanistic scaling in children. The developed paediatric PBPK model (Fig 2) represents the pharmacokinetics of a typical paediatric of European ethnic.

Based on model fitting, the result shows that the fraction unbound of bendamustine in a paediatric was 4%, which is lower than the fraction unbound in adult at 6%. The range is still within the reported range in the literature at 4-6% fraction unbound [31]. Theoretically, serum albumin would have reached adult value by approximately 2 years old [35]. Cancer status in children also did not seem to reduce the binding fraction in children [8]. Therefore, the reason for the slight reduction in fraction unbound is possibly due to a higher concentration of albumin in children or stronger binding affinity than in adults. Previously, it was reported that bendamustine total hepatic plasma clearance was 14.25 mL/min/kg, renal clearance at 0.46 mL/min/kg, 0.0034 mL/min/kg in an average 73kg European adult (unpublished manuscript by HPT). Here, we reported a higher renal and biliary plasma clearance at 1.38 mL/min/kg and 0.02 mL/min/kg respectively in a paediatric patient. The total hepatic clearance was lower than an adult at 13.76 mL/min/kg.

The previous study from Darwish and co-workers used a ‘top-down’ model building method, with allometric scaling of body weight for clearance and Bayesian estimates for a *priori* estimate of PK in the paediatric population. The estimated mean C_{max} was 6806 ng/mL (coefficient variation of 23%) and the mean AUC_{0-24h} was 8240 ng* h/mL (coefficient variation of 34%), t_{max} was 1.1 hour [8]. Our model predicted C_{max} of 6927.64 ng/mL, and AUC_{0-24h} of 7656.04 ng/mL and t_{max} of 1 hour in a typical European child, which is comparable to the mean *in vivo* population PK estimates. Therefore, the scaling method proves to be adequate and all underlying assumptions during the modelling process were reasonable.

There were deviations to the standard workflow for paediatric PBPK model construction. Ideally, individual data points in the *in vivo* study are needed to evaluate the performance of a population PBPK model, whereby the

proportion of data points that fall outside the two-fold range of mean prediction are quantified. In our simulation, the data points are unavailable. In the absence of data points, we chose to compare individual PK profiles (rather than the population-based PBPK model) that represents a wide range of paediatric age categories with the paediatric PBPK model as a reference and qualified the models with visual predictive checks (Fig 3a-3e). The predicted lines and shapes of curves were similar to the reference paediatric model, indicating the correct modelling process and ability of the paediatric model to be extended to other age groups.

As indicated by model predicted paediatric: adult ratios, our paediatric model is also comparable to the adult model, despite slightly higher C_{max} , AUC_{0-24h} and total plasma clearance observed in a child (Table 4). This is consistent with the population PK study, where the model predicted paediatric: adult ratios for mean C_{max} and AUC_{0-24h} were 1.18 and 1.16 respectively [8]. The non-linear changes in PK parameters in different age groups are expected and reflect all growth changes from neonate to adolescent. The greater difference in peak and exposure observed in the first month of life and a higher total plasma clearance in early infancy is on the account of the rapid anatomical and physiological changes in the first 2 years of life and delayed ontogenesis of the metabolic enzyme. Bodyweight, body surface area, body composition, organ volume and perfusion changes continuously throughout infancy to adolescence, often in a non-linear fashion [45]. Generally, the low activity of CYP enzymes soon after birth increase rapidly over the first 3 months of life [46]. In particular, CYP 1A2, which is the CYP enzyme responsible for the metabolism of bendamustine shows a delayed maturation in human compared to other isoforms, reaching approximation to the adult level at 1-2 years old [45–47]. Hepatic clearance of bendamustine is also influenced by the level of microsomal proteins per gram of liver (MPPGL), which is a measure of liver metabolic capacity, whereby newborn and geriatrics have 31% lower MPPGL from the average adult value of 40 mg/g liver attained at around 30 years old, indicating a lower capacity of drug clearance in very young and advanced age [48]. The interplay between lower MPPG, increased CYP 1A2 activity and liver blood perfusion influences the hepatic clearance of the drug in various age groups.

This study has a few limitations. As mentioned earlier, the biggest setback was the unavailability of individual data points from the *in vivo* clinical study. Consequently, extrapolating individual models of various age groups to population models would offer little value since the population models cannot be validated. As such, with the current paediatric models, strong conclusions cannot be drawn on the predictive performance of other age categories. Our PBPK model also does not allow the evaluation of dose optimisation based on age categories in a paediatric population. Due to limited knowledge on parameter input for paediatric, we estimated parameters through the fitting of the model to observed data from study sets with the same dosing regime. The limitation to

this method is that the individual paediatric parameters may not be uniquely defined, thus reducing the model's mechanistic meaning and applicability to an untested paediatric population outside of study condition [49]. Secondly, to our knowledge, only one published paper with the population PK profile of bendamustine is available [8]. Therefore, the developed paediatric model is yet to be verified against independent external datasets from *in vivo* studies. The modelling process also assumes that the disease state and genetic polymorphism did not significantly influence enzyme abundance and protein binding in children. However, Cheeti et al. discussed the lower level of albumin in cancer patients compared to normal healthy individual [50]. A meta-analysis has also shown that adjusting CYP 1A2 abundance by 20-33% lower in the cancer population gave a better prediction of a subset of oncology compound [51]. Although previously, it has been established that cancer status and albumin concentration did not significantly alter bendamustine PK in paediatric [8], future simulations may customise CYP 1A2 enzyme abundance and protein albumin for oncology population within PK-Sim, and test model robustness with the same observed data to increase predictive accuracy. It will also be interesting to assess the model predictive capacity in pre-term neonates and other special sub-population such as obese children and geriatric patients to better understand bendamustine disposition in these populations.

Despite limitations that weakened the model prediction, the developed PBPK model allows the prediction of systemic exposure in typical paediatric patients with relapsed/ refractory AML and ALL and PK prediction of various paediatric age categories.

Conclusions

We developed a paediatric PBPK model of bendamustine for childhood relapsed/refractory ALL and AML that allows for PK prediction in various paediatric age categories using the recommended paediatric dose at 120 mg/m² IV infusion for 60 minutes. The current model may be useful to estimate systemic exposure in individual children in different settings, but more *in vivo* studies are needed in paediatric patients to challenge the robustness of this model.

List of Abbreviations

ALL: Acute Lymphocytic Leukaemia

AML: Acute Myeloid Leukaemia

BCP-ALL: B cell precursor acute lymphoblastic leukaemia

CLL: Chronic Lymphocytic Leukaemia

344 C_{max}: Maximum concentration

345 CYP: Cytochrome P450

346 GFR: Glomerular filtration rate

347 ICRP: International Commission on Radiological Protection

348 IV: Intravenous

349 LLOQ: Lower limit of quantitation

350 MCL: Mantle cell lymphoma

351 MM: Multiple myeloma

352 NHL: non-Hodgkin Lymphoma

353 PBPK: Physiological-based pharmacokinetic

354 PK: Pharmacokinetic

355 R2PD: Recommended phase II dose

356 t_{max}: Time to reach maximum concentration (C_{max})

357 USFDA: United States Food and Drug Safety Agency

358 **Declarations**

359 **Ethics approval and consent to participate**

360 Not applicable

361 **Consent for publication**

362 Not applicable

363 **Availability of data and materials**

364 Physiochemical data of bendamustine is publicly available from Drugbank online,
 365 <https://go.drugbank.com/drugs/DB06769>. All other data generated and analysed during this study are included
 366 in this published article and its supplementary information files.

367 **Competing interests**

368 The authors declare that they have no competing interests.

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Authors' contribution

MK and TM contributed to the conception of this project. HPT provided guidance and technical support in PK-Sim software. LHW performed the simulation work and manuscript writing. All authors read and approved the final manuscript.

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Figures

Fig 1. Structure of bendamustine and its active metabolites

1a) Bendamustine

1b) γ -OH-bendamustine (M3)

1c) N-desmethyl-bendamustine (M4)

Fig 1 Structure of a) bendamustine and its active metabolites b) γ -OH-bendamustine (M3) and c) N-desmethyl-bendamustine (M4)

Fig 2 Observed vs predicted plasma bendamustine concentration in a typical European child

Fig 2 legend: Observed vs predicted plasma bendamustine concentration in a typical European child following a 60 minutes IV infusion of bendamustine at 120 mg/m². The solid black line is the model predicted concentration. Black dots are the observed mean concentration from the clinical study. The figure shows model prediction is in good agreement with observed data.

Fig 3a Model predicted plasma concentration in a 0-day term newborn and a typical paediatric individual

Fig 3a legend: Model predicted plasma concentration in a 0-day term newborn and a typical paediatric individual following 60 minutes IV infusion of bendamustine at 120 mg/m². The solid black line represents a 0-day term newborn. The dotted line represents the typical child. Black dots are the observed concentration in a typical child.

Fig 3b Model predicted plasma concentration in a 1-month-old infant and a typical paediatric individual

Fig 3b legend: Model predicted plasma concentration in a 1-month-old infant and a typical paediatric individual following 60 minutes IV infusion of bendamustine at 120 mg/m². The solid black line represents a 1-month-old infant. The dotted line represents the typical child. Black dots are the observed concentration in a typical child.

Fig 3c Model predicted plasma concentration in a 1-year-old toddler and a typical paediatric individual

Fig 3c legend: Model predicted plasma concentration in a 1-year-old toddler and a typical paediatric individual following 60 minutes IV infusion of bendamustine at 120 mg/m². The solid black line represents a 1-year-old toddler. The dotted line represents the typical child. Black dots are the observed concentration in a typical child.

Fig 3d Model predicted plasma concentration in a 2-year-old preschool child and a typical paediatric

Fig 3d legend: Model predicted plasma concentration in a 2-year-old preschool child and a typical paediatric following 60 minutes IV infusion of bendamustine at 120 mg/m². The solid black line represents the 2-year-old preschool child. The dotted line represents the typical child. Black dots are the observed concentration in a typical child.

Fig 3e Model predicted plasma concentration in a 16-year-old adolescent and a typical paediatric individual

Fig 3e legend: Model predicted plasma concentration in a 16-year-old adolescent and a typical paediatric individual following 60 minutes IV infusion of bendamustine at 120 mg/m². The solid black line represents a 16-year-old adolescent. The dotted line represents the typical child. Black dots are the observed concentration in a typical child.

Fig 3f The relative difference of model predicted concentration in the individual child and adult

Fig 3f legend: The relative difference of model predicted concentration in the individual child and adult following a 60 mins IV infusion bendamustine at 120 mg/m². The solid black line represents 0-day-old term newborn, the pink line represents 1-month-old infant, the blue line represents 1-year-old toddler, the black dash line represents 2 years old preschool child, the green line represents 16-year-old adolescent, the red line represents a typical child, red dots represent observed concentration in a typical child, black dotted line represents an adult.

604 **Additional files**

605 Additional file 1

606 File format: Microsoft Excel Worksheet (.xls)

607 Title: The mean concentration of paediatric population after bendamustine IV infusion at 120 mg/m² for 60
608 minutes

609 Description: The table shows the mean concentration-time of bendamustine extracted from a paediatric
610 population study. Data points were converted using WebPlotDigitizer Version 4.1.

611 Additional file 2

612 File format: PK-Sim Project File (.pksim5)

613 Title: PK-Sim Project File

614 Description: PK-Sim file contains all simulation works that have been carried out for the project.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AdditionalFile1.xlsx](#)
- [AdditionalFile2.pksim5](#)