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ORIGINAL RESEARCH ARTICLE

A Population Pharmacokinetic Modelling Approach to Unravel the Complex Pharmacokinetics of Vincristine in Children

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Abstract

Background Vincristine, a chemotherapeutic agent that extensively binds to β-tubulin, is commonly dosed at 1.4–2.0 mg/m^2 capped at 2 mg. For infants, doses vary from 0.025–0.05 mg/kg or 50–80% of the mg/m^2 dose. However, evidence for lower doses in infants compared to older children is lacking. This study was conducted to unravel the complex pharmacokinetics of vincristine, including the effects of age, to assist optimal dosing in this population.

Methods 206 patients (0.04–33.9 years; 25 patients < 1 years), receiving vincristine, with 1297 plasma concentrations were included. Semi-mechanistic population pharmacokinetic analyses were performed using non-linear mixed effects modelling.

Results A three-compartment model, with one saturable compartment resembling saturable binding to β-tubulin and thus, saturable distribution, best described vincristine pharmacokinetics. Body weight and age were covariates significantly influencing the maximal binding capacity to β-tubulin, which increased with increasing body weight and decreased with increasing age. Vincristine clearance (CL) was estimated as 30.6 L/h (95% confidence interval (CI) 27.6–33.0), intercompartmental CL (Q) as 63.2 L/h (95%CI 57.2–70.1), volume of distribution of the central compartment as 5.39 L (95%CI 4.23–6.46) and of the peripheral compartment as 400 L (95%CI 357–463) (all parameters correspond to a patient of 70 kg). The maximal binding capacity was 0.525 mg (95%CI 0.479–0.602) (for an 18 year old patient of 70 kg), with a high association rate constant, fixed at 1300 /h and a dissociation constant of 11.5 /h.

Interpretation A decrease of vincristine β-tubulin binding capacity with increasing age suggests that young children tolerate higher doses of vincristine.

Keywords oncology · pediatric · pharmacokinetics · population pharmacokinetics

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Introduction

Vincristine is used in the chemotherapeutic treatment of various pediatric malignancies. Its effect is caused by binding to tubulin and inhibiting microtubule formation, causing arrest of the cell at metaphase. Treatment with vincristine is mainly hampered by risk of developing vincristine induced peripheral neuropathy (VIPN). VIPN pathogenesis involves nerve cell mitochondria, endothelium and microtubules and has been shown to be dose-dependent [1, 2]. Younger children have been suggested to exhibit a lower risk of developing VIPN compared to adolescents, despite a higher dose per kg body weight administered, which underlines potential differences in pharmacokinetics (PK) and pharmacodynamics in the younger patient population [3].

Vincristine is usually dosed based on body surface area (BSA) (doses vary from 1.4–2.0 mg/m^2) [4]. Because of the dose-dependent VIPN, the absolute dose is capped to a maximum of 2 mg [4]. However, clear evidence for this maximum dose in children is lacking. Additionally, commonly used dose reductions in infants are not evidence based. For infants, several dosing regimens are used in current practice. Doses vary from 0.025–0.05 mg/kg or 50–80% of the usual dose per BSA [5–11], but none of these reductions are based on literature. Theoretically, younger children could be at risk for lower vincristine clearance values, due to incomplete maturation of cytochrome p450 (CYP) 3A4, however, findings on age-related differences in PK of vincristine in infants and children are not conclusive.

Recently, Barnett *et al.* did not report significant differences in BSA-normalized vincristine clearance values between infants and older children, apart from a trend towards lower clearance in neonates (0–4 weeks) as compared to infants (1–12 months) [12]. They showed that doses of < 0.05 mg/kg resulted in significantly lower area under the curve (AUC) values than observed in infants and children receiving doses of ≥ 0.05 mg/kg or 1.5 mg/m^2, showing that dose reductions to for example 0.025 mg/kg in infants could lead to underexposure. In a recently published in-depth literature review, based on these findings and the results of other PK studies that did not find a relationship between age and PK [13–21], we concluded that infants should be administered doses of 0.05 mg/kg or 1.5 mg/m^2 [11].

Even though age-related differences in the PK of vincristine have not been found in published studies, Lee *et al.* proposed that there is a fivefold higher β-tubulin binding capacity in children compared to adults [22]. Using a physiologically based pharmacokinetic (PBPK) modeling approach, they suggested that binding to β-tubulin in healthy tissue could play a key role in vincristine distribution, which might explain differences in toxicity. An increased fraction of the vincristine dose bound to β-tubulin in healthy tissue may lead to lower amounts of free vincristine and thus a lower risk of VIPN. Indeed, it is well known that vincristine binds to β-tubulin. Moreover, β-tubulin is abundant in thrombocytes, and, decades ago, both *in vitro* and *in vivo* studies showed that vincristine rapidly binds to thrombocytes, so it is hypothesized that thrombocyte levels could also have an effect on vincristine distribution [23–29].

This current study was conducted to unravel the complex PK of vincristine, including the effects of age, using a semi-mechanistic population PK modelling approach. Unravelling the complex PK of vincristine in (very) young children, alongside key clinical pharmacology data recently published in this area [12], will promote more rational vincristine dosing in this patient population.

Methods

Patients and Sampling

A prospective observational study was performed in Princess Máxima Center for Pediatric Oncology in the Netherlands. Patients up to the age of 18 years with a central venous line *in situ* were eligible for inclusion after written informed consent was obtained. No restrictions for types of tumors or malignancies were formulated, but patients with Down syndrome were excluded. Ethical approval by the institutional Medical Ethics Committee of the Erasmus MC was obtained (NL63037.078.18). The data generated from this study were combined with data from an ongoing prospective observational study in 20 clinical cancer centers across the UK. In the UK study, patients with Ewing sarcoma up to 24 years of age with a central venous line *in situ* were eligible for inclusion after written informed consent was obtained. Patients with a glomerular filtration rate < 60 mL/min/1.73m^2 were excluded. Ethical approval by the National Research Ethics Service committee North East-Newcastle and North Tyneside 1 was obtained (EudraCT 2013–000,052-17). Beside these two prospectively collected cohorts, data from three historical cohorts previously described by Lee *et al.* [22] (n = 24; only UK patients were included), van de Velde *et al.* [30] (n = 37) and Barnett *et al.* [12] (n = 26) were included in this analysis. All previous studies included patients up to the age of 18 years.

All patients were treated with vincristine as standard of care, with doses according to local protocols. Doses, varying from 1–2 mg/m^2 with a maximum of 2 mg, with specific reductions for infants, were administered either as bolus or 1 h infusion.

In total, 4–8 blood samples per patient were collected at various time points. Vincristine plasma concentrations were quantified using a previously described high-performance liquid chromatography tandem mass spectrometry (LC–MS/MS) method [31] or a validated LC–MS assay developed in Newcastle [32], with lower limits of quantification (LLOQ) of 0.25 ng/mL and 0.50 ng/mL respectively. Vincristine plasma concentrations of the Princess Máxima Center for Pediatric Oncology study were quantified using a validated LC–MS/MS method using 200 µL human plasma, with a LLOQ of 0.10 ng/mL [33]. First samples below LLOQ were included using ½ of the LLOQ value.

When data on covariates (age, body weight (BW) and height) were missing, values were imputed based on UK growth charts [34] and known variables. For all cases where age was missing, BW and height were documented, therefore, these values were used to find the corresponding age in the growth charts (using median BW and height curves). In the cases where BW and height were missing, age and BSA were available. The age and BSA were used to find the corresponding height, and this value and the BSA were used to calculate the BW, using the Du Bois Eq. [35].

Model Development

For the structural model, two- and three-compartment models with first order elimination were tested.

Saturable binding to β-tubulin was implemented by incorporating the maximal binding capacity (Bmax) in the differential equation as follows:

$$\frac{dA(bound)}{dt} = k_{on} \times A(Vc) \times \left(1 - \frac{A(bound)}{B_{max}}\right) - k_{off} \times A(bound) \quad (1)$$

where k_{on} is the association rate constant, k_{off} is the dissociation rate constant, A(Vc) is the amount of vincristine in the central compartment Vc, A(bound) is the amount of vincristine bound to β-tubulin and Bmax is the maximal binding capacity to β-tubulin. Bmax was estimated. See Supplementary Table S1 for differential equations of other compartments.

Interindividual variability (IIV) was evaluated for all PK parameters, and implemented as follows:

$$P_i = P_{pop} \times e^{(\eta_i)} \quad (2)$$

where P_i is the individual parameter estimate for individual i, P_{pop} is the typical population parameters estimate, and η_i is assumed to be normally distributed with a mean of zero and a variance of ω^2.

Since data of multiple cycles of therapy were available, interoccasion variability (IOV) was implemented similarly as IIV, with each dose and subsequent sampling defined as a separate occasion. This variability was evaluated for clearance parameters and Bmax to diagnose potential time-dependent trends and to allow for random unaccounted variability between dosing moments.

Residual unexplained variability was evaluated as a proportional error model or as a combination of a proportional and additive error model.

Covariate Analysis

The influence of patient-specific factors for variability in PK parameters were evaluated following structural model development. Allometric scaling was applied to implement the impact of body weight on PK parameters with a fixed exponent of 0.75 resp. 1 for clearances resp. volumes of distribution. PK parameters were normalized to a BW of 70 kg [36]. Other assessed covariates included age and thrombocyte levels, using a power function, normalizing to an age of 18 years and a thrombocyte level of 300×10^9/L, respectively.

Model Evaluation

Discrimination between models was guided by physiological plausibility, goodness-of-fit (GOF) plots, precision of parameter estimates and change in objective function value (dOFV). A drop of ≥ 3.84 points, corresponding to a $P < 0.05$ (χ^2-distribution with 1 degree of freedom (df)), was considered a significant improvement. The adequacy of the models was assessed by GOF plots and visual predictive checks (VPC) [37]. The sampling importance resampling (SIR) procedure was used for the assessment of parameter precision [38].

Software

Nonlinear mixed-effects modeling was performed using NONMEM (version 7.3.0, ICON development Solutions, Ellicott City, MD, USA) and Pearl-speaks-NONMEM (PsN, version 4.9.0) with First-Order Conditional Estimation with interaction (FOCE-I) as estimation method [39, 40]. Pirana (version 2.9.9) was used as graphical user interface for NONMEM [41]. R (version 3.4.3) was used for data handling and visualization [42].

Results

Patients and Sampling

In total, 206 patients with a median age of 8.3 years (range 0.04–33.9) were included. Detailed patient characteristics are presented in Table I. 25 patients, with 25 vincristine

Table I Patient Characteristics (Median (range), Unless Specified Otherwise)

	N = 206
Available data	
Total no. of occasions	253
Total no. of PK samples [n]	1297
No. of occasions per patient	1 (1–5)
No. of samples per occasion	5 (1–8)
Patient characteristics	
Age, years	8.3 (0.04–33.9)
No. of patients 0–1 yrs [n]	25
Actual body weight, kg	27.1 (2.9–126.0)
Female sex [n (%)]	98 (48%)
Thrombocyte levels	
Available occasions [n (%)]	137 (54%)
Thrombocyte levels, $\times 10^9$/L	224 (5–1063)
Not available occasions [n (%)]	116 (46%)
Vincristine treatment	
Dose, mg	1.6 (0.1–2.0)
Dose, mg/m^2	1.4 (0.4–2.5)
Dose, mg/kg	0.05 (0.02–0.09)
Infusion duration [n]	
Bolus	214
15–113 min	39

PK Pharmacokinetic(s)

cycles and 88 samples, were younger than 1 years of age (7 patients 0–3 months; 8 patients 3–6 months; 4 patients 6–9 months; 6 patients 9–12 months). In total, 1297 samples were available, of which 30 samples were below the LLOQ. Supplementary Figure S1 displays the observed plasma concentrations over time.

In total, for 8 patients the age was missing and for 2 patients BW and height were missing and were imputed based on UK growth charts. All these patients came from UK studies.

Model Development

The base model that best described the data was found to be a three-compartment model with first order elimination. Allometric scaling using BW was *a priori* included on all PK parameters. Following structural model development, a saturable compartment was incorporated, resembling saturable binding to β-tubulin and thus, saturable distribution, to test the hypothesis of Lee *et al.* [22]. They hypothesized that binding to β-tubulin has a significant impact on the PK of vincristine. This third, saturable compartment was incorporated as a compartment, driven by the concentration in the central compartment. Adjustment of the base three-compartment model to a three-compartment model containing one saturable compartment, resulted in a drop in OFV of 80 points. This model was parameterized in terms of volume of distribution of the central (Vc) and peripheral (Vp) compartment, clearance from the central compartment (CL) as well as intercompartmental CL between Vc and Vp (Q), Bmax, the association rate constant (k_{on}) and dissociation rate constant (k_{off}). k_{on} was considered to be too fast to estimate adequately, so was fixed at 1300 /h (the value that resulted in the lowest OFV). The model was further optimized by adding IIV on CL, Q, Vc, Vp, k_{on} and k_{off} and IOV on Bmax. No trends in IOV on Bmax *vs*. dosing occasions or age were observed.

Covariate Analysis

Subsequently, various covariates were tested for their influence on PK parameters and Bmax. In the PBPK model of Lee *et al.* [22], age was found to be a significant covariate for β-tubulin expression, defined as Bmax in our model. Furthermore, we expect Bmax to be dependent on BW, based on allometric scaling principles. For this reason, BW and age were tested as covariates on Bmax. Firstly, BW was included in Bmax using a power function with an estimated exponent. The exponent was estimated to be 0.707. This was thought to be a result of a combined, opposite effect of BW and age, where an increasing BW would lead to an increase in Bmax (allometric principles), but where an increase in age would lead to a decrease in Bmax (hypothesis Lee *et al.* [22]). For this reason, the exponent on BW was fixed to 1 (in accordance with allometric scaling for volumes of distribution) and age was included as covariate using a power function (normalization to a patient of 18 years), with an estimated exponent. This resulted in an exponent of -0.199 for age.

Furthermore, thrombocyte levels were tested as covariate on Bmax. Several studies showed that vincristine binds to thrombocytes, which is hypothesized to be related to tubulin, since β-tubulin isoforms are abundant in human thrombocytes [29]. Data on thrombocyte levels were not available for 46% of the occasions. When thrombocyte levels were not available, a thrombocyte count of 300×10^9/L was imputed, plus IIV to allow for variability on this imputed value. An IIV of around 30% was found. However, adding thrombocyte levels as covariate on Bmax resulted in unstable models with divergent OFV values, very sensitive to initial estimate changes. In addition, the IOV on Bmax did not decrease and IIV's on other parameters increased. Therefore, thrombocyte levels were not included as covariate on Bmax in the final model.

A graphical representation of the final model is presented in Fig. 1. Final PK parameters estimates are displayed in Table II. Figure 2 displays the typical Bmax and CL vs. age for patients until the age of 2 years. Data was based on typical weight and height values according to WHO growth

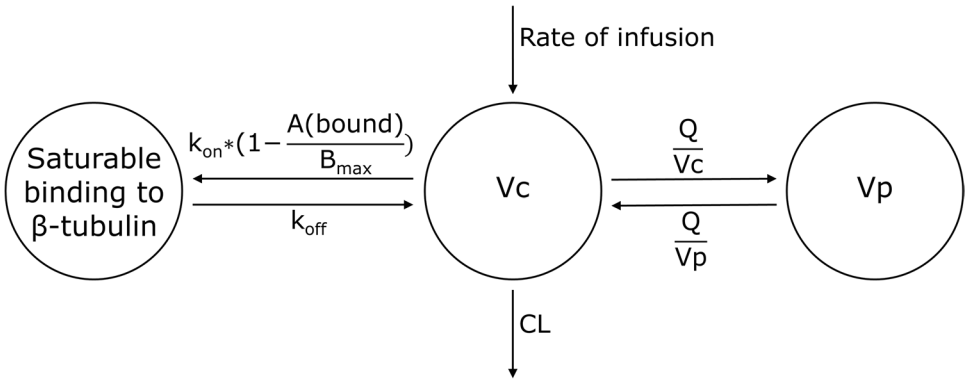

Fig. 1 Graphical representation of the final model for vincristine. k_{on} is driven by the amount of vincristine, bound to tubulin (A(bound)) and Bmax. Bmax Maximal binding capacity; CL Clearance; k_{off} Dissociation rate constant; k_{on} Association rate constant; Q Intercompartmental clearance; Vc Vincristine central compartment; Vp Vincristine peripheral compartment.

Table II Vincristine PK Parameters Estimates of the Final Model

Parameter	Estimate	95% CI
CL_{70kg} (L/h)	30.6	27.6 – 33.0
Q_{70kg} (L/h)	63.2	57.2 – 70.1
Vc_{70kg} (L)	5.39	4.23 – 6.46
Vp_{70kg} (L)	400	357 – 463
$Bmax_{18yrs,70\ kg}$ (mg)	0.525	0.479 – 0.602
k_{on} (/h)	1300 fixed	
k_{off} (/h)	11.5	9.2 – 14.5
Age on Bmax	-0.199	-0.304 – -0.090
IIV CL (%)	47.7	41.0 – 54.3
IIV Q (%)	38.1	26.2 – 49.0
IIV Vc (%)	122.5	98.7 – 158.3
IIV Vp (%)	57.1	48.8 – 69.7
IIV k_{on} (%)	126.5	108.7 – 147.8
IIV k_{off} (%)	24.1	11.1 – 33.8
IOV Bmax (%)	59.1	50.7 – 66.1
Proportional residual error (%)	30.1	28.9 – 31.4

Bmax Maximal binding capacity; *CI* Confidence interval obtained by sampling importance resampling; *CL* Clearance; *IIV* Interindividual variability; *IOV* Interoccasion variability; k_{off} Dissociation rate constant; k_{on} Association rate constant; *PK* Pharmacokinetic(s); *Q* Intercompartmental clearance; *Vc* central compartment; *Vp* peripheral compartment

Bmax corresponds to a subject of 18 years weighing 70 kg, other population estimates correspond to a subject weighing 70 kg and are adjusted to an individual value using allometric scaling

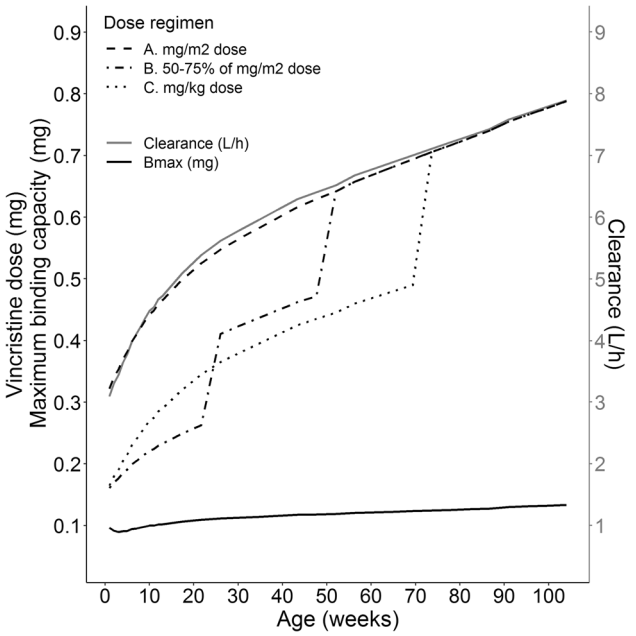

Fig. 2 Vincristine clearance (solid grey line), maximum binding capacity (solid black line) and absolute vincristine dose for three different infant dosing regimen over age: A. All ages: 1.5 mg/m^2 (dashed line). B. Children < 6 months: 50% of BSA dose (0.75 mg/m^2); Children 6–11 months: 75% of BSA dose (1.125 mg/m^2); Children ≥ 12 months: 1.5 mg/m^2 (dashdotted line). C. Children < 10 kg: 0.05 mg/kg/day; Children ≥ 10 kg: 1.5 mg/m^2 (dotted line).

charts [34]. Absolute doses according to three different dosing regimens were included:

A. All ages: 1.5 mg/m^2
B. Children < 6 months: 50% of BSA dose (0.75 mg/m^2); Children 6–11 months: 75% of BSA dose (1.125 mg/m^2); Children ≥ 12 months: 1.5 mg/m^2
C. Children < 10 kg: 0.05 mg/kg/day; Children ≥ 10 kg: 1.5 mg/m^2

Model Evaluation

The model performance was checked through GOF plots. Looking at population and individual predictions, conditional weighted residuals vs. plasma concentration and time after dose, no trends, or signs for over- of underprediction have been found (Supplementary Figure S2). Furthermore, the VPC showed no signs for structural over- of underprediction (Supplementary Figure S3). The PK parameters of the patients with imputed age or BW and height were not markedly different.

Discussion

This study successfully implemented saturable binding to β-tubulin in a population PK model of vincristine in children. A three-compartment model, including one saturable compartment, was found to best describe the available data from 206 patients. This saturable compartment resembles saturable binding to β-tubulin and thus, saturable distribution of vincristine.

Two covariates were identified to account for variability in the binding capacity to β-tubulin. BW was included as covariate to Bmax using allometric principles. Additionally, age was added as covariate using a power function. BW and age were found to have an opposite effect on Bmax. By using allometric scaling for volumes of distribution, a rise in BW resulted in an increase in Bmax, while we found that a higher age led to a decrease in Bmax. This is in line with the findings of Lee *et al.* [22]. With their PBPK analyses they showed that there is a fivefold higher β-tubulin binding capacity in children compared to adults.

Clinical information on the difference in β-tubulin exposition between children and adults is not available, but it has been shown that immunohistochemical distribution of β2-tubulin was higher in tissue of neonates compared to older children and adults, and that the expression decreased with increasing age [43]. The same is to be expected for other β-tubulin isotypes, since tubulin in microtubules play an important role in cell division, which is more prevalent in children. A higher β-tubulin expression in younger children is most likely the reason for a higher β-tubulin binding capacity of vincristine. However, evidence for the differences in β-tubulin expression between neonates and older children remains missing. Future studies should address β-tubulin expression of various β-tubulin isotypes in different types of tissue and different age categories.

A higher β-tubulin binding capacity leads to a faster drop of the vincristine plasma concentration and lower amounts of free vincristine present in the central compartment thus a lower risk of VIPN, since we assume that free vincristine is able to distribute to peripheral tissue, where it causes VIPN. In children, with a higher β-tubulin binding capacity, a lower amount of free vincristine is available to distribute to peripheral tissue, while in adults, with a lower β-tubulin binding capacity, the amount of free vincristine is higher as well as the risk of developing VIPN. This hypothesis is consistent with the findings that younger children seem to tolerate higher doses with regards to development of VIPN compared to older children and adults [1, 3].

These findings also raise the question whether children should be administered higher doses than adults to achieve the same effect. Children are usually treated with doses of 1.5–2.0 mg/m^2, with a maximum of 2 mg. Essentially, the capped dose comes into play for patients with a BSA > 1.3 m^2. It is unclear to what extend and over what age range the risk of developing VIPN is lower than in adults, so definitive advice for changing the maximum dose for children cannot be given. For younger children, however, we could make some remarks based on the current PK study and previous research. As mentioned previously, infants are treated with doses varying from 0.025–0.05 mg/kg or 50–80% of the usual dose per BSA [5–11]. Barnett *et al.* [12] showed that doses of < 0.05 mg/kg result in significantly lower AUC values than observed in infants and children receiving doses of ≥ 0.05 mg/kg or 1.5 mg/m^2. These exposure data, combined with our current results, strongly suggest that dose adjustments for infants may not be justified.

A major concern with current dosing approaches for vincristine, is that using different dosing regimens for infants leads to disproportional increases in the dose when the patient reaches a specific age or weight (see Fig. 2). While this widely used rudimentary approach to dosing is a concern for all drugs, it is of particular concern for a drug such as vincristine, for which no age-related differences in CL have been found. In the current study, we show that younger children have a higher β-tubulin binding capacity for vincristine. However, absolute Bmax values (taking BW and age into account) in patients up to 2 years of age, displayed in Fig. 2, do not seem to change markedly over time, except for the first weeks of age. Furthermore, Fig. 2 visually shows that a dose regimen of 1.5 mg/m^2 follows the curve of vincristine CL with increasing age. From a pharmacokinetic perspective, we would suggest administering the full mg/m^2 (e.g. 1.5 mg/m^2) dose to infants, except for neonates of 0–4 weeks (0.05 mg/kg according to Barnett *et al.* [12]).

Increasing the dose should, however, be done with caution. Besides VIPN, other adverse reactions, like vocal cord paralysis, respiratory distress or constipation, frequently occur. Preferably, a clinical trial in young patients investigating the exposure and toxicity profiles under the proposed mg/m^2 dosing regimen compared to the mg/kg dose is performed, before changing the dose in infants.

It would be interesting to perform simulations to explore different vincristine dosing regimens in patients of different ages in order to find the best dosing strategy for every patient. However, it is not known which PK parameter represents vincristine effectivity and toxicity. A parameter related to vincristine in the central compartment (like AUC or C_{max}) might not be the right way to compare doses between different groups, since vincristine does not show its effect in the central compartment. It would be interesting if future studies would address this important topic.

Furthermore, in order to decrease the IOV on Bmax, we aimed to look into the effect of thrombocyte levels on the β-tubulin binding capacity. We did not find an effect of thrombocytes levels on Bmax or the IOV of Bmax. This is probably due to missing thrombocyte counts in a large part of the dataset. Moreover, since β-tubulin is present in all cells (all types of blood cells as well as cells in peripheral tissue) [25, 27, 44, 45], it is possible that thrombocytes account for just a small part of the β-tubulin expression throughout the body. This will be studied further using a PBPK modelling approach. In addition, it is possible that the β-tubulin binding capacity is dependent on tumour type or disease state. Tumour cells also express β-tubulin, so tumour type and/or disease state could influence vincristine distribution [23, 43]. The saturable compartment should therefore be interpreted as vincristine binding to all β-tubulin that is available. The current dataset did not contain disease type or status, so this was not tested as covariate yet.

A limitation of the current study is that the data did not include the exact amount of β-tubulin in patients, since we were not able to measure β-tubulin exposition. We have explained the saturable distribution as being vincristine binding to β-tubulin, however, alternative explanations could be made. We have looked into other developmental changes that could explain the age-related findings of the current study, but did not find other pharmacological rationales.

Another limitation relates to the fact that the effect of CYP3A4/5-inductors and –inhibitors on the PK of vincristine was not studied, since information on the use of CYP3A4/5-inductors and –inhibitors was not available. Also, genetic variations in CYP3A4/5, which can vary with race, were not taken into account in the current study. Moreover, the metabolic capacity of CYP3A4/5 change during the first years of life. However, it is to be expected that variations in activity of metabolising enzymes CYP3A4/5 only effect vincristine CL, and does not influence vincristine distribution, which is the main topic of the current study. Furthermore, previous research did not find an effect of CYP3A4/5 polymorphisms on vincristine PK [16].

Conclusion

Vincristine binding to β-tubulin was found to be dependent of body weight and age. β-tubulin binding capacity decreases with increasing age, suggesting that children can tolerate higher doses of vincristine. Based on these results and previous literature we would suggest that administration of full mg/m^2 doses to infants from 4 weeks of age may be more appropriate than the currently used mg/kg dosing regimens.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11095-022-03364-1.

Author Contributions L.N., A.L., M.Z. and A.H. were responsible for protocol development and implementation of the study. L.N., E.d.V., C.E. and M.Z. were responsible for enrolling patients. Acquisition of the data was performed by all authors. Population PK modelling was performed by L.N. A.H. supervised this work. The first draft of the manuscript was written by L.N., and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of Interest There are no conflicts of interests to declare.

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