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A physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) model of the histone deacetylase (HDAC) inhibitor vorinostat for pediatric and adult patients and its application for dose specification

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Abstract

Purpose: This study aimed at recommending pediatric dosages of the histone deacetylase (HDAC) inhibitor vorinostat and potentially more effective adult dosing regimens than the approved standard dosing regimen of 400 mg/day, using a comprehensive physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling approach.

Methods: A PBPK/PD model for vorinostat was developed for predictions in adults and children. It includes the maturation of relevant metabolizing enzymes. The PBPK model was expanded by (i) effect compartments to describe vorinostat concentration-time profiles in peripheral blood mononuclear cells (PBMCs), (ii) an indirect response model to predict the HDAC inhibition, and (iii) a thrombocyte model to predict the dose-limiting thrombocytopenia. Parameterization of drug and system-specific processes was based on published and unpublished *in silico*, *in vivo*, and *in vitro* data. The PBPK modeling software used was PK-Sim and MoBi.

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Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the legal requirements and ethical standards of the responsible institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants before inclusion.

Conflict of interest

Daniel Moj, Hannah Britz, Thorsten Lehr and Clinton F. Stewart have reported no potential conflicts of interest. Walter E. Haefeli and Jürgen Burhenne have received financial and non-financial (analytical standards) support from Merck & Co., Inc., USA for the development and application of the vorinostat assay. Jürgen Burhenne has received travel support and lecture fees from MSD Sharp & Dohme GmbH, Germany.

Results: The PBPK/PD model suggests dosages of 80 and 230 mg/m² for children of 0-1 and 1-17 years of age, respectively. In comparison to the approved standard treatment, *in silico* trials reveal 11 dosing regimens (9 oral, 2 intravenous infusion rates) increasing the HDAC inhibition by an average of 31%, prolonging the HDAC inhibition by 181%, while only decreasing the circulating thrombocytes to a tolerable 53%. The most promising dosing regimen prolongs the HDAC inhibition by 509%.

Conclusions: Thoroughly developed PBPK models enable dosage recommendations in pediatric patients and integrated PBPK/PD models, considering PD biomarkers (*e.g.*, HDAC activity and platelet count), are well suited to guide future efficacy trials by identifying dosing regimens potentially superior to standard dosing regimens.

Keywords

Vorinostat; pediatrics; physiologically-based pharmacokinetics; histone deacetylase; thrombocytopenia; pharmacodynamics

1. Introduction

Histone deacetylases (HDACs) regulate gene transcription and cell signaling pathways. Changes in the structure, activity, or expression of HDACs can result in abnormal gene transcription and cell signaling leading to cancer [1]. HDAC inhibitors (HDACi), such as vorinostat, show antineoplastic effects *in vitro* and *in vivo* and offer therefore a new approach in chemotherapy [2]. Vorinostat is a fast, light-binding inhibitor with short residence time at the target that inhibits the enzymatic activity of Class I (HDACs 1-3) and Class II (HDAC 6) HDACs at nanomolar concentrations (IC₅₀ = 30-86 nM) [3,4]. It was approved by the U.S. Food and Drug Administration (FDA) for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma (CTCL) who have progressive, persistent, or recurrent disease on, or following, two systemic therapies [4,5].

Vorinostat shows a dose-proportional exposure increase after oral or intravenous doses of 100 to 800 mg or 75 to 900 mg/m², respectively, with an absorption-rate limited drug disposition in the gastrointestinal tract after oral dosing, leading to flip-flop pharmacokinetics (PK) [6]. Elimination primarily comprises metabolism, involving glucuronidation and hydrolysis followed by β -oxidation without contribution of CYP enzymes [7]. Renal excretion is negligible accounting for ~ 1% of total body clearance [8]. While the UDP-glucuronosyltransferases (UGTs) 1A9, 2B7, and 2B17 are the major enzymes of vorinostat glucuronidation, the enzyme responsible for hydrolysis and β -oxidation remains unidentified [7]. These enzymes exhibit nonlinear age-dependent maturations completed within 10 years after birth [9-14]. Genetic polymorphisms of UGT 2B17 might play a role in the clearance of vorinostat and in clinical outcomes [15-17]. In general, vorinostat exhibits a short half-life of 1 (intravenous) to 2 h (oral) and multiple-dose PK similar to single-dose administration [7].

Clinical studies of vorinostat in patients with stage Ib and higher CTCL and in patients with refractory CTCL demonstrated overall objective responses of 30 and 31%, respectively [18,19]. The most common adverse reactions, with an incidence ~ 10%, associated with

vorinostat treatment are anorexia, diarrhea, dysgeusia, fatigue, nausea, and thrombocytopenia with thrombocytopenia being the most common hematologic event [5,20]. In pediatric studies, vorinostat was well tolerated at 230 mg/m²/day (alone or in combination with bortezomib) or 300 mg/m²/day (in combination with temozolomide) and showed a drug disposition similar to that observed in adults [21-23].

While *in vitro* experiments suggest that vorinostat concentrations of 2.5 µmol/l lead to the maximum accumulation of acetylated histones, it has also been shown that the HDAC inhibition has to be maintained over a significant period of time to show antitumor activity [24,25]. Furthermore, vorinostat enhances the effect of other chemotherapeutics such as cisplatin and gemcitabine at concentrations > 2 µmol/l, which is, nevertheless, inconsistently achieved in patients at the approved 400 mg/day (qd) dose [6,26,27]. Little is known about the impact of different dosing regimens on its efficacy [6]. Hence, Dickson and co-workers attempted to achieve maximum vorinostat concentrations ($C_{\max}$) of > 2.5 µmol/l by an intermittent oral pulse dosing protocol of vorinostat in combination with the cyclin-dependent kinase inhibitor flavopiridol [28]. In this attempt, the $C_{\max}$ of vorinostat could be increased, but unfortunately the incidence of myelosuppression was also increased. However, these promising results demand further assessment of alternative vorinostat dosing strategies that might show more effectiveness and still tolerable toxicity compared to the vorinostat standard treatment.

Here, physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling and simulation enables the assessment of dosing regimens in *in silico* trials, while also being able to test clinical trial designs.

1.1 Objectives

1. Build and evaluate an adult whole-body PBPK model of vorinostat able to describe and predict the PK of varying doses of intravenously and orally administered vorinostat.
2. Develop and evaluate a pediatric PBPK model for vorinostat and estimate vorinostat doses for children between 0 and 17 years.
3. Build and evaluate a PBPK/PD model incorporating (i) a HDAC inhibition model and (ii) a thrombocytopenia model.
4. Identify potentially effective vorinostat dosing regimens while considering HDAC activity and the number of circulating thrombocytes.
5. Perform parameter sensitivity analysis for the developed whole-body PBPK model of vorinostat

2. Materials and Methods

2.1 Model development and evaluation

A schematic overview of the PBPK model development and evaluation steps is shown in supplemental Fig. S1. The detailed steps of the model building are outlined in the following sections. In brief, PBPK modeling was performed in a stepwise procedure. First, an

intravenous model of vorinostat was developed for adults. Second, an oral PBPK model for adults was established. Third, age-dependent physiological and anatomical changes were implemented enabling pediatric predictions and dose estimations. Fourth, PD models were incorporated into the PBPK model to describe HDAC inhibition and thrombocytopenia. Fifth, the PBPK/PD model was used to identify new dosing regimens. Finally, a parameter sensitivity analysis was performed.

Experimental datasets from 13 published and unpublished clinical studies were used to support the PBPK model development and evaluation (Table 1). The experimental datasets were split into an internal development (n = 4 trials) dataset and an external evaluation dataset (n = 9), such that wide dose ranges were covered in both datasets. The PBPK model was fitted to the development dataset and the prediction quality of the model was assessed by predicting the evaluation dataset. Parameter optimizations were carried out using either a Monte-Carlo or Nelder-Mead method [29]. An overview of fitted parameters and the applied optimization method is given in supplemental Table S1. The Monte-Carlo approach was used if no initial information on parameter values was available.

Model evaluation was carried out using (i) comparison of observed and simulated PK parameters and the fraction of vorinostat dose excreted unchanged into urine, (ii) mean prediction errors (MPE) of pharmacokinetic profiles [30] and (iii) visual predictive checks (VPC), which is a tool for evaluating the performance of PK and PD models, where percentiles of experimental and simulated data are compared [31]. PK parameter comparisons were deemed successful when they satisfied the 2-fold acceptance criterion.

Simulations were carried out using virtual PBPK individuals and populations. Virtual PBPK populations were created as described by Willmann and co-workers using ranges for age, height, and weight reported in the internal and external datasets [32]. In this approach the organ weights and the cardiac output (CO) of a target individual are allometrically scaled with height and the organ blood flows are scaled to the total CO [32]. To assess the inter-study variability of PK parameters, 10 virtual populations were created for each study. Applied dosing schedules and doses were used as published. The parameters of anatomy and physiology were varied as pre-defined in PK-Sim [33-36], parameters of the pharmacodynamic models were varied with a coefficient of variation (CV) of 20%.

An overview of important assumptions made during model development and evaluation is given in supplemental Table S2.

PBPK simulations were carried out using PK-Sim and MoBi (Version 6.3.2, Bayer Technology Services, Leverkusen, Germany). Statistical analyses of the results and graphics were compiled using MATLAB (Version R2013b, The MathWorks Inc., Natick, MA, USA).

2.1.1 Adult PBPK model - intravenous—Intravenous concentration-time profiles were used to establish a PBPK model for vorinostat. The partition coefficients for vorinostat were calculated using the PK-Sim standard model [37,38]. To describe the distribution phase correctly, the cellular permeability of vorinostat was estimated. The reference concentrations for UGT 1A9, 2B7, and 2B17 were kept at the PK-Sim default values of 1 $\mu\text{mol/l}$,

respectively. The UGT $V_{\max}$ values were gathered from the literature. The PK-Sim gene expression database was used to distribute the enzymes to the specific organs [39,40]. To fit the vorinostat half-life and concentration-time profiles, the unidentified hydrolytic and oxidative enzyme was implemented as a generic enzyme with a reference concentration of 1 $\mu\text{mol/l}$ assuming similar availability across organs. The clearance value for hydrolysis and oxidation was fitted. All metabolic reactions were modeled as first order processes. The unbound vorinostat was assumed to be filtered and excreted with the glomerular filtration rate (GFR).

2.1.2 Adult PBPK model – oral—The developed intravenous PBPK model of vorinostat was expanded by an oral formulation. The particle dissolution module of PK-Sim was used to describe vorinostat drug release in the intestine. This module calculates the dissolution kinetics of spherical particles with a predefined particle size distribution based on the Noyes-Whitney approach [41]. To describe the absorption of vorinostat correctly, the intestinal permeability coefficient was estimated. To capture the whole-blood-to-plasma ratio of 2.87, the red blood cell permeability was estimated [42].

Model evaluation of the intravenous and oral adult PBPK model was carried out comparing observed and predicted PK parameters and concentration-time profiles of the external evaluation dataset.

2.2 Pediatric PBPK model and dosage recommendations

The developed oral PBPK model of vorinostat was expanded by importing available age-dependent anatomical and physiological changes in human development from the PK-Sim databases [33]. The age-dependent maturation of UGT 1A9 and 2B7 was already available in PK-Sim, whereas the UGT 2B17 maturation was implemented using a Hill function (equation 1) based on recently published mRNA data [14],

$$\text{UGT 2B17 ontogeny factor (OF)} = \text{OF}_{\min} + \frac{\text{OF}_{\max} * \text{Age}^{\gamma}}{\text{OF}_{50}^{\gamma} + \text{Age}^{\gamma}} \quad (1)$$

where $\text{OF}_{\min}$ denotes the intercept with the y-axis and $\text{OF}_{\max}$ and OF_{50} represent the maximum adult ontogeny factor (= 1) and the post-menstrual age needed to reach 50% of the maximum ontogeny factor, respectively. Age denotes the post-menstrual age in years and γ denotes the Hill factor. The unidentified enzyme responsible for the hydrolysis and oxidation of vorinostat was assumed to be fully developed from birth on.

Model evaluation of the pediatric PBPK model was carried out comparing observed and predicted PK parameters and concentration-time profiles of the external evaluation dataset that consisted of 13 individual vorinostat plasma concentration-time profiles with individual ages ranging from 4.7 – 22 years.

The developed pediatric PBPK model of vorinostat was used to identify age-stratified $\text{mg/m}^2/\text{day}$ doses for children and adolescents from birth to 17 years of age. Pediatric doses were selected when the 25th, 50th, and 75th percentiles of predicted $\text{AUC}_{0-\text{inf}}$ or $C_{\max}$ values

for the pediatric age groups were comparable to the values of the adult population dosed with the approved oral dose of 400 mg/day. Two virtual populations were generated, first, for adults (age 18, n = 500 and second, for children (age 0 – 17, n = 10,000). The body surface areas were calculated using the DuBois equation [43].

2.3.1 Adult PBPK model – HDAC activity—The oral PBPK model of vorinostat was expanded by an indirect response model to describe the HDAC activity in peripheral blood mononuclear cells (PBMCs) during vorinostat treatment [6]. PBMCs are not offered as physiological compartments in the default PK-Sim PBPK model [42]. Therefore, two PBMC effect compartments were implemented in the default model (Fig. 1). First, the rate constants $k_{\text{effect},1}$, $k_{\text{effect},2}$, $k_{\text{effect,deep},1}$, and $k_{\text{effect,deep},2}$ of the PBMC effect compartmental model were estimated. Second, the HDAC activity in the central PBMC compartment was modeled using an indirect response model, where the HDAC activity R was described by equation 2 [44],

$$\frac{dR}{dt} = k_{\text{in}} - k_{\text{out}} * \left(1 + \frac{S_{\text{max}} * C_{V, \text{PBMC}}}{SC_{50} + C_{V, \text{PBMC}}} \right) * R \quad (2)$$

where k_{in} is the apparent zero-order rate constant for the production of the activity, k_{out} represents the first-order rate constant for activity loss, and R is assumed to be stationary with an initial value of $R_0 (= \frac{k_{\text{in}}}{k_{\text{out}}})$. The SC_{50} value represents the PBMC vorinostat

concentration ($C_{V, \text{PBMC}}$) producing 50% of the maximum k_{out} stimulation achieved at the effect site, whereas S_{max} is the maximum k_{out} stimulation achieved at the effect site. Predictions of HDAC activity following multiple oral vorinostat doses were carried out using intracellular vorinostat concentrations of the bone instead of PBMC vorinostat concentrations.

Model evaluation of the HDAC activity was carried out by comparing the observed and simulated HDAC activity-time profiles of the internal development dataset using a virtual population.

2.3.2 Adult PBPK model – platelet count—Thrombocytopenia was defined as a platelet count $< 150 * 10^9$ cells/l [45]. The adult PBPK model of vorinostat was expanded by a thrombocytopenia model as a pharmacodynamic toxicity biomarker. The platelet model was first published and comprehensively described by Friberg and co-workers and refined by du Rieu and co-workers [46,47]. The refined thrombocytopenia model was used in the presented PBPK modeling approach (Fig. 1).

In brief, the thrombocytopenia model consists of 5 compartments (cmts) describing proliferating cells (1 cmt), developing thrombocytes (3 transit cmts) and circulating thrombocytes (1 cmt). Both, the mean transit time (MTT) of the developing thrombocytes and the proliferation rate constant are influenced by the number of circulating thrombocytes by a feedback mechanism. Vorinostat reduces the proliferation or induces cell loss of thrombocytes.

Parameter values of the thrombocytopenia model were gathered from the literature (suppl. Table S3), except for the slope parameter of vorinostat, which was estimated using clinical study data of thrombocytosis patients [48].

The thrombocytopenia model was evaluated by comparing the observed and predicted fraction of patients with normal platelet counts that develop thrombocytopenia during a 24-week treatment with 400 mg vorinostat per day.

2.4 Identification of new vorinostat dosing regimens

The adult PBPK/PD model of vorinostat, including the HDAC activity and the thrombocytopenia model was used in *in silico* trials to identify vorinostat doses and dosing schedules that are potentially more effective than the standard dosing regimen of 400 mg qd, where ‘more effective’ is defined as causing a larger decrease in HDAC activity. Tested dosing regimens were chosen from a range of potential dosing frequencies per day (qd, bid, tid) and week (*e.g.* every 2nd week, 3 consecutive days per week). Intravenous and oral routes of administration were considered.

In silico trials – part 1: 25 dosing regimens with varying administration routes, administered doses, and dosing schemes of vorinostat were tested. New regimens were declared potentially more effective when they showed higher maximum (HDAC_{activity, bone} [%]) and longer HDAC inhibition than the standard treatment during a 10-week treatment period without decreasing the thrombocyte count [10^9 cells/l] more than the standard dosing regimen. A simulation time of 10 weeks was necessary to ensure that the circulating thrombocytes were at steady-state (MTT ~ 95 h).

In silico trials – part 2: Maximum doses were estimated for 15 dosing schedules from *in silico trials – part 1*. In trial 2, the minimum thrombocyte count was set at $50 \cdot 10^9$ cells/l and the maximum possible single vorinostat dose was set to 3000 mg.

All trial results were compared to the standard treatment based on the change of HDAC activity (%), the change in the accumulated time of HDAC activity < 50% (%), and the change in the thrombocyte count (%).

2.5 Parameter sensitivity analysis

A parameter sensitivity analysis of the adult PBPK model was performed to assess the impact of estimated model parameters on the predicted values of AUC_{0-inf}, C_{max}, half-life, and T_{max}. Assessed parameters were: Hydrolysis and β -oxidation clearance, intestinal permeability, cellular permeability, red blood cell permeabilities, and the unstirred layer of the particle dissolution. All parameters were varied individually over a wide range of values (via multiplication by factors between 0.1 and 10.0) and the changes in AUC_{0-inf}, C_{max}, half-life, and T_{max} were documented [49]. If a 10% change of a single parameter led to a > 1% change in AUC_{0-inf}, C_{max}, half-life, and T_{max}, the model was considered sensitive to this parameter [50].

3. Results

A comprehensive PBPK model for the prediction of vorinostat concentrations after different intravenous and oral doses of vorinostat has been successfully developed. The model can be applied in adult and pediatric patients and has been used to recommend doses for children from 0-17 years. Furthermore, the PBPK model has been expanded by (i) PBMC effect compartments and (ii) pharmacodynamic models of HDAC activity and thrombocytopenia. Finally, the vorinostat PBPK/PD model has been successfully used to identify new vorinostat dosing regimens that are potentially more effective than the approved standard treatment.

The developed vorinostat PBPK model shows good descriptive and predictive performance. In summary, 90 and 96% of the predicted AUC_{0-inf} and C_{max} values satisfy the 2-fold acceptance criterion, respectively (Fig. 2, suppl. Fig. S2). The MPE of the simulated and predicted adult and pediatric pharmacokinetic profiles is 44%. Trial specific MPEs are summarized in supplemental Tables S4. The final model parameters on the drug release, absorption, distribution, metabolism, and excretion of vorinostat are shown in Table 2.

Adult PBPK model - intravenous:

The intravenous model is able to describe and predict vorinostat concentration-time profiles in close agreement with the observed values over a wide range (75–900 mg/m²) of intravenous single dosages (Fig. 3, suppl. Fig. S3). The mean observed dose-independent half-life of vorinostat across available doses and studies is 0.65 h [4,51] where the PBPK model predicts 0.75 h, a slight over-prediction of 15%. The C_{max} and the AUC_{0-inf} have been predicted with mean deviations of 4% and 25% from the observed values, respectively [4,51]. The simulated fraction of unchanged vorinostat dose excreted in urine (f_e) has been consistently below 1% over all doses, which is also in good agreement with observed values [4].

Adult PBPK model – oral:

The oral PBPK model also shows a good descriptive and predictive performance (Fig. 3, suppl. Fig. S3). The AUC_{0-inf} , C_{max} , and T_{max} have been predicted with mean deviations of 5%, 1% and 22% from the observed values across available doses, respectively [6,8,28,42,52-56]. The absorption-rate limited PK is well predicted. The observed oral half-life is 1.75 h [6,8,28,42,52-56], where the PBPK model predicts a half-life of 1.66 h. The predicted bioavailability ($F_{pred} = \frac{AUC_{oral, pred}}{AUC_{IV, pred}} * \frac{Dose_{oral, pred}}{Dose_{IV, pred}}$) is comparable to the observed bioavailability (F_{obs}) with $F_{pred} = 30\%$ and $F_{obs} = 40\%$ [6]. The contribution of the implemented metabolic clearance processes via UGT 1A9, 2B7, 2B17, and hydrolysis/oxidation to the overall metabolized fraction (f_m) has been estimated at 3, 56, 11, and 30%, respectively.

Pediatric PBPK model and dosage recommendations:

The age-dependent maturation of UGT 2B17 has been successfully described and implemented using a Hill function with final parameter values of $OF_{max} = 0.46$, $OF_{50} = 6.0$

years, and $\gamma = 1.63$, and $OF_{\min} = 0.58$ (suppl. Fig. S4). The maturation time of the primary metabolizing enzymes of vorinostat can be ranked as follows: UGT 2B17 (longest) > 1A9 > 2B7. Given that there is (i) no clear relationship between pediatric doses and exposures and (ii) no inter-individual variability estimated, the individually predicted vorinostat concentration-time profiles are in adequate agreement with the observed data in the age range between 4.7 – 22.0 years (suppl. Fig. S5).

Because the, so far, proposed pediatric dosage of 230 mg/m²/day leads to very high exposure in children between 0-1 years (suppl. Fig. S6, a), the pediatric PBPK model has been used to identify dosages for all age groups from 0 to 17 years leading to $C_{\max}$ values similar to the ones in adults. The recommended dosages are 80 mg/m² for children from 0 - 1 year and 230 mg/m² for children and adolescents between 1 – 17 years (suppl. Fig. S6, b).

Adult PBPK model – HDAC activity:

The default PK-Sim PBPK model has been successfully expanded by two effect compartments describing PBMC concentrations and by an indirect response model of HDAC activity. The PBMC concentration-time profiles are best described using a 2-compartment model (Fig. 4, a). A comparison between vorinostat PBMC concentration-time profiles simulated using a 1- or 2-compartmental model is depicted in supplemental Figure S7. The observed half-life of oral vorinostat in PBMCs is 5.29 h, 3.0-fold longer than observed in plasma, and the predicted half-life in PBMCs (2-cmt) is 5.66 h, 3.4-fold longer than predicted in plasma (unpublished). The indirect response model is able to accurately describe the HDAC inhibition caused by a single dose of 400 mg vorinostat (Fig. 4, b). The minimum observed and predicted HDAC activities are 21.6 and 26.6%, respectively (unpublished). The final model parameters of both the PBMC effect compartment model and the HDAC indirect response model are given in supplemental Table S5. The structural model alterations of the default PK-Sim PBPK model are depicted in Fig 1.

Adult PBPK model – platelet count:

The PBPK model has been successfully expanded by the thrombocytopenia model. The integrated PBPK/PD model is able to capture the thrombocyte count over a 36-week standard vorinostat treatment period in a thrombocytosis population (Fig. 4, c). In a population with normal thrombocyte counts, the model has successfully predicted the thrombocytopenia prevalence of 27.9% which is in close agreement with the published observed value of 25.6% [57]. The model parameter values used in the simulations are compiled in supplemental Table S3. The manually estimated slope parameter for vorinostat is 0.22 μM^{-1} .

Identification of new vorinostat dosing regimens:

The adult PBPK/PD model of vorinostat including the HDAC activity and the thrombocytopenia model has been successfully used in *in silico* trials to identify new vorinostat doses and dosing schedules which are potentially more effective than the standard dosing regimen of 400 mg qd.

Although part 1 suggests that none of the tested 25 dosing regimens offers a higher maximum and/or prolonged HDAC inhibition when the target thrombocyte count is that of the approved standard treatment ($\sim 193 \times 10^9$ cells/l), the alternative treatment D (800 mg, every 2nd day) might be a replacement of the standard treatment showing a 14% higher maximum HDAC inhibition but a 10% shorter HDAC inhibition time (suppl. Table S6).

However, part 2 reveals that 11 (9 oral, 2 infusions) out of the 15 tested dosing schedules offer higher maximum and prolonged HDAC inhibitions (suppl. Table S7). Compared to the standard treatment, the potentially beneficial dosing regimens exhibit a mean maximum HDAC inhibition that is 27% higher (min: 16%, max: 62%) and the mean accumulated time of the HDAC inhibition being > 50% is 180% longer (min: 6%, max: 509%). Based on the accumulated time of HDAC inhibition, trial C with three daily doses of the maximum 3000 mg dose has shown the best results with a HDAC inhibition time increase of 509% and a platelet decrease of -72% which is close to the lower thrombocyte limit at -74%.

Parameter sensitivity analysis:

The parameter sensitivity analysis (suppl. Fig. S8) reveals that, among the tested parameters, the intestinal permeability, the clearance via hydrolysis and oxidation, and the unstirred layer of the particle dissolution have the biggest impact on the tested vorinostat PK parameters $AUC_{0-\infty}$, C_{max} , half-life, and T_{max} .

4. Discussion

A PBPK model for the prediction of vorinostat concentrations after different intravenous and oral vorinostat doses has been successfully developed. The model was able to accurately predict vorinostat concentrations in adult and pediatric patients and to recommend dosages for different pediatric age groups. After successful implementation of PBMC effect compartments, PD models of HDAC activity (measured in PBMCs) and thrombocytopenia were integrated in the PBPK model. The final integrated PBPK/PD model of vorinostat was successfully applied in *in silico* trials, thereby identifying potentially more effective vorinostat dosing regimens in comparison to standard treatment.

The cellular permeability drives the permeation rate of vorinostat across the interstitial - intracellular membrane in the PK-Sim PBPK model. The permeability was initially calculated within PK-Sim according to an empirically derived formula, using logMA and molecular weight as input variables, based on the procedure presented by Kawai and co-workers [58]. However, using the calculated cellular permeability value, the plasma concentration-time profiles of vorinostat were under-predicted. With the estimated permeability value, the model successfully predicted the intravenous profiles of the evaluation dataset.

In vitro tests found that UGT 2B17 is the major metabolizing enzyme of vorinostat. However, by compiling all available metabolic data of UGT 1A9, 2B7, and 2B17 in the model, the PBPK analysis suggested that UGT 2B17 is only of minor importance for the vorinostat clearance *in vivo*. This has important implications for the evaluation of other studies aiming to assess the impact of UGT 2B17 polymorphisms on survival of patients

treated with vorinostat. Furthermore, it was reported that the UGT 2B17*2 polymorphism, which leads to reduced vorinostat glucuronidation, was associated with a longer progression-free survival in Asian breast cancer patients [17]. Although, this polymorphism is present in 92% of Asian populations, based on the presented modeling results, it seems unlikely that the reported results are mainly due to the low glucuronidation capacity of UGT 2B17*2 as only a small fraction of the administered vorinostat dose is metabolized by UGT 2B17 ($f_m = 11\%$) [59,60]. The PBPK analysis suggests that UGT 2B7 ($f_m = 56\%$) polymorphisms, for instance, could play a vital role in the elimination, which is in accordance with the literature [16]. It is important to point out, that the clinical trial populations used in model development and evaluation were not tested for their UGT genotypes. Hence, additional information on their genotypes might alter the interpretation of these modeling results.

The PBPK analysis suggests that hydrolysis and oxidation is responsible for 30% of vorinostat metabolism and therefore more important than UGT 2B17 that accounts for 11%. Additionally, the sensitivity analysis underlined that the vorinostat PBPK model is sensitive to changes to the hydrolytic and oxidative clearance. In the model it was assumed that both hydrolysis and oxidation would be carried out by a single enzyme available in all tissues. Unfortunately, we do not know the contribution of either hydrolysis or oxidation to the metabolized fraction, which means that the tissue-specific expression of hydrolytic or oxidative enzymes cannot be accounted for in the model. It is known that carboxylesterases, responsible for hydrolysis of other drugs, are not equally expressed in all tissues [61]. In the model it was further assumed that hydrolysis and oxidation would be fully developed from birth on in all tissues whereas studies have shown that the developmental regulation of esterases occurs in a gene, organ, and age-dependent manner [62-64]. As no details on the oxidative enzymes are available it was decided to assume a scenario in which children would have adult enzymes level for hydrolysis and oxidation available and thus the highest possible clearance via this metabolic pathway. This might lead to an over-prediction of hydrolytic and oxidative clearance. Thereby the recommended dose of 80 mg/m² for children from 0-1 year of age might be over-predicted and should therefore be viewed as a maximum recommended dose.

The maturation of the UGTs should have the biggest impact on the pediatric vorinostat PK between the age of 0 to 3 years as the maturation progresses considerably. The youngest observed pediatric patient was 4.7 years old, thus it was not possible to evaluate the model prediction quality in the critical age below 3 years. Although there is uncertainty in the recommended doses for an age < 4.7 years, the PBPK methodology has demonstrated for several other drugs that the pediatric PK can be successfully predicted below the age of 5 [65,66].

Both, the PBMC concentration-time profiles and the HDAC inhibition could be very well described. Yet, the effect-compartment model and the indirect model were based on a single 400 mg dose only. Hence, there is some uncertainty to the *in silico* trials that were carried out using a wide range of doses. While most parameters of the thrombocytopenia model were gathered from the literature, the slope parameter of vorinostat had to be estimated. Because no other data were available, this parameter was estimated using a thrombocytosis population. It was assumed that all gathered parameters (*e.g.* MTT), but the baseline cell

counts (proliferating (Prol) and circulating (Circ) cells) were similar in patients with and without thrombocytosis, which might not be true. Nevertheless, the integrated PBPK/PD model was able to accurately predict the prevalence of thrombocytopenia in patients with normal thrombocyte counts.

Recommended doses for children were determined by targeting an exposure (AUC_{0-inf}) in children similar to the one observed in adults assuming the same efficacy and safety in these populations. Although data on the impact of vorinostat on the HDAC activity over time or the platelet count over time was not available in children the assumption appears reasonable. First, the pediatric dose of 230 mg/m² and the comparable approved standard dose of 400 mg/day for adults both lead to significant accumulation of acetylated H3 histones in PBMCs in children and adults, respectively [21]. Second, dose-limiting toxicities at comparable doses in children and adults are similar [21].

In part 2 of the *in silico* trials, the lower thrombocyte limit was set to 50×10^9 cells/l (part 1: 193×10^9 cells/l since platelet counts 50×10^9 cells/l typically do not lead to clinical interventions, for instance, prophylactic platelet transfusions are recommended for thrombocyte counts of 10×10^9 cells/l [67]). The upper vorinostat dose limit of 3000 mg was defined by two factors. First, the highest vorinostat dose administered *in vivo* was 1000 mg (suppl. Fig. S3). As the PBPK model could not be evaluated above doses of 1000 mg, there is some uncertainty in simulations above doses of 1000 mg. Second, vorinostat is typically available in 100 mg capsules, which makes dosing of high doses (> 400 mg) difficult in clinical practice. However, there are already promising new formulations under research that should simplify higher vorinostat dose administrations [68,69].

Finally, the parameter sensitivity analysis has shown that the vorinostat half-life mainly depends on the intestinal permeability and the unstirred water layer parameter of the particle dissolution, which underlines that the absorption rate-limited flip-flop PK of vorinostat has been correctly implemented. Although the absorption was adequately predicted with the particle dissolution module, there might be other reasons responsible for the limited absorption such as drug-disease interactions or transporters. Vorinostat was found to be a substrate of P-gp and BCRP as well as an inducer of P-gp at the blood-brain barrier [70-72].

Despite some limitations the presented work clearly demonstrates the applicability and usefulness of PBPK and PBPK/PD models in pediatric dose identification and in *in silico* trials. The PBPK modeling approach helps to integrate and leverage all available *in vitro*, *in vivo*, and *in silico* information while potentially reducing the number of clinical trials and study participants. Still, future clinical trials are needed to evaluate efficacy and safety of the newly proposed dosing regimens.

5. Conclusion

For the first time a comprehensive PBPK/PD model of vorinostat has been developed which can predict (i) vorinostat concentration-time profiles in adults and (ii) the impact of vorinostat on its major PD biomarkers, the HDAC inhibition and the dose-limiting decrease in thrombocytes. The PBPK/PD model was used to recommend (i) pediatric doses from 0 –

17 years and (ii) potentially more effective vorinostat dosing regimens for adults. However, further research on (i) the yet unidentified enzymes involved in vorinostat metabolism, (ii) the vorinostat PK in pediatric patients < 5 years of age, and (iii) the PK/PD relationship in pediatric patients is necessary to increase the confidence in the model predictions. These results exemplify a mechanistic modeling approach that might help researchers to design clinical studies and to potentially improve dosing regimens of newly developed and already established drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Schematic structural PBPK/PD model representation. **a** PBMC effect compartment model. **b** Schematic default PBPK model. **c** Thrombocytopenia model. **d** HDAC activity indirect response model. *PBMC* peripheral blood mononuclear effect compartment, *PBMC_{deep}* peripheral blood mononuclear deep effect compartment, $k_{effect,1/2}$ and $k_{effect,deep,1/2}$ concentration transfer rate constants, Q specific organ blood flow, CL clearance, *Prol* proliferating cells, *Transit 1/2/3* cells in transit state, *Circ* circulating cells, $Circ_0$ circulating cells baseline, k_{prol} proliferation rate constant, k_{tr} transit rate constant, k_{circ} elimination rate constant, MTT mean transit time, $slope$ slope parameter, $Drug_{conc}$ vorinostat concentration, E_{drug} vorinostat effect on the proliferating cells, FBP circulating cells feedback on proliferating cells, FBM circulating cells feedback on MTT , k_{in} HDAC activity synthesis rate, and k_{out} HDAC activity degradation rate.

Fig. 2.

Predicted versus observed PK parameters. **a** AUC_{0-inf} in adults ($n = 52$). **b** C_{max} in adults ($n = 52$). **c** AUC_{0-inf} in children ($n = 19$). Circles indicate adults and triangles children. Dashed line: 2-fold acceptance limits.

Fig. 3. Concentration-time profiles after intravenous (a) and oral administration of vorinostat single doses (b) [51,42]. Simulated data are represented as median (black lines), 90% prediction interval (grey shaded area), and minimum and maximum values (dotted lines).

Fig. 4. PBPK/PD model results. **a** PBMC concentration-time profiles [42]. **b** HDAC activity over time [42]. **c** Thrombocyte count in thrombocytosis patients during a 24 week vorinostat treatment with 400 mg qd [48]. **d** Thrombocyte count in patients with normal thrombocyte counts. Simulated data are represented as median (black line), 90% prediction interval (grey shaded area) and minimum and maximum values (dotted lines). The red dotted line represents the thrombocyte counts justifying the diagnosis of thrombocytopenia ($=150 \times 10^9$ cells/l). Single doses: sd, repeated once daily doses: qd.

Table 1

Clinical study data of vorinostat

Route of administration	Dose [mg]	Dosing schedule	n	Male patients [%]	Age median (range) [years]	Internal (in) or external (ex) dataset	Ref.
iv (inf. 2 h)	75, 150, 300, 600, 900 /m ²	sd	39	65	19-81	in	[51]
iv (inf. 2 h)	200, 400	sd	20	-	-	ex	[4]
po	400, 600, 800, 1000	qd	24	68	59 (31-85)	ex	[28]
po	100, 200, 400, 500	qd	18	83	58 (25-72)	in	[8]
po	200, 300, 400, 600	qd	73	69	(20-79)	ex	[6]
po	400	sd	10	50	62 (31-73)	in	[73,42] ^a
po	400	qd	27	44	(46-80)	ex	[52]
po	800	sd	24	52	(29-78)	ex	[53]
po	400	qd	28	75	64 (30-77)	ex	[54]
po	400	qd	23	48	(39-84)	ex	[55]
po	400	qd	6	100	53.5 (34-75)	ex	[56]
po	180, 230, 300/m ²	sd	13	60	14 (5-22)	ex	[21] ^a
Po	400	qd	63	49	64 (29-82)	in	[48]

^a unpublished*iv* intravenous*inf* infusion*po* per oral*sd* single dose*qd* once daily

Table 2

Vorinostat physicochemical and ADME parameters

Parameter	Unit	Literature value	Ref.	Value used for simulations	Parameter description
MW	g/mol	264.32	-	264.32	Molecular weight
logP	-	0.89	[74]	0.89	Lipophilicity
fu	[%]	28.8±2.9	[4]	28.8	Fraction unbound
pKa	-	9.2	[4]	9.2	Acid dissociation constant
Solubility	mg/ml	0.1-0.4	[4,70]	0.2	Solubility
UGT 1A9 CL _{in-vitro}	μl/min/mg	38.55	[15,16] ^a	38.55	UGT 1A9 <i>in-vitro</i> metabolic clearance
UGT 2B7 CL _{in-vitro}	μl/min/mg	580	[16]	580	UGT 1A9 <i>in-vitro</i> metabolic clearance
UGT 2B17 CL _{in-vitro}	μl/min/mg	53	[15,16] ^a	53	UGT 1A9 <i>in-vitro</i> metabolic clearance
UGT 1A9 content	pmol/mg	30.11	[75] ^b	30.11	Microsomal UGT 1A9 liver content
UGT 2B7 content	pmol/mg	78.49	[75] ^b	78.49	Microsomal UGT 2B7 liver content
UGT 2B17 content	pmol/mg	22.17	[75] ^c	22.17	Microsomal UGT 2B17 liver content
CL _{hydr,β-ox.}	l/h	NA	NA	1.8 ^d	Clearance via hydrolysis and β-oxidation
P _{intest}	dm/min	1.56E-07	[4]	2.20E-07 ^d	Intestinal permeability
P _{cell}	dm/min	6.55E-5	PK-Sim	9.60E-06 ^d	Cellular permeability
P _{RBC->PLS}	dm/min	6.48E-5	PK-Sim	4.20E-06 ^d	Blood-plasma permeability
P _{PLS->RBC}	dm/min	6.45E-5	PK-Sim	1.00E-04 ^d	Plasma-blood permeability
B:P	-	2.87	[42]	2.87	Blood-to-plasma concentration ratio
Layer _{PD,unstirred,water}	μm	20	PK-Sim	108 ^d	Particle dissolution unstirred water layer

^a mean of 2 reported values^b mean of 7 reported values^c mean of 3 reported values^d fitted

NA not available