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ORIGINAL ARTICLE

Pharmacokinetics and safety of erlotinib and its metabolite OSI-420 in infants and children with primary brain tumors

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Abstract

Purpose Erlotinib (Tarceva®), a potent small molecule inhibitor of the epidermal growth factor receptor tyrosine kinase, has been evaluated to treat infants and children with primary brain tumors. The pharmacokinetics of erlotinib and its primary metabolite OSI-420 were characterized and exposure–safety associations were investigated.

Methods This analysis involved patients enrolled in two clinical studies and receiving oral erlotinib once daily as part of treatment. Single-dose and steady-state erlotinib and OSI-420 plasma concentrations were assayed using HPLC–MS/MS methods. Population pharmacokinetic modeling and univariate covariate analysis evaluating demographic, clinical and selected *CYP3A5*, *CYP3A4*, *ABCB1*, and *ABCG2* genotypes were performed. Associations between erlotinib and OSI-420 pharmacokinetics, and with toxicities (diarrhea and skin rash) occurring post-dose were explored.

Results Data from 47 patients (0.7–19 years old) were collected and best fitted by one-compartment linear models. Erlotinib and OSI-420 apparent clearances (CL/*F* and CL_m/F_m) were higher in patients < 5 years compared to older patients (mean CL/*F*: 6.8 vs 3.6 L/h/m^2, and mean CL_m/F_m: 79 vs 38 L/h/m^2, $p < 0.001$), and were 1.62-fold and 1.73-fold higher in males compared to females ($p < 0.01$). Moreover, CL/*F* was 1.53-fold higher in wild-type patients than in patients heterozygous or homozygous mutant for *ABCG2 rs55930652* ($p < 0.05$). Most of the toxicities reported were grade 1. No associations were found between drug pharmacokinetics and drug-induced toxicities.

Conclusions Erlotinib therapy was well tolerated by pediatric patients with primary brain tumors. No dosing adjustments based on age or patient characteristics are recommended for this patient population.

Keywords Erlotinib · Pharmacokinetics · Pediatrics · Toxicity · Brain tumors

Introduction

Central nervous system (CNS) tumors remain the most common cause of cancer-related deaths among pediatric patients [1]. Several pediatric malignant brain tumors have been shown to overexpress the epidermal growth factor receptor (EGFR), which has become a promising target [2–5]. Erlotinib (Tarceva®) is a highly selective, potent small molecule inhibitor of EGFR tyrosine kinase, approved by the FDA for adult non-small cell lung cancer and advanced pancreatic cancer [6]. This compound has also been incorporated into several phase I/II clinical trials to determine its activity against glioma, which may overexpress EGFR [7], in both adults and pediatrics [8–13].

Erlotinib is administered orally, highly bound to plasma proteins and metabolized in humans mainly by hepatic cytochrome P450 (CYP) 3A4 and 3A5 [14, 15]. It is also a

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substrate for the efflux transporter P-glycoprotein (MDR1/ABCB1) and breast cancer resistance protein (BCRP/ABCG2) [16, 17]. The pharmacokinetic profiles of oral erlotinib and its primary pharmacologically active metabolite OSI-420 have been well described in adults including patients with malignant glioma [18–20]. High inter-individual variability in erlotinib and OSI-420 disposition was reported among the different studies.

Most common erlotinib-related adverse events include diarrhea and skin rash [6]. Conflicting results have been published with regards to the evaluation of erlotinib pharmacokinetic–toxicity associations in adults [20]. However, most of the studies performed in patients with various tumors have shown a higher incidence of severe skin rash in patients exhibiting higher erlotinib exposure or trough concentrations, suggesting evidence of a pharmacokinetic–pharmacodynamic relationship [20].

Only limited pharmacokinetic data have been published regarding erlotinib use in children with brain tumors. The comparison between adult and pediatric erlotinib and OSI-420 pharmacokinetic parameters have led to inconsistent results with either similar parameters [12, 21] or lower clearance reported in pediatrics compared to that observed in adults [11]. In this last study, the authors have also shown a similar association between erlotinib exposure and more severe skin toxicity for both children and adults [11]. Further evaluation of erlotinib and OSI-420 disposition and association with toxicity in the pediatric population is needed to inform effective dosing and treatment. Moreover, no data are currently available in patients less than two years of age who may represent a more vulnerable population, and for whom dosing adjustments may be necessary.

This study aimed to characterize the population pharmacokinetics of erlotinib and OSI-420 in a wide range of ages including infants less than two years of age with primary brain tumors to identify sources of inter-patient variability. Associations between erlotinib and OSI-420 pharmacokinetic parameters and both diarrhea and skin rash toxicities were also explored to determine the need for dosing alterations within the pediatric population.

Materials and methods

Study design and treatment

The patients involved in this analysis were treated on one of the two clinical trials SJYC07 (NCT00602667) or SJHG04 (NCT00124657). Both trials were approved by the St. Jude Children's Research Hospital Institutional Review Board, and informed written consent was obtained from the legal guardians or patients, as appropriate. SJYC07 was a multicenter phase II risk-adapted study that enrolled infants and young children less than 5 years old with a newly diagnosed CNS tumor [22]. The treatment plan consisted of an induction, consolidation, and a final maintenance phase which included three 28-day cycles of oral erlotinib (90 mg/m^2/day). SJHG04 was a single institution phase I/II study that enrolled patients between 3 and 26 years old with newly diagnosed high-grade glioma or unfavorable low-grade glioma [23, 24]. Along with and after radiotherapy, patients received continuous daily oral erlotinib at 70, 90, 120, 160, or 200 mg/m^2/day in the phase I and at 120 mg/m^2/day in the phase II component. In both clinical trials, the calculated erlotinib dosage was rounded to the nearest 25 mg to accommodate increments in the tablet dosage.

Blood sampling and bioanalysis

For consenting patients enrolled on SJYC07, pharmacokinetic studies were performed on day 1 of the second cycle of maintenance erlotinib. Serial blood samples (2 mL) were collected at pre-dose and at 1, 2, 4, 8, and 24 (± 2) hours post-dose. For consenting patients enrolled on the SJHG04, pharmacokinetic studies were performed on day 1 and day 8 of course 1 of concurrent chemotherapy and radiation treatment. On day 1, serial blood samples (2 mL) were collected pre-dose and at 1, 2, 4, 8, 24 (±2), 30 (±4), and 48 (±4) hours after drug administration. The erlotinib dose on day 2 was held during the pharmacokinetic studies. On day 8, blood samples were collected pre-dose, and at 1, 2, 4, 8, and 24 (±2) hours post-dose administration.

Blood was collected in heparinized tubes and centrifuged within 30–60 min after collection. The plasma was stored at − 80 °C until further analysis. Erlotinib and its metabolite OSI-420 were assayed using a validated method combining high-performance liquid chromatography and mass spectrometry [25]. The lower limits of quantitation of erlotinib and OSI-420 were 10 ng/mL and 1 ng/mL, respectively.

Genotyping assays

In consenting patients, genomic DNA was extracted and quantified from formalin-fixed paraffin-embedded tissues and blood with a Maxwell RSC DNA FFPE kit (#AS1450, Promega, Madison, WI, USA), and Qubit (Thermo Fisher Scientific, Waltham, MA, USA) using standard procedures. Genome-wide genotyping was performed in germline DNA with an Illumina Infinium Omni2.5Exome-8 BeadChip (Illumina Inc., San Diego, CA, USA). Selected single nucleotide polymorphisms (SNP) were assayed according to those previously published. Variants from the following genes involved in erlotinib metabolism and transport were included: *CYP3A5 (rs776746), CYP3A4 (rs2740574), ABCB1 (rs2032582* and *rs1045642),* and *ABCG2 (rs7699188).*

Pharmacokinetic and covariate Analysis

Population pharmacokinetic modeling was performed to analyze erlotinib and OSI-420 plasma concentration–time data using a nonlinear mixed-effects modeling approach (NONMEM 7.4.3 ICON Development Solutions, Ellicott City, MD, USA). The first-order conditional estimation with interaction method was used to estimate the parameters [26]. Single-dose and steady-state erlotinib and OSI-420 concentration–time data were simultaneously analyzed. Both one- and two-compartment models with first-order elimination were compared, defined by apparent pharmacokinetic parameters in the absence of intravenous data. Different absorption models were examined including first-order, zero-order and combined first- and zero-order absorption. The benefit of transit compartments and a lag time to describe the absorption phase was also explored.

The model parameters were assumed to be log-normally distributed, and the inter-individual variability and inter-occasion variability associated with the parameters were modeled as exponential terms. Different error models were examined for both the parent drug and metabolite including additive, proportional, and mixed additive and proportional error models. Error terms were assumed to be normally distributed with a mean of zero and variance of σ^2. Model selection was based upon changes in the minimum objective function value (OFV), precision of parameter estimates (relative standard error), and visual inspection of goodness-of-fit plots. The R-based tool Xpose 4.5.3 and GraphPad Prism 7.0 (GraphPad Software Inc, La Jolla, CA, USA) were used for graphical presentations and statistical analysis.

Covariate analysis

A covariate analysis was performed to identify the patient characteristics with a significant association with the pharmacokinetic parameters. The following covariates were analyzed: sex, age, body-surface area, actual bodyweight, α-1-acid glycoprotein, alanine aminotransferase, aspartate aminotransferase, total bilirubin, estimated glomerular filtration rate [27], concomitant dexamethasone administration, and genetic polymorphisms in *CYP3A5 (rs776746)*, *CYP3A4 (rs2740574)*, *ABCB1* (*rs2032582* and *rs1045642*), and *ABCG2* (*rs2231142, rs2622604, rs55930652,* and *rs7699188*). The continuous covariates were associated with the model parameters using a power model scaled to the population median covariate value. The categorical covariates were implemented on the parameters using a fractional change due to the covariate value. The covariate effects of SNPs were tested under dominant, additive, and recessive models.

The covariate analysis was performed using a univariate approach because of the small sample size. A covariate was considered to have a significant effect on a pharmacokinetic parameter if its addition to the model reduced the OFV by 3.84 units (corresponding to $p < 0.05$ based on the χ^2 test), if it showed a decrease in the inter-individual variability of the parameter, and if it improved the model fits.

Model evaluation

The predictive performance of the final model was evaluated using a prediction-corrected visual predictive checks [28]. We generated 1000 dataset replicates from the original dataset and simulated conditionally on the final pharmacokinetic parameters. The observed data were overlaid on the 5th, 50th, and 95th percentiles of the simulations to visually assess concordance between the observed and model-based simulated data. All the data were corrected using the median of the population prediction within the associated time bin to account for the different administered doses and the impact of potential covariates.

Relation of pharmacokinetics and polymorphisms to toxicity

The associations between the pharmacokinetics of erlotinib and OSI-420, genetic polymorphisms, and the common erlotinib-related toxicities were examined. Toxicities were defined as diarrhea and skin rash including rash/desquamation, rash/acneiform, and pruritus/itching, and occurred throughout the cycle during which the pharmacokinetic study was conducted. Toxicities were graded according to the common terminology criteria for adverse events version 3.0. Diarrhea was grouped by grade 0 vs grade 1+. Skin rash was grouped by grades 0–1 vs grade 2+.

The pharmacokinetic variables used in this analysis were derived for each patient from the population model and included: maximum concentration (C_{MAX}), trough concentration at 24 h, and area under the concentration–time curve from 0 to 24 h post-dose (AUC_{0-24h}). These variables were determined for both erlotinib and OSI-420, after single dose, and at steady state. For the SJYC07 patients, the steady-state pharmacokinetic parameters were obtained using model simulations in the absence of observed data. Wilcoxon–Mann–Whitney (WMW) tests were used to compare distributions of drug pharmacokinetic variables between two groups of patients (with vs. without toxicity). Genetic variables included the selected SNPs as previously described. Additive, dominant and recessive models were tested. Cochran–Armitage trend tests were used to examine the associations between genetic variables and toxicity. Statistical significance threshold was defined as $p < 0.05$, and no corrections were made for multiple comparisons.

Results

Data summary

A total of 867 erlotinib and OSI-420 plasma concentrations from 47 patients were available for pharmacokinetic analysis. Only two erlotinib and two OSI-420 data points in the terminal phase were below the limit of quantification and were excluded from the analysis. The patient population was mainly Caucasian with a median age of 3.6 years and included four very young infants (≤ 1-year old). The patient demographics and clinical characteristics are reported in Table 1, separately for the two studies. A total of 41 patients consented to pharmacogenetic studies. Among the patients enrolled in SJHG04 phase I component, seven patients received 70 mg/m^2/day erlotinib, two patients had 90 mg/m^2/day erlotinib, five patients had 120 mg/m^2/day erlotinib, and three patients were administered 160 mg/m^2/day.

Table 1 Patient characteristics

Study	SJYC07	SJHG04
No. of patients	24	23
Age (years)	2.36 (0.71–3.64)	10.0 (3.89–19.1)
Male/female *n* (%)	18 (75)/6 (25)	10 (43.5)/13 (56.5)
Ethnicity *n* (%)[a]		
White	15 (62.5)	17 (73.9)
Black	3 (12.5)	4 (17.4)
Other	6 (25)	2 (8.7)
Body surface area (m^2)	0.58 (0.33–0.71)	1.11 (0.67–2.26)
Weight (kg)	12.7 (5.50–17.4)	32.1 (15.8–106)
Height (cm)	87.9 (63.8–99.9)	132 (96.3–175)
Alanine aminotransferase (U/L)	20.5 (8.00–114)	30.0 (9.00–132)
Aspartate aminotransferase (U/L)	29.0 (12.0–56.0)	24.0 (15.0–39.0)
Total bilirubin (mg/dL)	0.20 (0.10–0.40)	0.20 (0.10–0.40)
Glomerular filtration rate (mL/min)	105 (74.7–151)	103 (59.5–205)
Concomitant dexamethasone *n* (%)	3 (12.5)	5 (22)
α-1-acid glycoprotein (mg/dL)[b]	19.7 (11.5–35.9)	126 (42.0–297)
Polymorphisms WT/HE/HOM (*n*)[c]		
*CYP3A5*3 rs776746*	10/7/2	13/6/3
*CYP3A4*1B rs2740574*	14/5/0	16/5/1
ABCB1 rs2032582	5/10/4	10/8/4
ABCB1 rs1045642	6/11/2	10/4/8
ABCG2 rs2231142	13/5/1	19/3/0
ABCG2 rs2622604	13/6/0	11/9/2
ABCG2 rs55930652	12/7/0	9/13/0
ABCG2 rs7699188	15/3/1	18/4/0

[a]Ethnicity was not genotypically determined. Other include Hispanic, Asian, Pacific, multi-race, or unknown

[b]α-1-acid glycoprotein concentration was not measurable in 3 SJYC07, and in 5 SJHG04 patients

[c]Genotypes were characterized in 19 SJYC07 patients, and in 22 SJHG04 patients. Polymorphisms are reported as the number of wild-type (WT), heterozygous (HE), and homozygous mutant (HOM) patients

Population pharmacokinetic modeling and covariate analysis

Erlotinib plasma concentration–time data were best described using a one-compartment with delayed first-order absorption and linear elimination, parameterized with an apparent clearance (CL/*F*), apparent volume of distribution (*V*/*F*), first-order absorption rate constant (K_a), and absorption lag time (ALAG1) (Online Resource Figure S1). OSI-420 plasma concentration–time data were best described using a one-compartment model parameterized with apparent clearance (CL_m/F_m) and volume of distribution (V_m/F_m). Because erlotinib was administered orally and no intravenous data were available, neither erlotinib bioavailability (*F*) nor the fraction of erlotinib converted into OSI-420 (F_m) could be identified. Proportional error models best described the residual variability for both erlotinib and OSI-420. Inter-individual variability parameters were estimated for CL/*F*, *V*/*F*, K_a, ALAG1, and CL_m/F_m. The pharmacokinetic model also included IOV estimated for CL/*F*, *V*/*F*, and CL_m/F_m. No correlation between random effects was found to be significant. The model parameters were well estimated and are summarized in Table 2. The associated shrinkages were all below 40% and supported an unbiased covariate inclusion. The goodness-of-fit plots did not show significant bias on the model (Online Resource Figure S2). The prediction-corrected visual predictive checks for erlotinib and OSI-420 showed that the central tendency was well predicted by the selected model (Fig. 1). The large inter-individual variability observed on both erlotinib and OSI-420 concentrations was slightly overpredicted by the model; however, no covariate was considered at this stage. Across the dosage range, erlotinib and OSI-420 AUC_{0-24h} after a single dose ranged from 7.22 to 101 μM h (median 34.4 μM h), and from 0.73 to 10.2 μM h (median 3.25 μM h), respectively. OSI-420 exposure was about 9.0% of that of erlotinib, and both drug exposures were well correlated ($r^2 = 0.63$, Online Resource Figure S3).

The univariate covariate analysis revealed the association of age, sex, and *ABCG2 rs55930652* with erlotinib and OSI-420 disposition (Fig. 2; Table 3). Age was categorized as < 5 and ≥ 5 years old and was negatively correlated with

Table 2 Population pharmacokinetic parameter estimates

Parameter (unit)	Estimate (RSE%)	IIV% (RSE%)	IOV% (RSE%)
Erlotinib			
Apparent clearance CL/*F* (L/h/m^2)	5.14 (10)	55.1 (28)	28.3 (35)
Apparent volume *V*/*F* (L/m^2)	71.9 (9.0)	45.2 (34)	45.7 (63)
Absorption constant K_a (/h)	2.16 (35)	150 (35)	–
Absorption lag time (h)	0.702 (8.0)	27 (46)	–
Proportional residual error RE_{prop}	0.29 (9.0)	–	–
OSI-420			
Apparent clearance CL_m/F_m (L/h/m^2)	57.2 (10)	57.6 (30)	38.2 (31)
Apparent volume V_m/F_m (L/m^2)	5.67 (28)	–	–
Proportional residual error REm_{prop}	0.36 (7.0)	–	–

RSE relative standard error, *IIV* inter-individual variability reported as coefficient of variation (%), *IOV* inter-occasion variability reported as coefficient of variation (%)

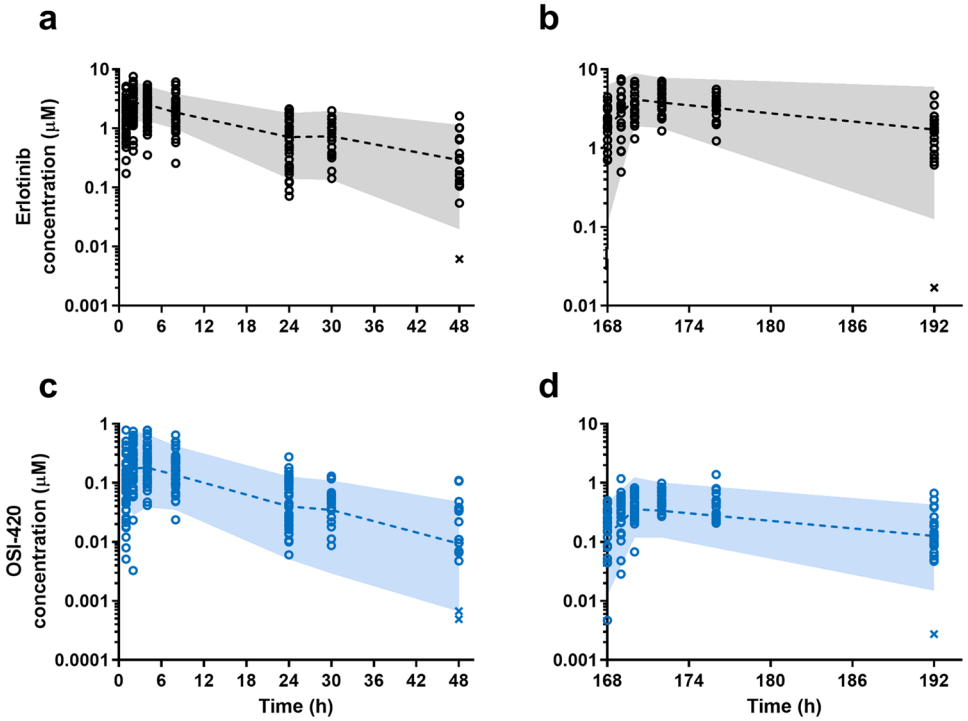

Fig. 1 Prediction-corrected visual predictive checks for the population pharmacokinetic model describing erlotinib (**a**, **b**) and OSI-420 (**c**, **d**) concentration–time data. Single-dose data and model predictions are depicted in **a**, **c**. Steady-state data and model predictions are depicted in **b**, **d**. The circles are the observed data, the crosses are data below the limit of quantification, the dashed line depicts the median of the model simulations, and the shaded area represent the 90th prediction interval

both erlotinib and OSI-420 apparent clearances ($p < 0.001$). Patients less than 5 years old exhibited 1.87-fold and 2.1-fold higher erlotinib and OSI-420 clearances, respectively, than did the older children. Males had 1.62-fold, and 1.73-fold higher erlotinib and OSI-420 clearances, respectively, than did female patients ($p < 0.01$). Wild-type patients for *ABCG2 rs55930652* exhibited 1.53-fold higher erlotinib clearance than did the patients with at least one variant allele ($p < 0.05$). The levels of α-1-acid glycoprotein were not quantified in 17% of the patients for analytical issues, and as such, this laboratory variable was not evaluated as a covariate. However, in the patients with an α-1-acid glycoprotein measure, a strong positive correlation was found between patient age and the α-1-acid glycoprotein values (spearman $r = 0.71$, $p < 0.0001$), which led to similar associations between erlotinib and OSI-420 clearances and α-1-acid glycoprotein values (Online Resource Figure S4).

Relation of pharmacokinetics and polymorphisms to toxicity

Out of 47 patients, two patients were not evaluable for toxicity analysis. Among the evaluable cohort, 30 patients did not report diarrhea, 14 patients had grade 1 diarrhea, and one patient experienced grade 3 diarrhea. Regarding the skin rash, 13 patients did not develop this toxicity, 18 patients

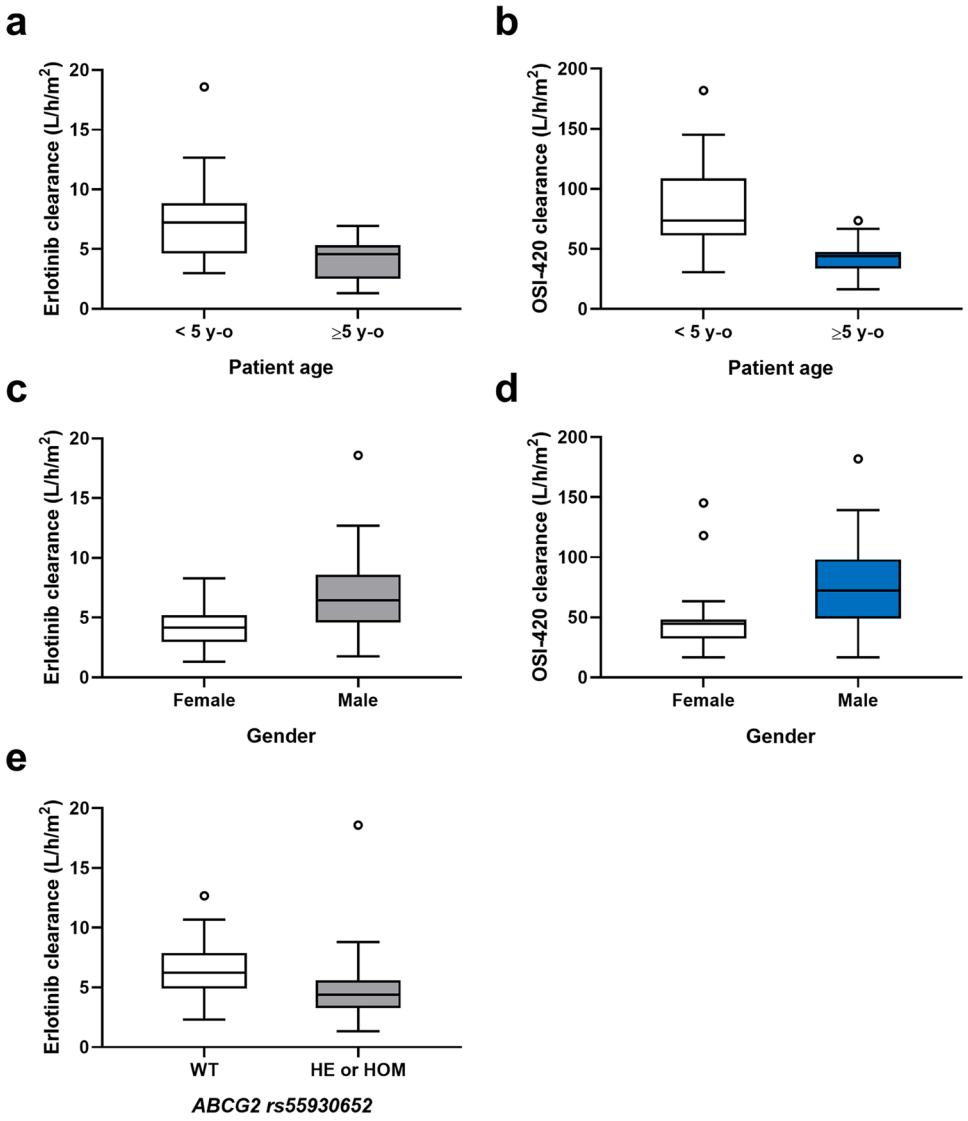

Fig. 2 Association between individual erlotinib and OSI-420 predicted clearances and patient covariates. Distribution of erlotinib (**a**) and OSI-420 (**b**) clearances in patients less than 5 years old vs older patients. Distribution of erlotinib (**c**) and OSI-420 (**d**) clearances in female *versus* male patients. **e** Distribution of erlotinib clearance in wild-type (WT), heterozygous (HE), and homozygous mutant (HOM) patients for *ABCG2 rs55930652*. The distribution of the variables is represented using Tukey boxplots

Table 3 Summary of univariate covariate analysis

Covariate	Parameter	Typical values	Reduction in IIV (%)	*P* value
Categorical age	CL/*F* (L/h/m^2)	< 5 years CL/*F* = 6.79 ≥ 5 years CL/*F* = 3.63	42	< 0.001
	CL_m (L/h/m^2)	< 5 years CL_m = 79.2 ≥ 5 years CL_m = 38.1	46	< 0.001
Sex	CL/*F* (L/h/m^2)	Male CL/*F* = 6.27 Female CL/*F* = 3.87	18	< 0.01
	CL_m (L/h/m^2)	Male CL/*F* = 71.3 Female CL/*F* = 41.2	20	< 0.01
ABCG2[a] *rs55930652*	CL/*F* (L/h/m^2)	WT CL/*F* = 6.32 Variant CL/*F* = 4.12	13	< 0.05

Variant includes both heterozygous and homozygous mutant for the *ABCG2 rs55930652* polymorphism

RSE relative standard error, *IIV* inter-individual variability reported as standard of deviation, *IOV* inter-occasion variability reported as standard of deviation, *WT* wild-type

[a]The univariate analysis was performed on a subset of 40 patients for whom genotypes were assayed

experienced grade 1 toxicity, 12 patients had grade 2 skin rash, and two patients had grade 3 toxicity. Diarrhea and skin rash were not associated with each other (Fisher's exact test $p = 1.00$) and were not associated with the clinical study SJYC07 vs SJHG04 (Fisher's exact test $p = 0.35$ and 0.21, respectively, for diarrhea and skin rash).

Figure 3 depicts the distribution of erlotinib and OSI-420 exposures ($AUC_{0–24h}$) after single dose by grade for these two toxicities. Based on WMW tests, the pharmacokinetic variables included in the analysis were not associated with whether toxicity (diarrhea or skin rash) occurred or not, nor with the different grades of toxicity events. Based on trend tests for diarrhea, the only significant pharmacogenetic variable was the *ABCB1 rs1045642* variant in additive mode (Online Resource Figure S5). A decreasing trend was observed between diarrhea and number of mutant alleles ($p = 0.044$). Based on trend tests for skin rash, none of the pharmacogenetic variables were associated with skin toxicity, regardless of genetic inheritance mode.

Discussion

A population pharmacokinetic model was developed to describe erlotinib and its active metabolite OSI-420 disposition in a population of children with CNS tumors that included infants less than 2 years of age. The analysis showed a strong significant influence of patient age on both erlotinib and OSI-420 clearances which seemed to be correlated with the differences in α-1-acid glycoprotein levels across age in pediatrics. In this population of children, erlotinib was overall well tolerated across the dosage range of 70–120 mg/m^2/day with no clear associations between treatment-related toxicities (diarrhea and skin rash) and erlotinib or OSI-420 pharmacokinetic parameters. Thus, no dosing alterations are proposed among children.

Our initial goal was to determine the feasibility of predicting erlotinib and OSI-420 pharmacokinetics in infants and young children based upon a population model developed in older children. First, a model was built using the data collected from the SJHG04 study that enrolled patients with a median age of 10 years old (3.9–19 years). Model simulations were performed based upon the SJYC07 patient characteristics, and the simulated data were overlaid with the observed concentrations collected in this study. The results were not satisfying with a trend to over-predict the concentrations observed in infants and young children, which could suggest strong pharmacokinetic differences related to patient age. Thus, the data from the two studies were combined and the model parameters re-estimated, as reported in this manuscript.

The covariate analysis that was performed may be considered exploratory due to the limited number of patients and the univariate approach; however, it revealed several interesting associations which were consistent with previous reports in both adults and pediatrics.

As expected, the patient age significantly influenced both erlotinib and OSI-420 clearances which largely explained the differences observed between the two studies. We tried to implement the age effect as a continuous covariate; however,

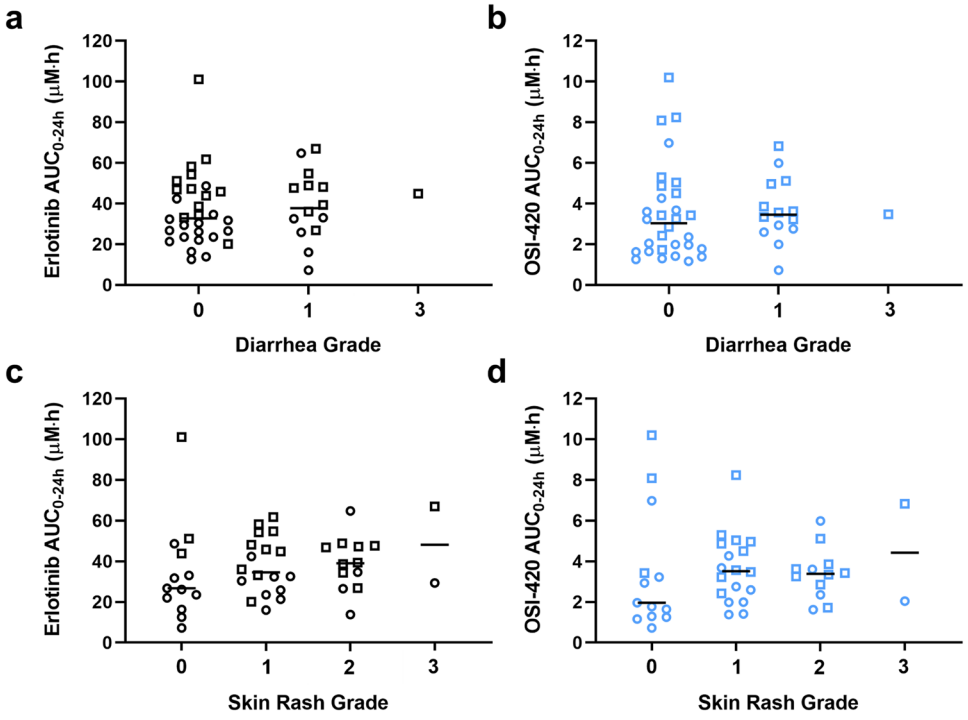

Fig. 3 Association between individual erlotinib and OSI-420 predicted exposures ($AUC_{0–24h}$) after single-dose and toxicity including diarrhea and skin rash. Individual erlotinib (**a**) and OSI-420 (**b**) $AUC_{0–24h}$ versus diarrhea grade toxicity. Individual erlotinib (**c**) and OSI-420 (**d**) $AUC_{0–24h}$ versus skin rash grade toxicity. The circles and squares represent patients enrolled in SJYC07 and SJHG04 studies, respectively

the covariate parameter was not well estimated. Thus, we included the age effect as a categorical covariate with a cut-off value of 5 years old based on visual inspection of age versus post hoc individual clearance estimates. The mean erlotinib clearance estimated for the older children (3.63 L/h/m^2–3.99 L/h) compared well with the other reported values in pediatrics at a similar age range (~3 to 20 years): 4.67 L/h (median 8.8 years) [11], 3.1 L/h/m^2 (11.5 years) [12], and 4.0 L/h (10 years) [13]. The mean OSI-420 clearance estimated for the older children as well as the mean metabolic ratio of 9% were also similar to previously reported values [13, 29]. However, the mean erlotinib and OSI-420 clearances of 6.79 and 79.2 L/h/m^2 estimated for the younger children (0.7–5 years) were much higher than any other reported values. In patients for whom α-1-acid glycoprotein levels were measurable, a strong correlation was observed between patient age and α-1-acid glycoprotein values (Online Resource Figures S3a). Higher protein levels were found in older patients, which was consistent with previous reports [30, 31]. Based on these results, we assume that the age influence on erlotinib and OSI-420 disposition can be largely explained by the α-1-acid glycoprotein plasma levels. The α-1-acid glycoprotein effect on erlotinib disposition has been previously reported in adults in several studies and is consistent with the high binding capacity of erlotinib [8, 32, 33].

Only two other covariates were identified as having significant associations with erlotinib and/or OSI-420 disposition in pediatrics: gender and *ABCG2 rs55930652* SNP. Males exhibited higher erlotinib and OSI-420 clearances than did females. In this study, males were slightly younger than females (median age of 5.3 years in males versus 8.0 years in females, $p = 0.071$), which may explain why we found this covariate effect that has not been previously reported. Patients with at least one variant allele of *ABCG2 rs55930652* SNP exhibited lower erlotinib clearance, which was consistent with previous findings in adults [34–36]. No significant impact of *CYP3A5, CYP3A4* and *ABCB1* variant on drug pharmacokinetics was found, similar to previous publications [20]. Other significant covariates have been identified in adults such as smoking, and hepatic function; however, conflicting results have also been reported such that no real consensus has been reached, and a large part of the inter-individual variability in erlotinib disposition remains unexplained [20].

Several studies have been performed in adults to evaluate the association between erlotinib pharmacokinetics and the occurrence of common adverse effects, such as diarrhea and skin rash [20]. Some evidence of pharmacokinetic–toxicity relationships has been reported for skin rash with an increasing risk associated with higher erlotinib exposure [34–37]. Furthermore, a similar relationship has been reported in pediatrics by White-Koning and colleagues [11]. However, no target exposure has yet been formally determined clinically. Interestingly, the grade of skin toxicity was shown to correlate with clinical efficacy, further suggesting that a grade 2 skin rash may be considered as a therapeutic target [38]. In the present studies, it was not possible to evaluate specific clinical efficacy of erlotinib which was administered in the context of combination therapy with other chemotherapeutic agents (SJYC07) or radiation (SJHG04).

Considering the large pharmacokinetic variability and the specific differences in drug exposure we observed between young *versus* older children, we were interested to evaluate potential pharmacokinetic–toxicity associations regarding diarrhea and skin rash. However, in our study, both erlotinib and OSI-420 exposures were similar in patients with or without toxicity. Although older children exhibited higher drug exposures due to lower drug clearance and/or higher dosage, they did not experience a higher incidence of toxicities compared to younger children with lower drug exposures. The median erlotinib exposure observed in our study after a single dose was 34.4 μM h (~13.5 mg h/L), which was approximately 1.4- to 2.2-fold lower than those reported in the studies that found an association between erlotinib exposure and skin rash [11, 34–36]. Thus, at the dosage range from 70 to 120 mg/m^2/day, we may not reach sufficiently high drug exposures to identify erlotinib exposure and toxicity association in our patient. As such, based upon our data, similar dosages can be used across children of different ages. Overall, erlotinib was well tolerated in our population of infants, young and older children in the dosage range from 70 to 120 mg/m^2/day with most adverse events observed at grades 1 or 2. No associations were detected between toxicity and patient characteristics, except between one *ABCB1* variant and diarrhea, which will need to be validated. These results did not provide the rationale for deriving any dosing adjustments based on age or other patient characteristics.

We recognize that the main limitation of this study is the relatively small sample size ($n = 47$) which could have restricted our ability to identify finer trends in the data. Nonetheless, relevant findings were shown for a young pediatric population, which has not been studied so far. These results support the safe use of erlotinib in the pediatric population including infant patients with no dosing alterations based on age.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in this work.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the St. Jude Children's Research Hospital Institutional Review Board and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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