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Population Pharmacokinetics of Oral Topotecan in Infants and Very Young Children with Brain Tumors Demonstrates a Role of ABCG2 rs4148157 on the Absorption Rate Constant

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Running Title: Pharmacokinetics of Oral Topotecan in Very Young Children

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P: 901-595-3665

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Text pages: 13

Tables: 3

Figures: 4

References: 50

Words in Abstract: 242

Words in Introduction: 670

Words in Discussion: 1244

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Abbreviations: BCRP/ABCG2 – breast cancer resistance protein/ATP-binding cassette sub-family G member 2; BSA - body surface area; BSV – between subject variability; CL/F – apparent oral clearance; C_{max}- maximal concentration; eGFR – estimated glomerular filtration rate; IWRES – individual weighted residuals; K_a – absorption rate constant; MCMC - Markov Chain Monte Carlo; NPDE – normalized prediction distribution errors; OFV – objective function value; P-gp/ABCB1 – P-glycoprotein/ATP binding cassette Sub-Family B member 1; PWRES – population weighted residuals; RUV – residual unexplained variability; SAEM - stochastic approximation expectation maximization; SCr – serum creatinine; V/F – apparent volume of distribution

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Abstract

For infants and very young children with brain tumors, chemotherapy after surgical resection is the main treatment due to neurological and neuroendocrine adverse effects from whole brain irradiation. Topotecan, an anticancer drug with antitumor activity against pediatric brain tumors, can be given intravenous or orally. However, high inter-patient variability in oral drug bioavailability is common in children less than 3 years. Therefore, this study aimed to determine the population pharmacokinetics of oral topotecan in infants and very young children, specifically evaluating the effects of age and *ABCG2* and *ABCB1* on the absorption rate constant (K_a), as well as other covariate effects on all pharmacokinetic parameters. A nonlinear mixed effects model was implemented in Monolix 4.3.2. A one-compartment model with first-order input and first-order elimination was found to adequately characterize topotecan lactone concentrations with population estimates as (mean (s.e.)); $K_a = 0.61$ (0.11) h^{-1} , apparent volume of distribution (V/F) = 40.2 (7.0) L, and apparent clearance (CL/F) = 40.0 (2.9) L/h. After including body surface area on V/F and CL/F as a power model centered on the population median, the *ABCG2* rs4148157 allele was found to play a significant role on K_a . Patients homozygous or heterozygous for G>A demonstrated a K_a 2-fold higher than their GG counterparts, complemented with a 2-fold higher maximal concentration as well. These results demonstrate a possible role for the *ABCG2* rs4148157 allele in the pharmacokinetics of oral topotecan in infants and very young children, and warrants further investigation.

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Introduction

The current standard of care that has improved overall survival for children with brain tumors consists of surgical resection of the tumor, radiotherapy, and chemotherapy (Karajannis et al., 2008). However, infants and very young children less than 3 years old with primary central nervous system tumors have a poor prognosis due to the increased likelihood of disseminated disease at diagnosis and the morbidity of whole brain irradiation, among other factors (Duffner, 1983; Gajjar et al., 1994; Walter et al., 1999; Mulhern et al., 2004; Laughton et al., 2008). Little is known about the disposition of many anticancer drugs in infants and young children, which often leads to increased risk of morbidity, poor tumor control, and increased risk of late effects. Thus, it is essential that thorough pharmacokinetic studies be performed in this patient population to provide the data upon which rational dosing schedules can be developed that will increase patient survival and decrease toxicity.

One promising anti-cancer drug, topotecan (structure at Beijnen et al., 1990), is a topoisomerase I inhibitor and camptothecin analogue that has shown effective cytotoxic activity in previous pediatric solid tumor trials (Pratt et al., 1994; Stewart et al., 1997; Takimoto et al., 1998; Santana et al., 2003). Topoisomerase I is an intracellular enzyme that binds to DNA, introducing single strand DNA breaks. Topotecan binds to and stabilizes the topoisomerase I/DNA complex, preventing DNA replication and causing apoptosis (Hsiang and Liu, 1988; Kingsbury et al., 1990; Taudou et al., 1993). At physiologic pH topotecan exists in an equilibrium between the active lactone (closed form) and the inactive hydroxy acid (open-ring form) (Creemers et al., 1994). Topotecan is effective in mouse xenograft models of various types of pediatric tumors and preclinical studies have shown that the protracted dosing schedules were associated with greater antitumor effect (Houghton et al., 1992; Houghton et al., 1995;

Tubergen et al., 1996; Nitschke et al., 1998; Guichard et al., 2001; Kretschmar et al., 2004; Minturn et al., 2011). It is most commonly administered intravenously but clinical studies have shown evidence of efficacy with low-dose oral topotecan given once or twice daily on a protracted schedule in adults (Schellens et al., 1996; Creemers et al., 1997). In infants and very young children especially, oral administration provides an attractive alternative to intravenous administration due to the decreased discomfort to the patient and the overall convenience for the long term care of the patient during maintenance therapy.

A confounding factor of oral chemotherapy, however, is the extensive inter- and intra-patient variability in oral bioavailability (Hande et al., 1993; Zhou et al., 1998; Zamboni et al., 1999). Medications with a low bioavailability, like topotecan, can have a significantly higher inter-patient variability as compared to compounds with a high bioavailability (DeMario and Ratain, 1998; Daw et al., 2004). Infants demonstrate even greater variability in the bioavailability of orally administered agents due to the physiological maturation of factors affecting drug absorption, such as higher gastric pH and slower gastric motility (Berseth, 1996; Alcorn and McNamara, 2003). Furthermore, topotecan is a substrate for the drug efflux ATP-binding cassette transporters ABCB1 (P-glycoprotein; P-gp) and ABCG2 (breast cancer resistance protein; BCRP) both in vitro and in vivo (Chen et al., 1991; Hendricks et al., 1992; Brangi et al., 1999; Maliepaard et al., 1999; Erlichman et al., 2001; Maliepaard et al., 2001; de Vries et al., 2007), suggesting that polymorphisms in these transporters could also affect the oral absorption of topotecan from the gut. Therefore, several factors could contribute to the inter-patient variability with oral topotecan in infants and very young children. As a result, this study aimed to describe the population pharmacokinetics of oral topotecan in infants and very young children, specifically focusing on the influence of age and/or efflux transporters (ABCB1, ABCG2) on the absorption rate constant (K_a) of oral topotecan. In addition, other common patient characteristics

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(i.e., weight, height, etc.) were evaluated on all parameters describing the oral topotecan pharmacokinetics to determine if other covariate effects could explain the inter-individual variability in oral topotecan for infants and very young children.

Patients and Methods

Patients and study design

Risk-Adapted Therapy for Infants and Young Children with Embryonal Brain Tumors, High Grade Glioma, Choroid Plexus Carcinoma or Ependymoma (SJYC07; NCT00602667) is a multicenter protocol that is carried out in accordance with the Declaration of Helsinki and approved by our institutional review board. Parental permission and written informed consent was obtained before conducting any study procedures. This study is currently ongoing. Inclusion criteria included newly diagnosed tumor of the central nervous system (CNS), age <5 at the time of diagnosis, and no previous radiotherapy or chemotherapy. Additional criteria included adequate organ function, performance status with a Lansky Score ≥ 30 , and patients beginning their treatment within 31 days of definitive surgery. Patients were stratified into one of three risk arms (low, intermediate, and high) based on their stage of metastasis, diagnosis, extent of resection, histologic subtype, and age at diagnosis. Each treatment arm consists of an induction, consolidation, and a final optional maintenance phase. During the maintenance phase, patients on all treatment arms receive oral topotecan (0.8 mg/m^2) for ten days and oral cyclophosphamide (30 mg/m^2) for 21 days on a 28-day cycle for three cycles. Oral topotecan was administered as a liquid mixed in a flavored vehicle of the patient's choosing. Additional parental consent had to be obtained before inclusion into the oral topotecan pharmacokinetic analysis, which only included patients from St. Jude Children's Research Hospital. Additionally, patients who were simultaneously enrolled in and consented to the ongoing Pharmacogenetic

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Determinants of Treatment Response in Children study (PGEN5; NCT00730678) were used in the genotyping analysis for genetic polymorphisms in *ABCG2* and *ABCB1*.

Genotyping

Genome-wide genotyping was completed in germline DNA using the Illumina Infinium Omni2.5Exome-8 BeadChip (Illumina Inc., San Diego, CA). Variants were filtered on the basis of Hardy-Weinberg disequilibrium, minor allele frequency (MAF;0.1) and call rate (<95%). Within variants that passed these quality control criteria, a candidate gene approach was implemented to specifically evaluate *ABCG2* and *ABCB1* single nucleotide polymorphism (SNP) genotypes following those reported in PharmGKB (www.pharmgkb.org). The alleles reported in PharmGKB that met with above criteria in this analysis included rs4148157 (intron 11, G>A, population MAF 0.1006), rs2622628 (intron 9, C>A, population MAF 0.2362), rs2725252 (intron 1, C>A, population MAF 0.4145), rs1045642 (exon 27, A>G, Ile>Ile, population MAF 0.3952), rs2032582 (exon 22, A>C, Ser>Thr, population MAF 0.3343), and rs1128503 (exon 13, A>G, Gly>Gly, population MAF 0.4161) (dbSNP).

Pharmacokinetic sample collection and analysis

Whole blood (2 mL) was collected before the dose was administered and at 15 minutes, 90 minutes, and 6 hours after treatment, as indicated in a limited sampling model (Turner et al., 2006). Samples were placed in a heparinized tube, immediately centrifuged at 10,000 RPM for two minutes to separate plasma and 0.2 mL of plasma was added to 0.8 mL of ice-cold methanol. The methanolic mixture was vortexed for 10 seconds and centrifuged for 2 minutes at 10,000 RPM. The supernatant was retained and assayed for topotecan lactone by an isocratic high performance liquid chromatography assay (HPLC) with fluorescence detection, as previously described (Beijnen et al., 1990). Briefly, a Shimadzu RF 10AXL detector was used at

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370 nm excitation and 520 nm emission wavelengths and a lower limit of quantitation of 1 ng/mL.

Population pharmacokinetic analysis

Topotecan lactone concentrations were estimated using a nonlinear mixed-effects model implemented in Monolix 4.3.2 (Lixoft, Orsay, France) using stochastic approximation expectation maximization algorithm (SAEM) (Kuhn and Lavielle, 2005) combined with a Markov Chain Monte Carlo (MCMC) procedure. The minimum sample size was set to 50, resulting in the number of chains set to one. Concentration measurements that were below the limit of quantitation were marked as left-censored for the analysis, meaning actual concentrations were set to the lower limit of quantitation (1 ng/mL) and the extended SAEM approach simulates concentrations below the limit of quantitation according to a right-truncated normal distribution (Samson et al., 2006). A Bayesian prior of 0.69 h^{-1} with a deviation of 0.50 was applied to K_a as previously described (Daw et al., 2004). One- and two-compartment models were evaluated and the final model selection was based on their goodness of fit. Models that did not converge and those with erroneous results (e.g., negative parameter values) were disregarded. Diagnostic plots were used to assess the model's fit. Population weighted residuals (PWRES) and individual weighted residuals (IWRES) were plotted versus time and population or individual predictions, respectively. Models were compared using statistical criteria ($-2 * \log$ likelihood or the objective function value (OFV)), variability of the parameter estimates (e.g., $< 50\%$), diagnostic plots (e.g., graphical representation of the goodness of fit), and assessment of the physiological relevance of the parameter estimate.

Model variability and random effects were classified as between subject variability (BSV) or residual unexplained variability (RUV). BSV was assumed to be log-normally distributed according to an exponential equation:

$$P_i = \theta_{pop} * \exp(\eta_{pop}) \quad (1)$$

where P_i is the pharmacokinetic parameter of the i th individual, θ_{pop} is the population mean for P , and η represents the normally-distributed between subject random effect with a mean of zero and a variance of ω^2 . Additive, proportional, combined additive and proportional, and exponential equations were evaluated for RUV. The final model used an additive error model:

$$Y_{ij} = \hat{Y}_{ij} + \varepsilon_1 \quad (2)$$

where Y_{ij} is the observed concentration for the i th individual at time j , \hat{Y}_{ij} is the individual predicted concentration, and ε_1 represents additive error, which is a normally-distributed error term with a mean of zero and variance of σ^2 .

Several patient characteristics were tested for their influence on topotecan lactone pharmacokinetic parameters. The covariates tested included sex, age, estimated glomerular filtration rate (eGFR; calculated using the Schwartz equation (Schwartz et al., 1976)) body surface area (BSA), weight, height, *ABCG2* alleles (rs4148157, rs2622628, and rs2725252) and *ABCB1* alleles (rs1045642, rs2032582, and rs1128503). Only 42 patients had relevant serum creatinine measurements (serum creatinine measured within 24 hours of the pharmacokinetic analysis); therefore, the average of those measured was imputed for those missing a serum creatinine to calculate eGFR. Additionally, only 52 patients had gene data, so those with missing gene information were handled as a missing covariate within Monolix. Power and linear models were evaluated for the continuous covariates (i.e., weight, height, age, eGFR, BSA) and exponential models were evaluated for categorical covariates (i.e., sex and genotype). In addition, each continuous covariate was evaluated centered on the median population value.

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Forward addition was utilized to determine significant covariates. A decrease in the OFV ≥ 3.84 was considered significant for one degree of freedom at $p = 0.05$ based on the χ^2 distribution. Backward elimination was used to remove covariates from the model with an increase in the OFV ≥ 6.63 corresponding to 1 degree of freedom at $p = 0.01$.

The final model was evaluated using diagnostic plots to assess the fit of the model. Observed drug concentrations were evaluated against their predicted concentrations to evaluate their correlation. PWRES as well as IWRES were plotted versus time and population or individual predictions. Models were compared by assessing the variability of the parameter estimates in addition to the OFV. Normalized prediction distribution errors (NPDE) were plotted against time and population-predicted topotecan lactone concentrations to assess for model misspecification.

Results

Patient characteristics

The initial data set included 69 patients with 207 concentration measurements enrolled on the trial at the time of analysis. Eight patients with 24 concentration measurements were eliminated from the analysis because they did not have at least 1 concentration measurement above the lower limit of quantitation. Another concentration measurement from an individual was eliminated because the PWRES was > 6 . Thus, the final data set for the population analysis contained 61 patients with 182 concentration measurements. Seventy-one concentration measurements (39%) were below the limit of quantitation and were marked as left-censored for the pharmacokinetic analysis. The demographics for the study subjects at the time of the pharmacokinetic study are depicted in **Table 1**. The age range for those enrolled spanned from about 6 months to ~4.5 years, with 20 of the 61 patients less than 2 (33%) at the time of study.

In addition, about 2/3 of the patients enrolled were male. Pharmacogenomic data were available from 52 patients of the population and the frequencies of germline mutations examined were all in Hardy-Weinberg equilibrium. Alleles specifically evaluated in the model for *ABCG2* were rs4148157, rs2622628, and rs2725252, and for *ABCB1* were rs1045642, rs2032582, and rs1128503 with frequencies described in **Table 2**.

Population pharmacokinetic analysis

A concentration-time plot of the data is represented in **Figure 1**. The data were well described by a one-compartment model with first-order input and first-order elimination as assessed by visual inspection of diagnostic plots, physiological relevance of the estimates, and a significant reduction of the OFV.

The covariate analysis revealed a significant influence of BSA (centered on the population median) on apparent clearance (CL/F). Additionally, the inclusion of the *ABCG2* allele rs4148157 dichotomously as either GG homozygous or AG heterozygous and AA homozygous for the variant allele (represented as a 0 or 1, respectively) resulted in a significant effect on K_a . Heterozygous or homozygous G>A patients were grouped together because there was only 1 homozygous G>A patient in the analysis. Including this covariate in the model resulted in a K_a of 0.610 (s.e. 0.11) h^{-1} for GG and 1.76 (s.e. 0.49) h^{-1} for AG or AA patients. Further analysis of the effect of the *ABCG2* allele on K_a demonstrated a significant difference in observed maximal concentrations (C_{max}) between the two groups as assessed by the 1.5 hour concentration ($p=0.0001$; student t-test). An almost 2-fold difference in the C_{max} was observed in patients with GG genotype compared to those with AG or AA genotype (3.10 ng/mL and 5.34 ng/mL, respectively), supporting the inclusion on K_a . No *ABCB1* alleles significantly affected the K_a . Additionally, after including these covariates, no other covariates significantly affected oral

topotecan lactone pharmacokinetics. However, BSA on apparent volume of distribution (V/F) was included in the model for biological plausibility (Anderson et al., 2006). The final model estimates are presented in **Table 3** and were derived from the following final model:

$$Ka_i = Ka_{pop} * \exp(\theta^1 * GENE_{CAT}) \quad (3)$$

$$V/F_i = V/F_{pop} * \left(\frac{BSA_i}{BSA_{pop}} \right)^{\theta^2} \quad (4)$$

$$CL/F_i = CL/F_{pop} * \left(\frac{BSA_i}{BSA_{pop}} \right)^{\theta^3} \quad (5)$$

where Ka_i is the individual absorption rate constant, Ka_{pop} is the estimated population absorption rate constant, θ^1 is the estimated coefficient of the effect of the *ABCG2* allele rs4148157 on Ka , and $GENE_{CAT}$ is the categorical covariate of the GG allele compared to AG and AA allele (dichotomously defined as 0 or 1, respectively). V/F_i is the individual apparent volume of distribution, V/F_{pop} is the estimated population apparent volume of distribution, BSA_i is the individual body surface area, BSA_{pop} is the median population body surface area, and θ^2 is the estimated exponent for the effect of body surface area on topotecan lactone apparent volume of distribution. Similarly, CL/F_i is the individual apparent clearance, CL/F_{pop} is the estimated population apparent clearance, and θ^3 is the estimated exponent for the effect of body surface area on topotecan lactone apparent clearance. Diagnostic plots were visually inspected to confirm the selection of the final model (**Figures 2A** and **2B**). To further evaluate the model, PWRES, IWRES, and NPDE plots were examined (**Figures 3A-D**, **Figures 4A**, and **4B**). The data were equally distributed around zero and the majority of the data were in the -2 to +2 range, indicating acceptable agreement between observed and predicted concentrations.

Discussion

This study is the first to specifically evaluate the population pharmacokinetics of oral topotecan in infants and very young children with primary CNS tumors. Furthermore, it is the first study to implicate the *ABCG2* rs4148157 allele as a possible contributor to variations in the pharmacokinetics of oral topotecan. This study demonstrated that oral topotecan pharmacokinetics in this population of infants and very young children was well described by a one-compartment model with a population K_a equal to 0.61 h^{-1} , a V/F equal to 40.2 L (70.5 L/m^2), and a CL/F equal to 40.0 L/h (70.2 L/h/m^2). Covariate analysis indicated that after the inclusion of BSA on CL/F and V/F , the *ABCG2* rs4148157 allele also played a significant role on K_a , with patients either heterozygous or homozygous with the G>A allele resulting in a K_a 2-fold higher than their homozygous GG counterparts. Through the forward addition covariate analysis, age, weight, and height were also significant covariates on CL/F . However, after accounting for BSA, weight and height were no longer significant as assessed by the change in OFV due to the high correlation between these covariates, supporting the use of a single covariate to capture the effect of body size on CL/F . In addition, weight was no more significant than BSA in univariate analysis, supporting the current approach of normalizing oral topotecan dosage to BSA. Furthermore, after accounting for BSA, age was no longer significant on CL/F . However, these results may be confounded with the sparse sampling strategy due to small blood volume in infants and very young children, which could decrease the power to find multiple covariates in a small population size. Renal function has been shown in other studies to play a significant role on topotecan CL (Leger et al., 2004; Devriese et al., 2015); however, all patients included in this study demonstrated normal renal function, possibly explaining why eGFR was not a significant covariate on CL/F . Surprisingly, age was not a significant covariate on K_a , though having concentrations below the limit of quantitation for some of the patients at 15 min could have decreased the power of the model to find age as a significant covariate on K_a .

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Further covariate analysis evaluating *ABCG2* and *ABCB1* on K_a showed only one SNP, the *ABCG2* rs4148157 allele, to have any significant effect on K_a in this patient population. A literature review, including information found in PharmGKB, described this allele as intronic and to date, this allele has not been demonstrated to have clinical significance. The only publication specifically discussing the rs4148157 G>A allele was a study in patients with epilepsy, which showed no significant resistance to antiepileptic drugs in 441 Asian patients with the A allele, though no alleles addressed in this study demonstrated any significance (Kwan et al., 2011). Further analysis indicated strong linkage disequilibrium between rs4148157 and the well-known *ABCG2* rs2231142 allele (exon 6; MAF 0.119). It is possible the intronic allele (i.e., rs4148157) is serving as a surrogate for rs2231142. Gene imputation was performed to identify the genotype for the rs2231142 allele for our patient cohort and included in the model on K_a , CL/F, and V/F as a categorical covariate. For CL/F, including rs2231142 was not a significant covariate, and including it on K_a and V/F resulted in a model where the Fisher Information Matrix could not be estimated. Although the *ABCG2* rs4148157 allele was a significant covariate on K_a , when we included it as a categorical covariate on CL/F or V/F it resulted in a model where the Fisher Information Matrix could not be estimated. Therefore, these covariates were not included in further model development. Several explanations could explain this observation including our limited sampling of 3 plasma samples were per patient; we studied a relatively small population sample size, or the extent of data that were below the limit of quantitation. Therefore, further in vitro and in vivo research is required to determine if the *ABCG2* rs4148157 G>A allele does play a significant role in the decreased efflux of topotecan into the gut, and therefore would impact the clinical use of oral topotecan in infants and very young children who expressed the G>A rs4148157 allele, or if it is truly serving as a surrogate for the *ABCG2* rs2231142 allele.

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It is possible that the expression of *ABCG2* differs between adults and very young children, which could contribute to pharmacokinetic differences in oral topotecan. While the majority of the research has focused on CNS and hepatic *ABCG2* expression, no difference between adults and term neonates was observed (Daood et al., 2008; Yanni et al., 2011). Therefore, it is unlikely that ontogeny of *ABCG2* is playing a role.

In the analysis, it was noted that a number of concentrations that were marked as censored and therefore simulated in Monolix using a right-truncated normal distribution resulted in negative concentrations (**Figures 2A and 2B**). Simulations resulting in negative data likely mean that these concentrations were probably zero. However, instead of treating these concentrations as zero as the simulations suggested and removing them from the analysis, we believed it important to leave these in the model to not overly bias the estimation of the pharmacokinetic parameters.

Other population pharmacokinetic models in adults and children have published oral topotecan as a 2-compartment model. However the majority of these models also included intravenous data as part of their analysis, which was not included in our analysis. A study in adults estimated the K_a as 1.7 h^{-1} and a CL/F as about 63 L/h with creatinine clearance and WHO performance status included as significant covariates (Leger et al., 2004). Comparing to the population means in this study, K_a was about 2 times higher than those wild-type for rs4148147 but CL/F was very similar. However, patients who did have the G>A rs4148147 allele did have a K_a closer to that of adults (1.76 h^{-1}). In a pediatric study, CL/F was estimated to be about 20.82 L/h in older pediatric patients using a two-compartment model (Wagner et al., 2004), which was about half that found in this study. In another pediatric study completed the same year, Daw *et al.* found CL/F to be about 79.7 L/h/m², which is almost identical to the apparent clearance found

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in our population (Daw et al., 2004). In addition, the K_a of 0.69 h^{-1} was similar to our population mean of 0.61 h^{-1} , which is not surprising since the K_a reported by Daw *et. al* was used as a Bayesian prior in our model. Finally, comparing our CL/F to a study completed in 1999 showed very similar determinations, with a CL/F reported as about 64.4 L/h/m^2 , which was also determined using a one-compartment model (Zamboni et al., 1999). Comparing all the studies together, it seems that the patients from this study with GG rs4148157 allele had an estimated K_a similar to older pediatric patients, but those expressing the AG or AA allele had a K_a closer to adult values. In addition, CL/F in infants and very young children is similar to other pediatric patients as well as adults based on the results from this study.

This is the first study to evaluate oral topotecan pharmacokinetics in infants and very young children, and our results have implicated a possible role for the ABCG2 rs4148157 allele on K_a . BSA was also a significant covariate on CL/F, with BSA also included on V/F for biological plausibility. Future analyses investigating the clinical implications of ABCG2 rs4148157 allele on oral topotecan chemotherapy in infants and very young children are warranted.

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Acknowledgments

The authors would like to thank the clinical nursing team, including Sheri Ring, Paula Condry, and Terri Kuehner, for their assistance in this study; the Stewart lab for bedside collection and processing of the samples; The Hartwell Center for performing the Illumina Chip; Wenjian Yang for his expertise in creating the database which housed the genetic information for the patients; and Jun J. Yang for his valuable expertise in genetic analyses.

Authorship Contributions

Participated in research design: Broniscer, Robinson, Gajjar, and Stewart

Conducted experiments and performed data analysis: Roberts, Birg, Lin

Wrote or contributed to the writing of the manuscript: Roberts, Birg, Lin, Daryani, Panetta, Broniscer, Robinson, Gajjar, and Stewart

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Footnotes

This work was supported by the National Cancer Institute [Grants CA154619, CA21765], and the American Lebanese Syrian Associated Charities at St. Jude Children's Hospital.

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Figure 1 Concentration-time data for the patients included in the analysis represented as topotecan lactone concentrations (ng/mL) versus time (hour). Patients with wild type rs4148157 and variant rs4148157 are represented in gray circles and black open circles, respectively. The dashed line at 1 ng/mL represents the lower limit of quantitation for the assay.

Figure 2 Observed topotecan lactone concentrations versus population predicted and individual predicted topotecan lactone concentrations in **A** and **B**, respectively, for the final topotecan lactone model. The closed circles represent data above the lower limit of quantitation, the open circles represent data below the limit of quantitation or simulated data, the solid line represents the line of identity, and the dashed gray line represents the spline of the model.

Figure 3 The population weighted residuals (PWRES) and the individual weighted residuals (IWRES) versus time and predicted topotecan lactone concentrations are presented in panels **A-D**. The closed circles represent data above the lower limit of quantitation, the open circles represent data below the limit of quantitation or simulated data, and the solid line is the reference line at zero.

Figure 4 The normalized prediction distribution errors (NPDE) plotted against time and population predicted topotecan lactone concentrations are presented in **A** and **B**, respectively. The closed circles represent data above the lower limit of quantitation, the open circles represent data below the limit of quantitation or simulated data, and the solid line is the reference line at zero.

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Table 1 Patient Demographics

Patient Characteristic	n	Mean	Median (Range)
Age (y)	61	2.4	2.37 (0.48 to 4.59)
Weight (kg)	61	12.6	12.60 (5.20 to 17.50)
Height (cm)	61	87.1	89.10 (59.50 to 102)
BSA (m ²)	61	0.57	0.57 (0.31 to 0.72)
eGFR (mL/min/1.73 m ²)	42	148.1	139.79 (100.67 to 200.01)
SCr (mg/dL)	42	0.25	0.24 (0.14 to 0.40)
Sex			
Male	38		
Female	23		

BSA – body surface area; eGFR – estimated glomerular filtration rate; SCr – serum creatinine;

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Table 2 Pharmacogenomic Analysis

ABCG2	n	ABCB1	n
rs4148157 (G>A)		rs1045642 (A>G)	
GG	42	AA	8
AG	9	AG	22
AA	1	GG	22
Not available	9	Not available	9
rs2622628 (C>A)	n	rs2032582 (A>C)	n
CC	43	AA	10
AC	7	AC	20
AA	2	CC	22
Not available	9	Not available	9
rs2725252 (C>A)	n	rs1128503 (A>G)	n
CC	14	AA	9
AC	26	AG	18
AA	12	GG	25
Not available	9	Not available	9

ABCG2 – breast cancer resistance protein/ATP-binding cassette sub-family G member 2;

ABCB1 – P-glycoprotein/ATP binding cassette Sub-Family B member 1

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Table 3 Final Population Model Estimates

Parameters	Mean Parameter Estimate	S.E.
K _a (1/h; wild type rs4148157)	0.61	0.11
θ^1	1.06	0.25
K _a (1/h; variant rs4148157)	1.76	0.49
V/F (L)	40.2	7.0
θ^2	0.78	0.48
CL/F (L/h)	40.0	2.9
θ^3	1.25	0.39
ω - BSV		
K _a	0.517	0.12
V/F	0.338	0.17
CL/F	0.465	0.11
σ - RUV		
Additive (ng/mL)	0.592	0.44

S.E.- standard error; K_a – absorption rate constant; θ^1 - estimated coefficient of the effect of ABCG2 rs4148157 G>A heterozygotes and homozygotes on the absorption rate constant; V/F – apparent volume of distribution; θ^2 - estimated exponent of the effect of body surface area on apparent volume of distribution; CL/F- apparent clearance; θ^3 – estimated exponent of the effect of body surface area on apparent clearance; BSV- between subject variability; RUV- residual unexplained variability

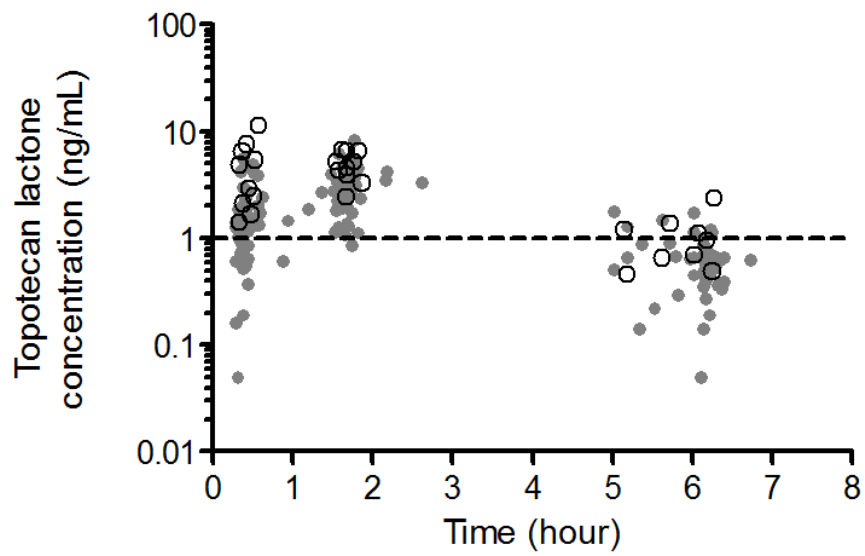


Figure 1

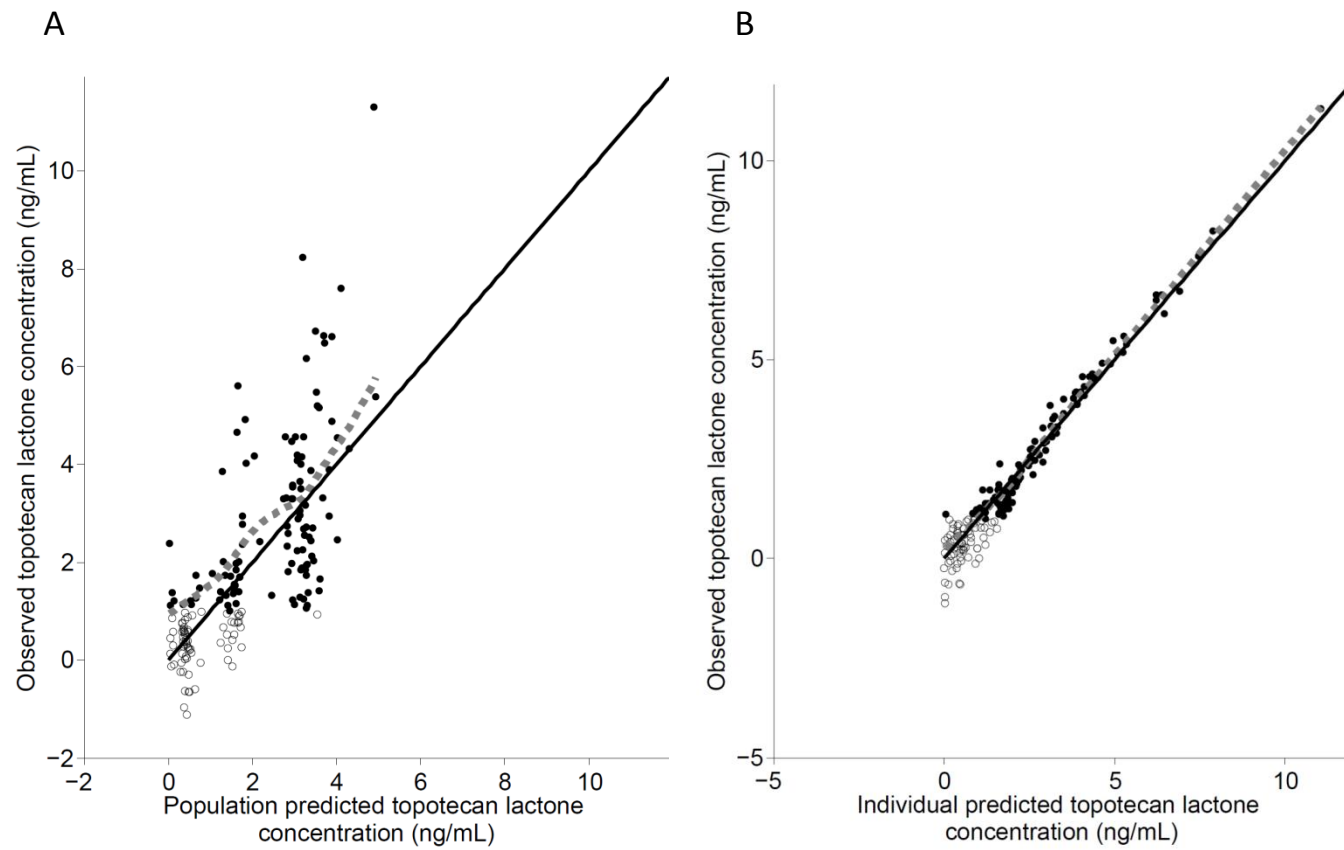


Figure 2

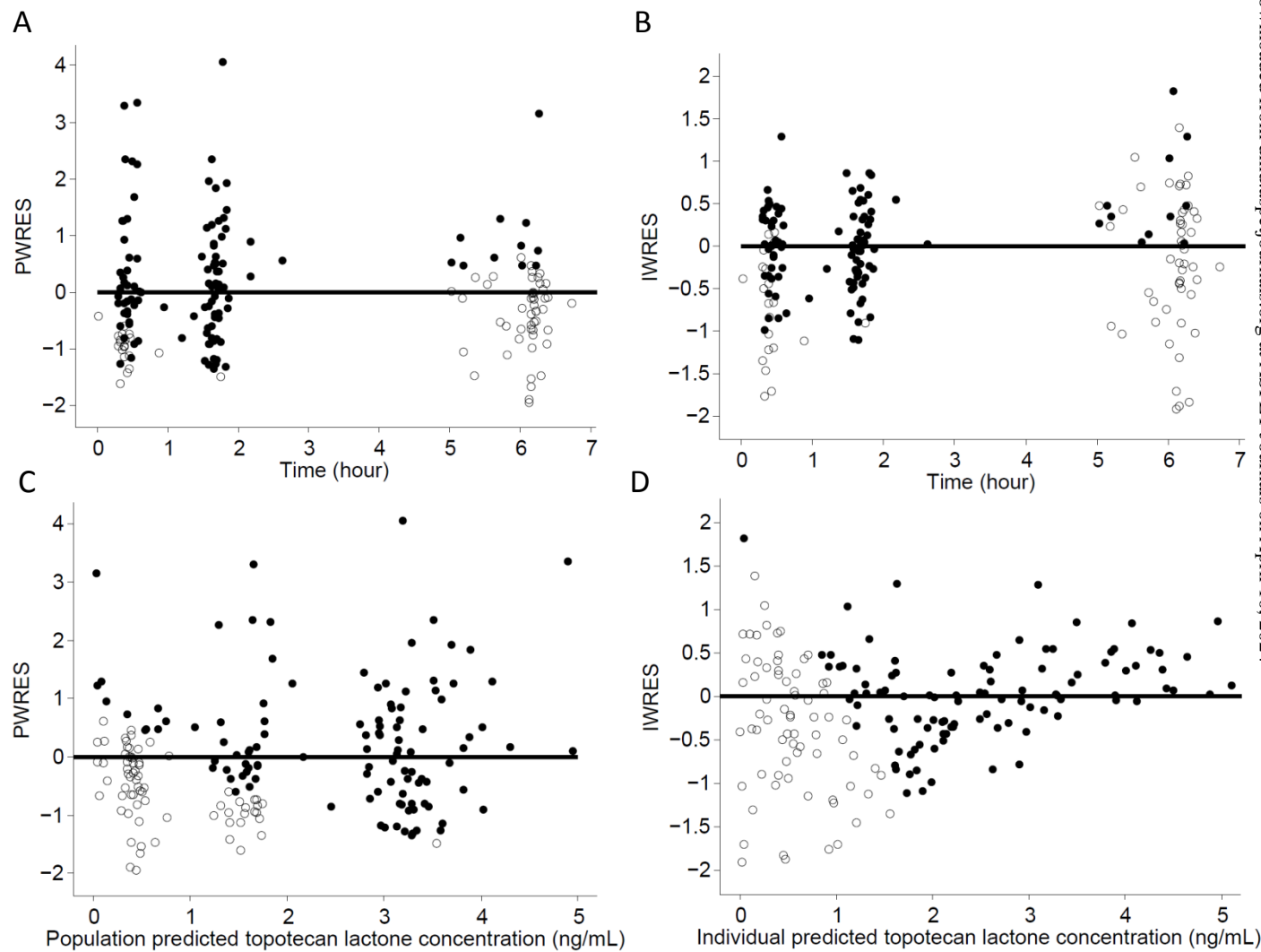


Figure 3

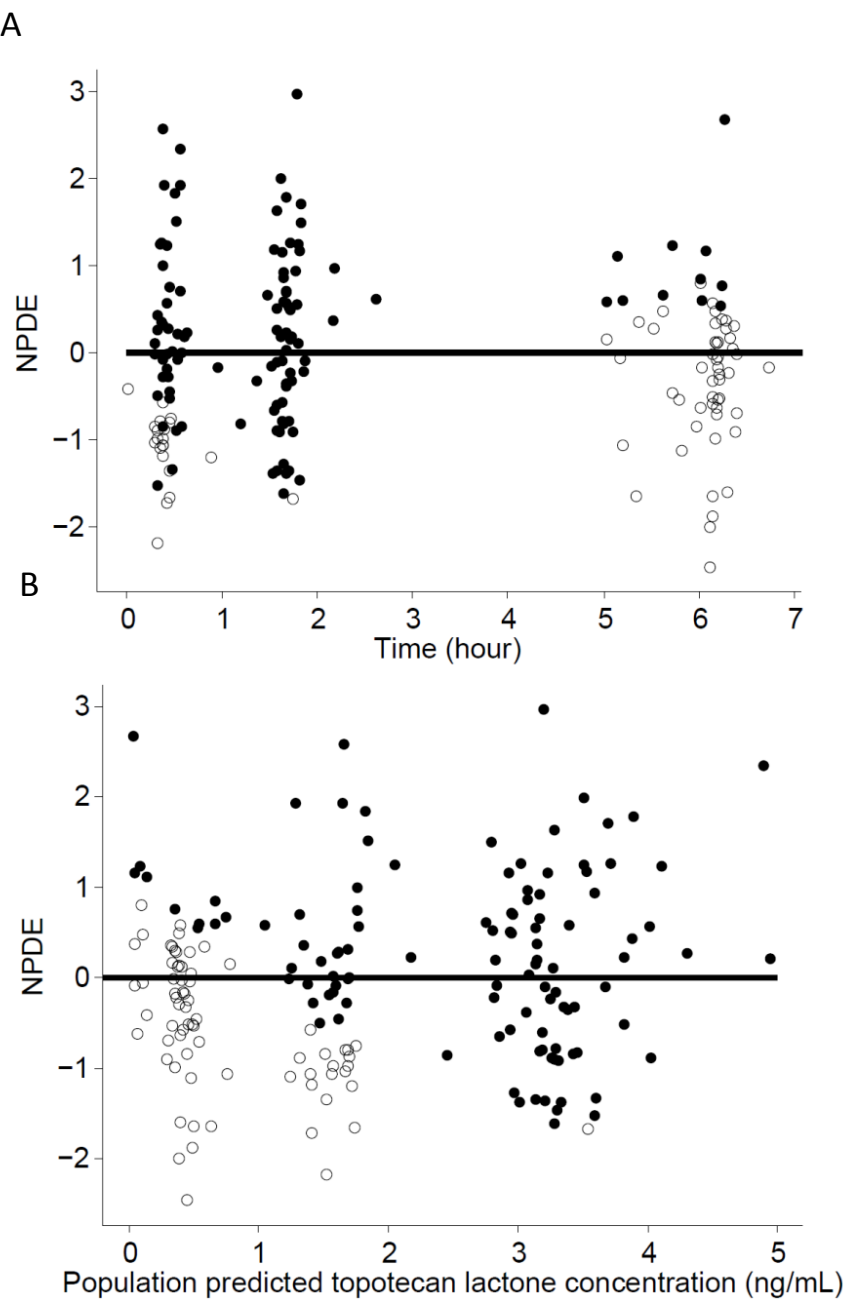


Figure 4