Cancer Chemother Pharmacol (2006) 57: 475–482
DOI 10.1007/s00280-005-0062-z

ORIGINAL ARTICLE

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Development and validation of limited sampling models for topotecan lactone pharmacokinetic studies in children

Received: 25 January 2005 / Accepted: 7 June 2005 / Published online: 27 July 2005

Abstract *Purpose*: To develop and validate a pharmacokinetic limited sampling model (LSM) for intravenous and oral topotecan pharmacokinetic studies in children. *Methods*: Topotecan lactone concentration-time data from five trials were used to develop and validate LSM for intravenous and oral topotecan. Based on full sampling from one intravenous study (30 patients; 195 studies), a LSM for intravenous topotecan was determined using a modification of the D-optimality algorithm. For oral topotecan we used full sampling data from one oral topotecan study (27 patients; 47 studies) to develop an LSM. Accuracy and bias of each LSM were determined relative to the full sampling method. Predictive performance of the LSM was validated using additional data and Monte–Carlo simulations based on these data. *Results*: LSM for intravenous topotecan includes: 5 min, 1.5, and 2.5 h after the end of the 30 min infusion. The median accuracy (absolute predicted error) and bias (predicted error) are $\leq 8\%$ and $\leq 6.1\%$, respectively. For oral topotecan, the optimal LSM includes: 15 min, 1.5, and 6 h. The median accuracy and bias are 6% and 4%, respectively. *Conclusions*: Our results indicate that the optimal sampling times for the intravenous LSM for topotecan in children consist of: predose, and 5 min, 1.5, and 2.5 h after the end of infusion. For oral topotecan the sample times are predose, 15 min, 1.5, and 6 h after dose administration. These LSM are invaluable to children receiving topotecan because it minimizes inconvenience and blood collection.

Keywords Topotecan · Pharmacokinetics · Limited sampling model

Supported in part by US Public Health Service award CA23099 and the American Lebanese Syrian Associated Charities (ALSAC).

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Introduction

An increasingly important area of clinical investigation is focused on studies designed to gain a better understanding of the variability in drug metabolism and elimination, and how this variability translates into varied pharmacologic effects. However, unique challenges and limitations remain, which complicate the performance of pharmacokinetic studies in the child with cancer. The frequency and volume of blood collected from a child with cancer for clinical pharmacology studies must be minimized, especially for very young children who have small total blood volumes [12, 15, 16]. The frequency and timing of blood levels can inconvenience patients and families, requiring them to remain in the clinic for long periods of time after drug administration (e.g., 6–8 h). Significant costs are associated with the personnel, equipment, and time required to obtain, process, and interpret drug concentrations in children [12]. Therefore, a need exists to collect samples at the most appropriate and informative time points to obtain accurate pharmacokinetic information, while minimizing blood volume collected from children with cancer [16]. Finally, recent legislation (i.e., Pediatric Research Equity Act 2003) in addition to existing laws (i.e., Best Pharmaceuticals for Children Act) will likely increase the need for clinical pharmacokinetic studies in children in all phases of drug testing. Thus, the need for

well-designed pharmacokinetic studies in children is greater now than ever.

Topotecan, a topoisomerase I interactive agent, is active in several pediatric cancers including neuroblastoma, medulloblastoma, rhabdomyosarcoma, and acute leukemia [6, 8, 10, 18]. In preclinical models we have shown that this antitumor activity is both schedule-dependent and related to topotecan systemic exposure (area under the plasma concentration-time curve; AUC). Topotecan cytotoxicity is specific to the S phase of the cell cycle [5]; thus, protracted administration has been associated with increased cytotoxicity [9]. Therefore, protracted, repeated administration of topotecan (e.g., 10 doses over 12 days) is the preferred dosing regimen for clinical development.

A steep systemic exposure-tumor response curve has been observed for tumor xenograft models [26]. This observation is clinically relevant because we have demonstrated significant interpatient variability in topotecan clearance, which at fixed topotecan dosages results in wide interpatient variability in topotecan lactone AUC [18, 23]. To address this in the clinic we have proposed the use of pharmacokinetically guided topotecan, which we have shown is feasible [18]. Moreover, this approach to dosing topotecan is associated with promising clinical outcomes in children with high-risk neuroblastoma and high-risk medulloblastoma, with acceptable side effects [17, 22]. Further studies of this approach to dosing topotecan are planned in larger cohorts of patients to evaluate the antitumor efficacy. Although topotecan pharmacokinetic studies are planned in these patients, limited sampling models would enhance our ability to conduct these studies.

Thus, in the present study we used topotecan lactone concentration–time data from children receiving either intravenous or oral topotecan. The objectives of this study were to develop and validate pharmacokinetic limited sampling models for use after intravenous or oral topotecan administration.

Patients and methods

Patients

Patients less than 22 years of age with histologically documented solid malignancies or hematological malignancies were evaluated in prospective phase I/II studies from 1994 to 2002 (Table 1) [4, 17, 22, 27]. These studies were approved by the St. Jude Children's Research Hospital Institutional Review Board, and informed consent was obtained from patients, parents, or guardians, as appropriate, according to institutional guidelines. The IV data consisted of three studies with 54 patients and 255 datasets, and the oral data consisted of two studies with 47 patients and 73 datasets.

Topotecan preparation and administration

For intravenous administration, topotecan (Hycamtin; GlaxoSmithKline, Philadelphia, PA, USA) was reconstituted with 2 ml sterile water, USP, and further dilutions were made in 50 ml of D_5W. Each dose was administered by a syringe pump through either a peripheral or a central venous line as a 30-min infusion. For oral administration, the topotecan intravenous solution was mixed with juice and administered orally to all children in studies 4 and 5 [4, 27].

Sampling strategy and sample analysis

Serial plasma samples for pharmacokinetic analysis were obtained at specific times for each clinical trial as listed in Table 1. At each time point, 3 ml of whole blood was collected into a heparinized tube, blood was immediately centrifuged at 7,700 ×*g* to separate plasma, and 200 µl of plasma were added to 800 µl of cold (−30°C) methanol. Blood was collected from a site contralateral to the

Table 1 Topotecan clinical protocols

Study number	Disease	Topotecan route and schedule of administration	Topotecan sampling times (h)[a]
Study 1 [17] (30/195)[b]	Advanced stage high-risk neuroblastoma	30 min i.v. infusion daily ×5 for 2 weeks	Pre, 0.25, 1, 6
Study 2 [22] (9/35)[b]	Newly diagnosed medulloblastoma and supratentorial PNET	30 min i.v. infusion daily ×5	Pre, 0.25, 0.5, 1, 3, 6
Study 3 (CF Stewart, in preparation) (24/25)[b]	Relapsed acute lymphoblastic leukemia	30 min i.v. infusion daily ×5	Pre, 0.25, 0.5, 1, 3, 6
Study 4 [27] (27/47)[b]	Recurrent/ refractory solid tumors	i.v. solution given orally daily ×21 or daily ×5 for 3 weeks	Pre, 0.25, 0.5, 1.5, 3, 4, 6, 8
Study 5 [4] (20/26)[b]	Recurrent/refractory solid tumors	i.v. solution or gelatin capsule given orally daily ×5 for 2 weeks	Pre, 0.25, 0.5, 1.5, 3, 4, 6

[a]Listed times of sampling after intravenous (i.v.) topotecan are calculated after the end of 30-min infusion
[b] Number of patients used in LSM development or validation/ number of pharmacokinetic sampling days used in LSM development or validation

topotecan infusion in patients receiving intravenous topotecan. This methanolic mixture was vortexed for 10 s and centrifuged for 2 min at 7,700×*g*. The supernatant was retained and analyzed by an isocratic HPLC method with fluorescence detection (RF551; Shimadzu, Columbia, MO, USA) at 370 nm excitation and 530 nm emission wavelengths [20, 28]. This method has been validated and has the following performance characteristics: 90–103% accurate; within- and between-day variation <10%. The lower limit of quantitation (LLOQ) for plasma topotecan lactone was 0.25 ng/ml.

Structural pharmacokinetic model

We estimated topotecan lactone population pharmacokinetic parameters in our patient population with a two-compartment model using a nonlinear mixed effects modeling approach (via NONMEM) [1, 21]. Model parameters estimated for intravenous dosing included volume of distribution (V), elimination rate constant (k_e), and intercompartmental rate constants (k_{12} and k_{21}). For the oral dosing the parameters included apparent volume of distribution (V/F), where F is bioavailability, elimination rate constant (k_e), intercompartmental rate constants (k_{12} and k_{21}), and absorption rate constant (k_a). Standard equations [7] were used to calculate systemic clearance (CL) for the intravenous dosing and apparent systemic clearance (CL/F) for the oral dosing.

Selection of LSM

Although data derived from intravenous and oral sampling in the same patient could be used to generate a single LSM for topotecan, our clinical application for a LSM required that we have separate models, one for use after intravenous topotecan dosing and one for use after only oral topotecan dosing. The sampling time points for the intravenous and oral LSMs were determined using the estimated population pharmacokinetic parameters (study 1 for intravenous and study 5 for oral, Table 1), and a modification of the D-optimality algorithm [2], which accounts for the prior distribution of these parameters. These two studies were chosen to develop their respective LSM since they each had the largest number of courses/samples for describing the population pharmacokinetics of TPT lactone. The additive model was used to account for model variance [3]: $\sigma = [\ \sigma_{inter} + \sigma_{slope}\, c(t)\ ]^2$ where $c(t)$ is the plasma concentration of the drug, σ_{inter} represents the absolute error (e.g., LLOQ), and σ_{slope} represents the relative error (e.g., assay error). The relative error was fixed at 10% [20, 28] and the absolute error was set at three levels, small, medium, and large, which equated to 50% of the lower limit of quantitation, the LLOQ, and fourfold LLOQ, respectively. By setting the absolute error at these three levels we represented three possible magnitudes of error at or near the LLOQ. Furthermore, we limited the window of sampling times to 6 h due to the relatively short terminal half-life for topotecan (2–3 h), which yields concentrations close to the LLOQ after oral dosing or after low intravenous dosages.

LSM validation

The LSM for intravenous topotecan administration was validated using topotecan concentration-time data from studies 2 and 3 (Table 1). The LSM for oral topotecan administration was validated using topotecan concentration-time data from study 4 (Table 1). No data from these three studies were used in the development of the LSM models.

Two methods were used to evaluate the accuracy and bias for the LSM: (a) comparison of results from the full sample set to LSM (which are subsets of the full sampling set) using patient data, and (b) Monte–Carlo simulation. To perform the validation with the patient data, the pharmacokinetic parameters were first determined using all available data with maximum likelihood estimation and then compared to the parameters determined from subsets of data. Since some time points in the optimal intravenous LSM (5 min, 1.5 h, and 2.5, 3.5, or 5.5 h) did not match the samples obtained from patients (Table 1), we used the samples that were closest to the time points in the LSM for validation purposes. The limited sampling parameters for individuals were estimated using maximum a posteriori probability estimation (MAP) as implemented in ADAPT II using the above described population priors (study 1 pharmacokinetics were the priors for intravenous and study 5 pharmacokinetics were the priors for oral).

The Monte–Carlo simulations were used for an additional method of validation. It was useful since (a) actual clearance is known a priori, and (b) data points can be simulated at the specific times of the LSM. For the Monte–Carlo simulations 1,000 datasets were generated based on the distribution of pharmacokinetic parameters from each of the validation studies 2, 3, and 4 (Table 2) using a fixed dosage of 3.3 mg/m^2 for the intravenous group or 1 mg/m^2 for the oral group. We estimated the pharmacokinetic parameters for each Monte–Carlo simulated data set with all the simulated samples using the maximum likelihood method in ADAPT II. This set of parameters is referred to as the "full" set and will be used for comparison to the various LSMs. In the same manner as with the patient data, we fit subsets of the simulated data only including samples in the LSM and compare these to the parameters from the above described "full" set.

Bias (in terms of the prediction error) and accuracy (in terms of the absolute prediction error) of each model were calculated for each comparison as follows [19]:

Table 2 Topotecan clearance and volume estimates determined using nonlinear mixed effect modeling

Route	Protocol	CL (l/h/m^2) IIV* (CV%)	Volume (l/m^2) IIV* (CV%)	Intra individual CV%
IV	Study 1	28.4 (22)	35.9 (16)	25
	Study 3	26.5 (40)	33.6 (29)	6.5
	Study 2	44.1 (23)	48.8 (24)	22
		CL/*F* (l/h/m^2) IIV* (CV%)	Volume/*F* (l/m^2) IIV* (CV%)	Intra individual CV%
PO	Study 4	29.8 (56)	151.0 (34)	26
	Study 5	80.5 (35)	218.0 (63)	39

*IIV inter-individual variability

$$\%\text{Bias} = \frac{100\left(X_{\text{actual}} - X_{\text{predict}}\right)}{X_{\text{actual}}}$$

$$\%\text{Accuracy} = \frac{100\left|X_{\text{actual}} - X_{\text{predict}}\right|}{X_{\text{actual}}}$$

where X_{actual} represents the value of the estimated pharmacokinetic parameter of interest based on the full sampling scheme, and X_{predict} represents the value of the estimated pharmacokinetic parameter of interest based on the LSM.

Results

Intravenous LSM derivation and validation

Using D-optimality methods with study 1 population pharmacokinetics yielded three possible LSM for validation. Based on larger absolute error (relative to the LLOQ), the most optimal sampling time points are 5 min, 1.5, and 2.5 h after the end of the 30-min infusion. Based on medium absolute error, the most optimal sampling time points are at 5 min, 1.5, and 3.5 h after the end of the 30 min infusion. Based on small absolute error, the most optimal sampling time points are at 5 min, 1.5, and 5.5 h after the end of the infusion. As expected, the predicted LSM with small residual error includes later sampling times, which assumes that plasma concentrations at 6 h are $>$LLOQ. Based upon the dosages that patients in our studies received this is, in fact, the case. In particular, only 14 of 273 topotecan plasma concentrations at 6 h were less than 1 ng/ml (four times the LLOQ), and only one was below the LLOQ of 0.25 ng/ml. Therefore, the LSM with small absolute error is acceptable.

The concentration-time data from studies 2 and 3 were used to validate the intravenous LSM; these data were independent of the data (i.e., study 1) that were used to develop this LSM. Samples for topotecan pharmacokinetic analysis were drawn at five time points after the end of the infusion in studies 2 and 3 (Table 1). For validation, the pharmacokinetic parameters were determined in two subsets of the full set of samples, (0.25, 1, and 3 h after the infusion; 0.25, 1, and 6 h after the infusion; or those samples closest to the proposed LSM) and compared to the pharmacokinetic parameters based on the full sample set. Although the LSM with the 6 h time point was more accurate and less biased than the other model (see Table 3), both models performed well with median accuracy $\leq 8\%$ and median bias of $\leq -6.1\%$ for topotecan lactone systemic clearance.

Next we used the Monte Carlo simulation to evaluate the optimal LSM, as well as several interim time points (e.g., 3.5 and 5.5 h). The results of this validation based upon the intravenous topotecan population pharmacokinetics in studies 2 and 3 are summarized in Table 4. In particular, the LSM was more accurate when validated against study 3 ($\leq 7.5\%$ median accuracy), compared to the study 2 ($\leq 12.3\%$ median accuracy) distribution since the study 3 population pharmacokinetic parameters were more comparable to those of study 1. However, the accuracy and bias in both validation sets were $<15\%$ (see Fig. 1) and were similar to the validation results generated using the patient samples.

Table 3 Accuracy and bias of clearance estimates for two different intravenous LSM validated using data from patients on study 2 or study 3

Protocol	LSM[a] (h)	CL Accuracy (%)		CL bias (%) Median (quartiles)
		Median (quartiles)	90th percentile	
Study 2	0.25, 1, 3	7.7 (4.6, 9.5)	17.3	−5.1 (−8.1, −1.9)
	0.25, 1, 6	4.1 (3.0, 6.5)	18.0	−2.9 (−4.3, 2.5)
Study 3	0.25, 1, 3	6.6 (4.7, 8.6)	10.4	−6.1 (−7.8, −3.9)
	0.25, 1, 6	4.5 (1.6, 4.8)	9.6	−0.2 (−4.5, 3.5)

[a]Time after the end of topotecan 30-min infusion

Table 4 Accuracy and bias of clearance estimates for three different intravenous LSM validated using Monte–Carlo simulation based on topotecan population pharmacokinetic analysis

Protocol	LSM[a]	Accuracy (%)		Bias (%) Median (quartiles)
		Median (quartiles)	90th percentile	
Study 2	(5 min, 1.5, 2.5 h)	12.3 (5.8, 25.8)	47.5	8.4 (−4.0, 25.6)
	(5 min, 1.5, 3.5 h)	11.1 (5.0, 21.3)	41.5	4.9 (−5.0, 19.9)
	(5 min, 1.5, 5.5 h)	7.3 (3.5, 14.4)	30.3	2.2 (−4.4, 12.5)
Study 3	(5 min, 1.5, 2.5 h)	7.4 (3.2, 12.0)	17.4	−2.8 (−8.9, 3.7)
	(5 min, 1.5, 3.5 h)	7.5 (3.7, 11.7)	15.8	−6.2 (−10.7, −0.7)
	(5 min, 1.5, 5.5 h)	5.6 (2.4, 9.1)	12.9	−3.8 (−8.1, 0.8)

[a]Time after the end of topotecan infusion

Oral LSM derivation and validation

Three oral LSM were developed for validation. The three models included sampling topotecan plasma levels at the following times after oral topotecan administration: 0.25, 1.5, and 3 h; 0.25, 1.5, and 6 h; or 0.25, 0.5, and 3 h. Validation of the oral LSM using the study 4 clinical data (Table 5) demonstrated that extending sampling to 6 h is more accurate than stopping at 3 h (6 vs. 15%; Table 5). These results also indicate that if the topotecan lactone concentrations at 6 h are greater than the LLOQ, then sampling at later time points should yield more accurate clearance estimates. Similar to the data from the intravenous studies, 37 of 62 topotecan lactone concentrations at 6 h after the oral dose were less than 1 ng/ml, but none were below the LLOQ of 0.25 ng/ml. Validating the oral LSM based upon a Monte–Carlo simulation of the study 4 population showed that sampling at 0.25, 1.5, and 6 h has an accuracy measure of 8% and a bias measure of 2% in estimating topotecan clearance (Table 6, Fig. 2).

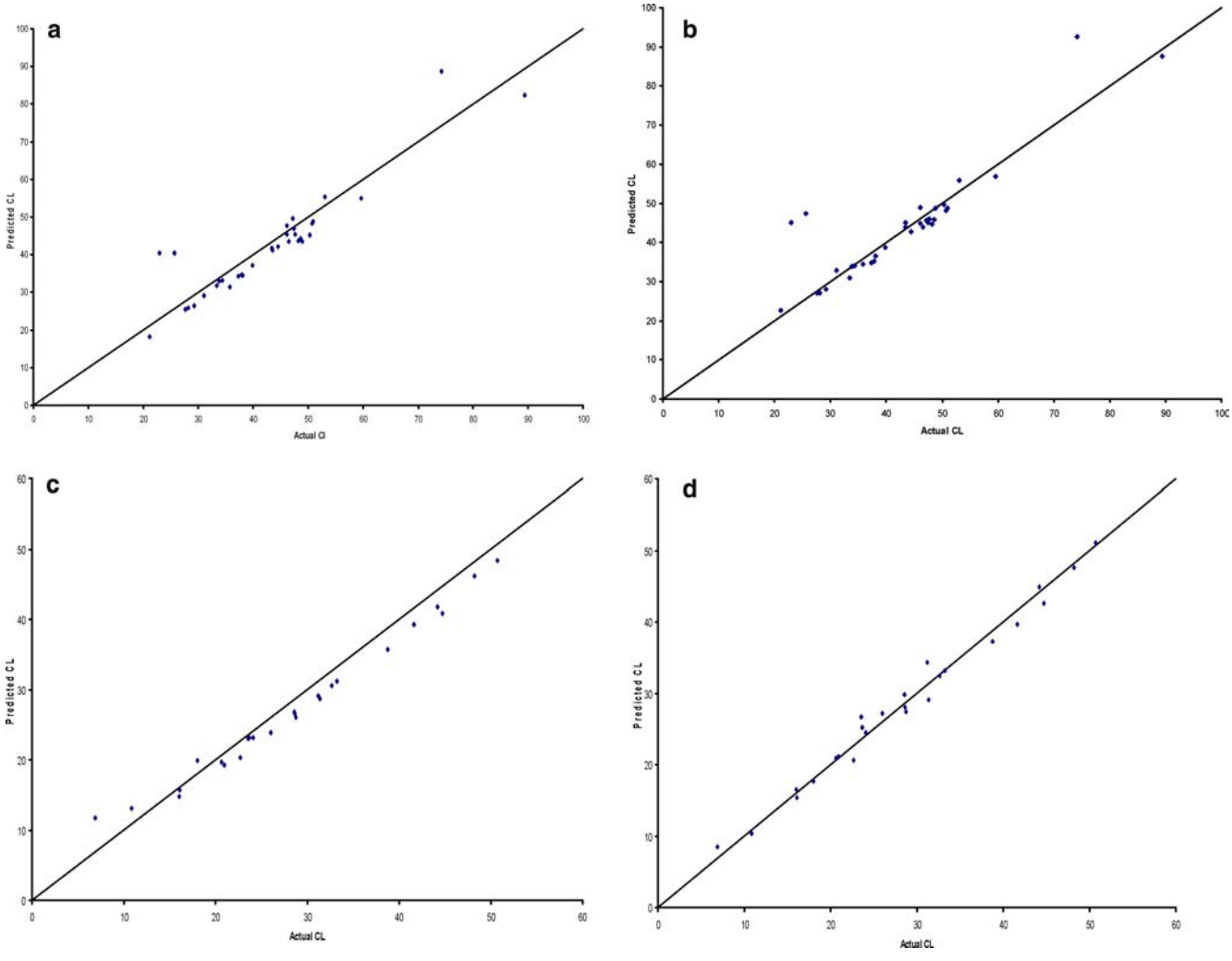

Fig. 1 Predicted intravenous topotecan clearance versus actual clearance. Study 2 results are in **a** and **b** and study 3 results are in **c** and **d**. The LSM consists of 0.25, 1, and 3 h after the end of the 30-min infusion (**a** and **c**) and 0.25, 1, and 6 h after the end of the 30-min infusion (**b** and **d**)

Table 5 Accuracy and bias of clearance estimates for three LSM for oral topotecan validated using pharmacokinetic data sets from patients on study 4

LSM (h)	Median (quartiles)	
	Accuracy (%)	Bias (%)
0.25, 1.5, 3	15 (7, 32)	6 (−8, 32)
0.25, 1.5, 6	6 (2, 11)	4 (0, 11)
0.25, 0.5, 3	18 (9, 28)	10 (−6, 26)

Table 6 Accuracy and bias of clearance estimates for three different LSM for oral topotecan validated using Monte–Carlo simulation based on the population pharmacokinetics from patients in study 4

LSM (h)	Median (quartiles)	
	Accuracy (%)	Bias (%)
0.25, 1.5, 3	10 (5, 18)	5 (−4,15)
0.25, 1.5, 6	8 (4, 15)	2 (−6, 11)
0.25, 0.5, 3	11 (5, 19)	5 (−4, 16)

Discussion

This is the first report of the derivation and validation of LSM for short intravenous infusions and oral administration of topotecan in children. These LSM have excellent performance characteristics (i.e., accuracy and bias), and can be applied to future clinical pharmacokinetic studies of topotecan in children with cancer. Based upon our validation results and for patient convenience, we recommend using the LSM with samples at 5 min, 1.5, and 2.5 h after the end of a 30 min. topotecan intravenous infusion, and the LSM with samples at 0.25, 1.5, and 6 h after oral topotecan.

Previously we proposed a LSM to study the pharmacokinetics of topotecan in an early phase I trial of topotecan in children with recurrent solid tumors [23]. The model, developed from pharmacokinetic data from a 72-h continuous infusion, consisted of sampling prior to the infusion and at 30 min, 3, and 23.5 h after the end of the infusion. However, we noted that at the topotecan dosages used in that study many of the plasma concentrations at 23.5 h were below the assay LLOQ. Although

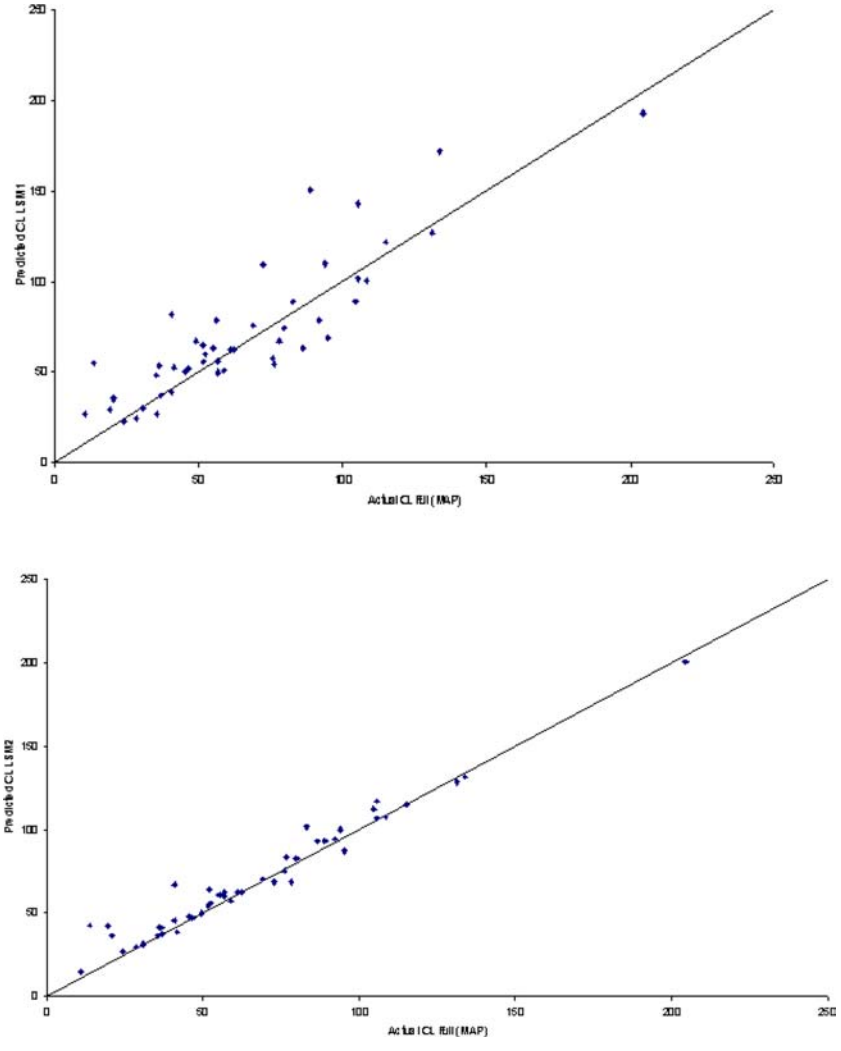

Fig. 2 Predicted oral topotecan clearance versus actual clearance for study 4. The LSM consists of sampling time points at 0.25, 1.5, and 3 h (**a**) and at 0.25, 1.5, and 6 h after oral topotecan administration (**b**)

this sample time point was convenient for the patient and clinic staff, it is unlikely to yield useful results in future studies. Thus, in the current analysis we constrained our LSM development to times within 6 h of topotecan administration.

In recent phase II clinical trials we have demonstrated the feasibility and clinical relevance of individualizing topotecan dosage in children with cancer [18]. The rationale for using this approach to dose topotecan is based primarily on the wide interpatient variability in topotecan clearance, and the relation between topotecan lactone systemic exposure and pharmacologic effect (e.g., myelosuppression) [20, 24]. After demonstrating the feasibility of pharmacokinetically guided topotecan in children with cancer [18], we have now completed two phase II pediatric clinical trials in which we used this approach [17, 22]. Based upon the results from our phase II study we have extended the use of pharmacokinetically guided topotecan dosing to a cooperative group study in children with high-risk neuroblastoma, and the proposed LSM for intravenous topotecan has been used to reduce the technical and clinical costs associated with this approach to dosing topotecan.

Several reports [13, 14, 25] of LSM for topotecan in adults have been published. Whereas Montazeri and colleagues used total topotecan concentrations, our report used topotecan lactone concentrations. Their sampling strategy included sampling 5 min before the end of a 30 min infusion and at 4 h after the end of the infusion. While drawing a sample during the infusion may add to patient convenience since the patient must be in the clinic for the infusion, this is impractical particularly for children. For children who do not have double lumen catheters, the topotecan infusion must be stopped for blood collection or the child must be subjected to an extra peripheral needle stick. Stopping the infusion may confound pharmacokinetic modeling and interpretation of results and thus the clinical decision about potential changes to the topotecan dosage. In addition, drawing the topotecan sample from the same line as the infusion may result in falsely elevated concentrations due to the potential for contamination of plasma with residual drug in the line. Moreover, our approach using D-optimality permits selection of the most informative time points within a given sampling window regardless of inclusion of a particular time point in the training set; whereas, the Montazeri limited sampling development methodology constrains sampling times to those used in the training sets.

Using a stepwise regression approach and data from nineteen patients, van Warmerdam et al. [25] proposed LSM for topotecan. They considered LSM with one, two, or three plasma samples. For the three sample model their sampling times were 0.25, 1.5, and 2.5 after the 30-min infusion, which is very similar to the times recommended in the present study. Furthermore, the bias and accuracy results for that model were similar to what we report, with a bias of −2% and accuracy ~15%. Minami et al. [13] proposed a LSM using two plasma samples at 15 min and 6 h after the end of the 30 min topotecan infusion. Both the van Warmerdam and Minami models were developed using linear regression and constrained LSM time points to those used in the training sets. Compared to these LSM in adults, our intravenous LSM shortens the time a patient spends in the clinic for pharmacokinetic studies, has demonstrated validity in a large population of children with cancer, and is robust over a wide range of topotecan clearances.

More recently Leger et al. [11] proposed a LSM for oral topotecan in adults. Their LSM includes two possible models consisting of either two or three sampling times. Because of a lack of concentration-time data before 1 h after oral administration in their LSM training set, and limitations of the LSM development methods, the first proposed sampling time is 1.5 h after oral administration, and the last sampling time in both models is 6 h after administration. Postponing sampling until 1.5 h after oral administration may potentially obscure interindividual variation in topotecan absorption. The oral LSM proposed in the current study contains a sample at 0.25 h, which will allow for observation of interindividual differences in absorption. In addition, our validated LSM will minimize the time that patients and families must spend in the clinic for collection of samples for pharmacokinetic studies.

We have demonstrated the robustness of the proposed intravenous LSM by validating the model in two populations independent of the population from which the model was developed. Despite variation in clearance between development and validation populations, our proposed LSM for children receiving intravenous topotecan is accurate and unbiased. The intravenous LSM has been validated across a diverse patient population. In addition, we have developed an LSM for oral topotecan that has been validated in an independent population with a topotecan clearance that is different from that used to develop the model. Further validation of this model will be pursued in future studies. Our proposed LSM will give accurate and unbiased pharmacokinetic results and will decrease cost of performing pharmacokinetic studies.

Acknowledgements The authors would like to thank Lisa Walters, Terri Kuehner, Paula Condy, Margaret Edwards, and Sheri Ring for assistance in obtaining plasma samples, Geeta Nair, Feng Bai, Suzan Hanna, Mark Leggas, and Brad Johnston for their invaluable technical support in the laboratory, and Nancy Kornegay for her support with data management.

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