Abstract

Purpose: The study aims to use mathematical modeling and simulation to assess the relative contribution of topotecan systemic exposure and scheduling in the activity and myelosuppression of topotecan in pediatric patients with neuroblastoma.

Experimental Design: Pharmacokinetic and pharmacodynamic data were obtained from a phase II study for pediatric patients with high-risk neuroblastoma. The topotecan dosage was individualized to attain a topotecan lactone area under the plasma concentration-time curve between 80 and 120 ng/mL h and given over a protracted schedule (i.e., 10 days). Four mathematical models describing topotecan pharmacokinetics, tumor growth, and neutrophil and platelet dynamics were developed. The models were combined to simulate and compare different topotecan treatment strategies with respect to systemic exposure and schedule.

Results: The median change in tumor volume was significantly different between schedules (5% increase for D/\(\times\)5 versus 60% decrease for D/\(\times\)5/\(\times\)2; \(P < 0.0001\)) when administering the same total systemic exposure. Whereas protracted schedules showed increased neutropenia (median of 7 versus 12 days below an absolute neutrophil count of 500/\(\mu\)L; \(P < 0.0001\)) and thrombocytopenia (median of 3 versus 10 days below a platelet count of 20,000/\(\mu\)L; \(P < 0.00001\)), simulations showed that delays in topotecan therapy would not be required. Simulations showed that an increase in topotecan exposure on the D/\(\times\)5 schedule by 2.4-fold resulted in a modest decrease in tumor volume (i.e., median percentage change tumor volume of 24% versus 3%).

Conclusions: The present mathematical model gave an innovative approach to determining relevant topotecan schedules for possible evaluation in the clinic, which could lead to improved tumor response with minimized toxicities.

Topotecan, a water soluble semisynthetic analogue of camptothecin, has been active against several pediatric cancers, including neuroblastoma, medulloblastoma, rhabdomyosarcoma, and acute leukemia (1 – 6). Topotecan acts as a specific inhibitor of topoisomerase I, which results in DNA damage during cell replication and transcription, and ultimately leads to apoptosis.

On the basis of its cell cycle-specific mechanism of action and previous preclinical data, the antitumor activity of topotecan has been considered highly schedule-dependent (7, 8). Using a panel of human colon adenocarcinomas and rhabdomyosarcoma xenografts, Houghton and colleagues showed that protracted topotecan therapy given as a daily dose for 5 days for three consecutive weeks resulted in greater antitumor efficacy than when topotecan was given on a more intense schedule (every 4 days for four doses; ref. 9). Therefore, the authors showed a relationship between the duration of drug administration and the tumor response in these xenograft models. Moreover, results of these preclinical studies showed that increasing dose intensity beyond some threshold offered no advantage for the antitumor effect but rather led to increased toxicity (10).

Based on the preclinical data, it is expected that protracted and intermittent topotecan exposure would optimize its antitumor effect in the clinical setting. Initial clinical trials with topotecan in pediatric patients used 24 h and 72 h of continuous infusions with minimal antitumor efficacy (11, 12). However, when topotecan was used as a short infusion (30 min) daily for 5 days every 21 days, promising antitumor activity was noted, particularly in patients with relapsed neuroblastoma (13, 14). Using single-agent topotecan on this schedule in children with untreated disseminated neuroblastoma, Kretschmar and colleagues showed a 67% response rate (i.e., response rate defined as complete, partial, and mixed responses) after only...
two courses of therapy (15). Furman and colleagues found that more clinically significant responses were observed in patients with refractory leukemia who received topotecan during 12 consecutive days every 3 weeks, regardless of dosage, compared with children who received shorter regimens (16). Altogether, these studies suggested that protracted topotecan systemic exposure was associated with greater antitumor responses in the treatment of pediatric cancers than higher short-term systemic exposures.

Protracted topotecan administration has also been associated with normal tissue toxicity, such as myelosuppression and diarrhea. A narrow therapeutic window exists between the topotecan systemic exposure associated with antitumor effect and that associated with toxicity. Thus, it is advantageous to determine the topotecan schedule and systemic exposure that will maximize tumor response yet allow for manageable toxicity.

Mathematical models provide useful guidance to develop chemotherapeutic schedules to maximize efficacy and effectively manage toxicities. Many mathematical modeling approaches have been proposed to describe the kinetics of tumor growth and the effects of anticancer drugs on that growth (17). We have reported a mathematical model that described the effects of a cell cycle–specific drug on cancer tissues (18). Moreover, considering the importance of ensuring a desired rate of tumor cell kill while minimizing toxicity, we have developed a model to describe the pharmacodynamics of neutrophils after metozolamide administration (19). This model could be particularly useful to describe the cytotoxic effects of topotecan on tumor tissues, as well as hematopoietic tissues (e.g. neutrophils and platelets), because these effects are dependent on both the duration of drug administration and topotecan lactone systemic exposure.

Therefore, the objective of the current study was to use pharmacokinetic and pharmacodynamic modeling to investigate the contribution of topotecan lactone systemic exposure and schedule on antitumor effect and myelosuppression (i.e., neutropenia and thrombocytopenia) and to use simulations to compare various topotecan systemic exposures and schedules. This modeling and simulation approach provided rapid evaluation of different therapeutic regimens and enabled the identification of those with the highest likelihood for further preclinical or clinical testing.

Patients and Methods

Patients and treatments. Pharmacokinetic and pharmacodynamic data were obtained from an institutional phase II study (SINB97) for children with previously untreated high-risk neuroblastoma (20). This study used two cycles of single-agent topotecan as window therapy before induction and intensification phases. The median age at enrollment was 3.1 years (range, <1 month to 16.9 years) and 40% of patients (12 of 30) had MYCN amplified tumors. The median topotecan dosage in the cycles, in which the target topotecan area under the plasma concentration-time curve (AUC) was achieved, was 2.7 mg/m^2/day (range, 0.95–3.8 mg/m^2). The St. Jude Institutional Review Board approved the protocol and written informed consent, or permission was obtained from all patients, parents, or guardians, as appropriate.

Topotecan was given as an i.v. infusion over 30 min daily for five consecutive days over two consecutive weeks. Using pharmacokinetically guided dosing, the topotecan dosage was individualized to attain a topotecan lactone systemic exposure (depicted as AUC) of 80 to 120 ng/mL h. Briefly, the initial topotecan dosage on day 1 of therapy was 3 mg/m^2/day, and the dosage on subsequent days was adjusted linearly based upon the patient’s topotecan lactone clearance value to achieve the target topotecan systemic exposure on the following day of treatment. Pharmacokinetic studies were repeated each day until the patient’s AUC was within the target range. The second cycle of topotecan was given at a median (range) of 25 (21-33) days after the start of the first cycle. The dosage given on day 1 of the second cycle was that determined to attain the target AUC during the first course of therapy. Decisions about dosing were made as described for the first treatment cycle.

Filgrastim [granulocyte colony-stimulating factor (G-CSF)] was given s.c. at 5 µg/kg/day beginning 24 h after the last topotecan dose of each cycle for a minimum of 10 days or until the absolute neutrophil count (ANC) exceeded 500/µL in two consecutive measurements after the expected nadir. Topotecan was not continued if disease progression or nonhematologic grade 4 toxicity (excluding diarrhea lasting <72 h) occurred in patients during the initial topotecan therapy. Prophylactic antiemetic agents were given to all patients at the treating physician’s discretion. All patients were started on trimethoprim-sulfamethoxazole for Pneumocystis carinii prophylaxis at a dosage of 150/750 mg/m^2 per day of trimethoprim-sulfamethoxazole in two divided oral doses on Monday, Tuesday, and Wednesday of each week. Patients did not receive trimethoprim-sulfamethoxazole during the 2 weeks that encompassed the topotecan therapy days (days 1-12). Most patients reinitated their trimethoprim-sulfamethoxazole therapy on the Monday or Tuesday after a cycle of topotecan therapy (i.e., on day 15 for most patients). Platelet transfusions for topotecan-induced thrombocytopenia were given when the patient’s platelet count was <20,000/µL.

All patients were examined at least weekly and complete blood counts were determined twice weekly or more during topotecan therapy. The ANC was calculated from measurements of the total number of white cells and the percentage of neutrophils and bands.

Pharmacokinetic data and sample analysis. Plasma samples were collected for pharmacokinetic analysis after days 1 and 8 during both cycles of topotecan and when pharmacokinetically guided topotecan dosing was required. Blood samples were collected from a site contralateral to topotecan infusion before and at 0.25, 1, and 6 h after completion of the infusion. Heparinized tubes were used to collect 3 mL of whole blood at each of these time points. Blood was centrifuged in a microfuge for 2 min at 7,000 × g for 2 min at 7,000 × g. plasma was separated, and 200 µL of plasma was added to 800 µL of cold (-30°C) methanol. This mixture was vortex mixed for 10 s and centrifuged for 2 min at 7,000 × g, the supernatant was decanted into a screw-top tube, and topotecan lactone plasma concentrations were determined by high performance liquid chromatography with fluorescence detection as previously described (20–22).

Pharmacodynamic data. Tumor status was determined before starting topotecan by physical examination and diagnostic imaging, which were part of the diagnostic workup. Patients underwent diagnostic imaging of the primary and metastatic sites after completing two cycles of chemotherapy. The absolute primary tumor volume was obtained by using the following formula to calculate the ellipsoid volume on the basis of the length, width, and depth of the tumor: 

\[ V = \left(4/3\pi\right) \times \left(0.5 \times \text{length}\right) \times \left(0.5 \times \text{width}\right) \times \left(0.5 \times \text{depth}\right) = 0.52 \times \text{length} \times \text{width} \times \text{depth}. \]

Additionally, complete blood counts, which include ANC and platelet count, were also measured at diagnosis and approximately every other day during topotecan therapy.

The models. Four mathematical models describing the topotecan plasma pharmacokinetics, tumor growth, neutrophil dynamics, and platelet dynamics were defined. These models were then used in combination to simulate and compare treatment strategies in terms of the topotecan systemic exposure and schedule.

Topotecan plasma pharmacokinetic model. A two-compartment pharmacokinetic model was used to describe the topotecan plasma data (20, 21). Model parameters included clearance (CL, L/h/m^2), volume of the central compartment (\( V_C \), L), and intercompartmental parameters (\( k_{12} \) and \( k_{21} \), L/h).

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**Tumor growth model.** The growth kinetics of the tumor and antitumor effects of topotecan were depicted in Fig. 1A and mathematically described by the following model previously reported (18).

\[
\frac{dP}{dt} = (\gamma - \alpha - \delta - f(t))P + \beta Q \\
\frac{dQ}{dt} = 2P - \beta Q
\]

\(P\) and \(Q\) represent the proliferating and quiescent cells in the tumor mass, respectively, \(\gamma\) was the proliferation rate of cycling or proliferating cells, \(\delta\) was the natural cell decay rate, and \(\alpha\) and \(\beta\) described the transition rates between the proliferating and quiescent compartments. During chemotherapy, it was assumed that the kinetics of tumor growth were perturbed by the action of topotecan via the function \(f(t)\) only in the proliferating compartment due to the cell cycle-specific chemotherapy. Specifically, we considered constant cell kill described by the equation \(f(t) = DC_{\text{plasma}}\), wherein \(D\) was the drug effect parameter and \(C_{\text{plasma}}\) was the plasma topotecan lactone concentration (from the topotecan plasma pharmacokinetic model).

**Neutrophil time course model.** To quantitate the serial ANC data after topotecan therapy along with the effects of G-CSF support, we developed a mechanistic mathematical model based on our previous reports (19, 22). This model accounted for the production of neutrophils in the bone marrow (from stem cell production and differentiation to release into circulation), pharmacodynamic effects of topotecan on neutrophils in the bone marrow, effects of endogenous G-CSF, and effects of exogenous G-CSF support. The model was shown schematically in Fig. 1B and described by the following system of ordinary differential equations.

\[
\frac{dN_p}{dt} = \left( k_{in}(N_{circ}) \frac{IC_{50}}{IC_{50} + C_{\text{plasma}}} - k_{out} \right) N_p \\
\frac{dN_{d1}}{dt} = k_{bp}(N_P - N_{d1}) \frac{dN_{d2}}{dt} = k_{bp}(N_{d1} - N_{d2}), \frac{dN_{d3}}{dt} = k_{bp}(N_{d2} - N_{d3}) \\
\frac{dN_{circ}}{dt} = k_{bp}N_{d3} - k_{out}N_{circ}
\]

The parameters were defined as follows: \(C_{\text{plasma}}\), topotecan plasma concentration; \(N_{p}\) concentration of proliferating cells including stem cells, colony-forming units, myeloblasts, promyeloblasts, and myelocytes; \(N_{d1-d4}\), concentration of nonproliferating blasts at increasing stages of differentiation, including metamyelocytes, bands, and segmented neutrophils; \(N_{circ}\), concentration of circulating neutrophils. The model parameters are defined as follows: \(k_{in}(N_{circ})\), production rate of stem cells that is defined as follows.

\[k_{in}(N_{circ}) = k_a \frac{K_m}{K_m + N_{circ}}\]

This equation described the inverse relationship between circulating neutrophils (\(N_{circ}\)) and stem cell production and took into account the endogenous effects of circulating neutrophils on G-CSF (i.e., lower neutrophil counts stimulate G-CSF, which in turn stimulate the production of stem cells). \(k_a\) was the half saturation parameter, which was fixed so that the steady-state solution to the system without treatment was equal to the baseline ANC level. The transition rate of blasts between the various phases of differentiation was modeled as \(k_{bp}\). Lastly, \(k_{out}\) was the elimination rate of the circulating neutrophils and \(IC_{50}\) was the topotecan concentration in the bone marrow that causes a 50% decrease in stem cell production (e.g., \(k_a\)). The transition time from the initial production of blasts in the bone marrow to their release into circulation was defined as \(4/k_{bp}\). Finally, the effects of exogenous G-CSF support were modeled by an increase in the stem cell production rate, \(k_{icr}\), and the differentiation rate, \(k_{bp}\) (23, 24). For parsimony, this change was made discrete. In particular, we defined \(k_{icrGCSF}\) as the change in the stem cell production rate, \(k_{icr}\), in the presence of G-CSF and likewise \(k_{bpGCSF}\) as the change in the differentiation rate, \(k_{bp}\), in the presence of G-CSF.

**Platelet time course model.** The model describing the platelet dynamics was identical to that of the neutrophils. In the case of platelets, the various compartments represented the stages of differentiation of the precursors of this type of cells, and endogenous G-CSF was replaced by the platelet growth factor thrombopoietin (i.e., \(k_{icr}(N_{circ})\)). Finally, platelet transfusions were accounted for with an instantaneous increase in the circulating platelets.

**Parameter estimation.** Topotecan plasma pharmacokinetic parameters were estimated in each individual using the Bayesian estimation method (maximum \textit{a priori} estimation) via ADAPT II (25) due to the limited sampling nature of the data. These parameters were then fixed for each individual when estimating the pharmacodynamic parameters. Given that data was only available on changes in tumor volume from the start of topotecan treatment to the end of the second course, tumor growth rates were not explicitly available. Therefore, we considered

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**Fig. 1.** A, schematic of the tumor efficacy model. Model parameters: \(P\), proliferating cells; \(Q\), quiescent cells. Model parameters \(\gamma\), proliferation rate of cycling cells; \(\delta\), natural cell decay rate; \(\alpha\) and \(\beta\), transition rates between the proliferating and quiescent compartments; and \(f(t) = DC_{\text{plasma}}\), drug effect function wherein \(D\) was the drug effect parameter and \(C_{\text{plasma}}\) was the plasma topotecan lactone concentration (from the topotecan plasma pharmacokinetic model). B, schematic of topotecan pharmacokinetics, a first order two-compartment model (top) and pharmacodynamic effect on the neutrophil production in the bone marrow (bottom). Pharmacodynamic model parameters: \(k_{in}\), stem cell production rate; \(k_{bp}\), transition rate of blasts between various phases of differentiation; and \(k_{out}\), elimination rate of circulating neutrophils. Dashed line, indicates the negative feedback effects of endogenous G-CSF on neutrophil production.
doubling times from human neuroblastoma xenografts, with a median (range) of 13 days (5-18 days) along with values twice and thrice this maximum value (8). The parameters \( \alpha \) and \( \beta \) were fixed so that there was a fixed ratio between the proliferation and quiescent compartment. Specifically, several studies in neuroblastoma cell lines have shown (using the labeling index method) that the percentage of the proliferating compartment can range from 2% to 26% with a median of 12% (26, 27). Therefore, we considered two different values (12% and 20%) for the percentage of proliferating cells. Finally, the efficacy function \( f(t) \) was estimated for each individual by using their change in tumor volume from pretreatment to the end of the second topotecan course (20).

The parameters describing the ANC and platelet dynamics were estimated in each individual using maximum likelihood estimation methods implemented in Matlab (Version 7.2, The Math Works, Inc.). As noted above, the topotecan plasma pharmacokinetic parameters for each individual were fixed when these parameters were being estimated.

**Simulations.** Simulations of various topotecan systemic exposure values and schedules were done with each individual’s set of model parameters by varying either the topotecan systemic exposure or treatment schedule to observe how these changes affected tumor efficacy and myelosuppression (e.g., neutropenia and thrombocytopenia). In our simulations, we included G-CSF dosing for 8 days after the last topotecan infusion, which is consistent with current clinical practice in our institution. From the results of our simulation for each course, we estimated the number of days the ANC was below 500/µL. The descriptive statistics of median and quartiles were used to describe the changes, given a fixed schedule, due to varying the topotecan systemic exposure or treatment schedule to observe how these changes affected tumor efficacy and myelosuppression (e.g., neutropenia and thrombocytopenia). In our simulations, we included G-CSF dosing for 8 days after the last topotecan infusion, which is consistent with current clinical practice in our institution. From the results of our simulation for each course, we estimated the number of days the ANC was below 500/µL. In the present analysis, we chose not to include platelet transfusions for situations where the platelet count was below 20,000/µL, although this is a commonly accepted clinical practice. Instead, we estimated the number of days where the ANC was below 20,000/µL, with the understanding that platelet transfusions could be used to transiently increase platelet counts.

The descriptive statistics of median and quartiles were used to describe the results. Specifically, we were interested in (a) comparing the differences in shorter, more intense schedules with longer, more fractionated schedules and (b) comparing the differences, given a fixed schedule, due to varying the topotecan systemic exposure (i.e., topotecan lactone AUC). To accomplish the first goal, we simulated the response to daily doses for five consecutive days of two consecutive weeks (D × 5 - 2) of a topotecan lactone AUC of 100 ng/mL/h/day, daily doses for 10 consecutive days (D × 10) of a topotecan lactone AUC of 100 ng/mL/h/day, and daily doses for four consecutive days, rest 2 days, daily doses for three consecutive days, rest 2 days, daily doses for three consecutive days (D × 4 - 3 × 3) of a topotecan lactone AUC of 100 ng/mL/h/day. Regarding the second aim proposed, we simulated and compared daily exposures for both five consecutive days (D × 5) and 10 days (D × 5 × 2) after a topotecan lactone AUC of 100 ng/mL/h/day. The sensitivity analysis was performed by using the current clinically targeted AUC, 140, 200, and 240 ng/mL/h/day.

**Sensitivity analysis.** Because we could not estimate the parameters that describe tumor growth or percentage of proliferating cells in each individual, we chose to fix these parameters to specified values. In these cases, we did sensitivity analyses to determine how changes in these parameters affected the results. This was done by simulating the results obtained over a physiologically accepted range (13-54 days for tumor doubling time and 12% and 20% for proliferating fraction) and observing the changes that occurred in the outcome with respect to the baseline value.

**Table 1.** Descriptive statistics of the tumor efficacy model parameter subdivided by the percentage proliferating (12% or 20%) and the doubling time (13, 18, 36, or 54 d; n = 25)

<table>
<thead>
<tr>
<th>Doubling time (d)</th>
<th>12%</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.52</td>
<td>0.66</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>204%</td>
<td>193%</td>
</tr>
<tr>
<td>25th percentile</td>
<td>0.099</td>
<td>0.018</td>
</tr>
<tr>
<td>Median</td>
<td>0.52</td>
<td>0.0621</td>
</tr>
<tr>
<td>75th percentile</td>
<td>5.43</td>
<td>0.44</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.091</td>
<td>0.017</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>194%</td>
<td>165%</td>
</tr>
<tr>
<td>25th percentile</td>
<td>0.024</td>
<td>0.009</td>
</tr>
<tr>
<td>Median</td>
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<td>0.017</td>
</tr>
<tr>
<td>75th percentile</td>
<td>0.014</td>
<td>0.006</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.014</td>
<td>0.006</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>163%</td>
<td>484%</td>
</tr>
<tr>
<td>25th percentile</td>
<td>0.008</td>
<td>0.004</td>
</tr>
<tr>
<td>Median</td>
<td>0.014</td>
<td>0.004</td>
</tr>
<tr>
<td>75th percentile</td>
<td>0.031</td>
<td>0.003</td>
</tr>
<tr>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.011</td>
<td>0.005</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>155%</td>
<td>262%</td>
</tr>
<tr>
<td>25th percentile</td>
<td>0.005</td>
<td>0.003</td>
</tr>
<tr>
<td>Median</td>
<td>0.011</td>
<td>0.006</td>
</tr>
<tr>
<td>75th percentile</td>
<td>0.031</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Results**

**Topotecan lactone plasma pharmacokinetics.** The topotecan lactone plasma pharmacokinetics in this study have been previously reported (20).

**Tumor efficacy model.** The tumor efficacy model was used to describe the change in tumor volume from pretreatment to the end of the second topotecan course. Because serial measurements were not obtained before topotecan therapy, the growth rate parameter \( (\gamma) \) could not be determined for each individual. Therefore, we used the doubling time (median, 13 days; range, 5-18 days) previously reported for human neuroblastomas grown as xenografts (8), along with twice and thrice the maximum doubling time of 18 days. Additionally, the percentage of the tumor proliferating was unknown in our patients. Thus, the parameters \( \alpha \) and \( \beta \) were fixed to give a ratio of both 12%:88% and 20%:80% proliferating to quiescent cells in untreated tumor growth. Table 1 shows the descriptive statistics of the tumor efficacy model parameters for our population of 25 pediatric neuroblastoma patients.

**ANC model.** The results of the ANC model fit to the serial ANC data in our population of 25 pediatric neuroblastoma patients were summarized in Table 2, and representative fits are shown in Fig. 2A and B. One test of the validity of the model was to determine if estimated model parameters were within published physiologic and pharmacologic ranges. First, the median (range) transient time (4/\( k_{bp} \)) was 2.5 (1.4-5.4) days for blasts to proceed through the various differentiation stages and to enter the circulation. Whereas this time was shorter than the range of 4 to 10 days in normal bone marrow (24, 28-30), it was reflective of the shortened transition time associated with increased endogenous G-CSF due to decreased ANC as a result of topotecan therapy (31, 32). Second, the median (range) IC\(_{50}\) was 0.54 (0.001-2.4) ng/mL, which was consistent with the in vitro determination of the IC\(_{50}\) of 1.2 ng/mL in human granulocyte-macrophage colony-forming unit cells (33). This was particularly true given that our IC\(_{50}\) was based on lactone topotecan and the IC\(_{50}\) previously reported was based on total topotecan, which was typically 30% to 70% larger than topotecan lactone (34).

The effects of exogenous G-CSF on the stem cell production rate (\( k_{in} \)) and transition rate (\( k_{bp} \)) in the bone marrow were
the transition rate ($k_{IC50}$ for the platelet model was 1.27 (0.85-2.85) ng/mL, which of thrombopoietin in the model. Also, the median (range) normal tissue (5-7 days) but reasonable, given the likely effects model was 2.2 (1.6-2.7) days, which was shorter than in section. The median (range) transition time for the platelet experimentally obtained values given in the previous Table 3 and Fig. 3A and B. In a similar manner as with the population of 25 pediatric neuroblastoma patients are shown with a median dosage of 2.7 mg/m² topotecan D × 5 × 2 with G-CSF (5 mg/kg/day) from days 12 to 20 relative to the start of topotecan (20). Using these parameters, we simulated the effects of other topotecan systemic exposures and schedules in an effort to determine effective treatments that will maximize efficacy and minimize toxicities.

As shown in Fig. 4, given the tumor doubling time of 18 days and an equivalent systemic exposure of 200 ng/mL h/day for the D × 5 and 100 ng/mL h/day for the remaining schedules, the change in the total tumor volume over two courses of therapy was significantly different relative to the schedules ($P < 1e-5$, Kruskal-Wallis ANOVA). The median changes in tumor volume were 19%, -49%, -57%, -64% for D × 5, D × 10, D × 5 × 2, and D × 4 × 3 × 3, respectively, with a proliferating fraction of 12%. When a proliferating fraction of 20% was used, the median changes in tumor volume were 5%, -51%, -60% and -67% for D × 5, D × 10, D × 5 × 2, and D × 4 × 3 × 3, respectively. These results clearly showed that more protracted schedules lead to more antitumor effect with topotecan.

We observed increased neutropenia for the more protracted schedules relative to the D × 5 schedule (median days below ANC of 500/µL: 7, 11, 12, 13 days for D × 5, D × 10, D × 5 × 2, and D × 4 × 3 × 3, respectively; $P < 0.0001$). However, the ANC toxicity was still within a range that would not require a delay in the next course of therapy. Thrombocytopenia was also greater for the more protracted schedules relative to D × 5 (median days below a platelet count of 20,000/µL: 3, 11, 10, 10 for D × 5, D × 10, D × 5 × 2, and D × 4 × 3 × 3, respectively; $P < 1e-5$), although the addition of platelet transfusions would likely ameliorate this toxicity.

The results of our simulations of varying topotecan lactone systemic exposure on a D × 5 or D × 5 × 2 schedule showed that an increased exposure did not result in a clinically significant increased antitumor effect. Specifically, Fig. 5 showed that increasing topotecan exposure 2.4-fold (i.e., from 100 to 240 ng/mL h/day) resulted in only a modest improvement in antitumor response given the D × 5 schedule (e.g., 24% median change in tumor volume with 100 ng/mL h/day versus

<table>
<thead>
<tr>
<th>$k_{in}$ (1/h)</th>
<th>IC$_{50}$ (ng/mL)</th>
<th>$k_{bp}$ (1/h)</th>
<th>$k_{ingcsf}$ (1/h)</th>
<th>$k_{topgcsf}$ (1/h)</th>
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<tbody>
<tr>
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<td>0.009</td>
<td>1.27</td>
<td>0.068</td>
<td>0.082</td>
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<tr>
<td>Coefficient of variation</td>
<td>125%</td>
<td>42%</td>
<td>125%</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>0.0008</td>
<td>0.85</td>
<td>0.082</td>
<td>0.052</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.0013</td>
<td>2.85</td>
<td>0.082</td>
<td>0.052</td>
</tr>
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</table>

Table 2. Descriptive statistics of the ANC model parameters considering topotecan administration and G-CSF (n = 25)

Table 3. Descriptive statistics of the platelet model parameters (n = 25)
with 240 ng/mL h/day for a proliferating fraction of 20% or 33% versus 17% given a proliferating fraction of 12%). These results suggested that increasing topotecan systemic exposure may not be as critical as administering topotecan on a protracted schedule. In fact, results of our simulation of two schedules, D/C2 and D/C2, showed that, even with 20% more topotecan lactone systemic exposure (e.g., 1,200 ng/mL h versus 1,000 ng/mL h) on the D/C2 schedule, the antitumor effect was significantly less than the D/C2 schedule (3% versus -60%, respectively; \( P < 0.00001 \)) given a doubling time of 18 days and a proliferating fraction of 20%.

The sensitivity analysis showed that shorter, more intense schedules were more affected by changes in the proliferating fraction compared with the protracted schedules (Fig. 4). The sensitivity analysis showed that the antitumor effects of topotecan were sensitive to the doubling time of the tumor. Specifically, as observed in Fig. 6, the longer the doubling time the smaller the effect schedule has on the change in tumor volume. These results may indicate the need to determine the proliferating fraction and doubling time for each individual tumor. Thus, in a more aggressive faster growing tumor, attention to schedule of topotecan administration would be more important than if it were a slower growing tumor.

**Discussion**

In the present study, we have presented a mathematical model that simultaneously describes topotecan pharmacokinetic analysis, the temporal variation of topotecan-induced tumor response, and the effects of the chemotherapeutic regimen on the time course of neutropenia and thrombocytopenia based on data obtained in a pediatric clinical trial in children with high-risk neuroblastoma. Using this mathematical model, we explored alternate topotecan schedules and systemic exposures to evaluate the relative contribution of these two factors on the tumor cell kill and the myelosuppressive effects (e.g., neutropenia and thrombocytopenia) in the pediatric patient with neuroblastoma.

In children with high-risk neuroblastoma, we have shown that topotecan was highly active on a protracted schedule when it was dose individualized to obtain a systemic exposure between 80 and 120 ng/mL h/dose (20). A variety of topotecan schedules have been evaluated to treat children with high-risk neuroblastoma, and the results of our simulations were consistent with the results of those clinical trials (15, 20).

Basically, all these results showed increased duration of therapy was important for topotecan efficacy and leads to better response rates in pediatric patients as opposed to fixed BSA-normalized dosages given over shorter schedules. Therefore, when evaluating the antitumor efficacy of topotecan when treating high-risk neuroblastoma, one must consider the extent of topotecan systemic exposure and schedule as clinically relevant factors.

**Fig. 3.** Platelet counts versus time for two representative patients (A and B) from SJNB97. Open symbols, platelet data; line, model fit to the data; arrows, platelet transfusions; rectangular boxes, indicate when TPT was given.

**Fig. 4.** Simulation results with a targeted topotecan lactone exposure of AUC = 100 ng/mL h/d (200 ng/mL h/d for D × 6) and a doubling time of 18 d show the effects of schedule on the change in tumor volume (i.e., a negative percentage represents reduced tumor volume relative to diagnostic sample) over two courses of topotecan. Line, median; boxes, quartiles; whiskers, range of the simulated results for all the patients. Open boxes, proliferating fraction of 12%; shaded boxes, proliferating fraction of 20%.

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One approach we evaluated in the present study was to consider the influence of different topotecan systemic exposures on tumor response and toxicity, primarily myelosuppression (e.g., neutropenia and thrombocytopenia). The simulations showed that an improvement in tumor response was observed when increasing topotecan systemic exposure under a fixed schedule. However, the benefits gained in tumor response by increasing topotecan systemic exposure were less notable than those found when fixing the systemic exposure and considering more protracted schedules. Our results coincide with those previously reported in mice, which indicated no therapeutic advantage to increasing dose intensity beyond some critical point because no further tumor reduction was observed (35).

Considering topotecan cell cycle specificity and the preclinical data obtained using a panel of neuroblastoma xenografts, protracted topotecan therapy resulted in a greater antitumor effect than intermittent administration over a shorter schedule when comparing the same total systemic exposure (8). These observations are supported by the clinical data reported by Furman and colleagues who reported that more clinically significant responses were observed in children with refractory leukemia who received 12 days of topotecan compared with patients that underwent a shorter schedule (16). The simulations discussed in our approach support the previous preclinical and clinical observations that, at the same cumulative topotecan exposure of 1,000 ng/mL·d (i.e., 100 ng/mL·d for 10 days), the more protracted schedule presented the greatest decrease of the tumor volume compared with shorter schedules.

As presented in our results, a certain period of time between topotecan doses was necessary to achieve the greatest tumor cell kill. Presumably, this was to allow resting tumor cells to reenter the cell cycle and become sensitive to topotecan again. Although the myelosuppressive effects were greater with the protracted schedule, in all cases, the toxicities were consistent with those observed during our recently completed clinical trial of pharmacokinetically guided topotecan in children with high-risk neuroblastoma (20).

We also report that the schedule effect on the tumor response was highly dependent on the growth rate of cycling cells. The faster the growth rate the more sensitive the tumor response is with respect to the schedule. The results imply that a well-defined schedule would be relevant, in particular to those patients with a more aggressive tumor growth.

The mathematical models we report accurately described the time course of neutrophils and platelets after topotecan

![Fig. 5. Simulation results of the effects of two hypothetical targeted exposures of topotecan lactone AUC (100 or 240 ng/mL·h/day) on the tumor volume (i.e., a negative percentage represents reduced tumor volume relative to diagnostic sample) given a fixed schedule of D × 5 and D × 5 × 2, a doubling time of 18 d, and a proliferating fraction of 20%. Line, median; boxes, quartiles; whiskers, range of the simulated results for all the patients. A, D × 5 schedule. B, D × 5 × 2 schedule.]

![Fig. 6. Simulation results with a targeted topotecan lactone exposure of AUC = 100 ng/mL·h/d (200 ng/mL·h/d for D × 5). The effect of extremes of tumor doubling times (13 and 54 d) on the change in tumor volume (i.e., a negative percentage represents reduced tumor volume relative to diagnostic sample) over two courses of topotecan. Line, median; boxes, quartiles; whiskers, range of the simulated results for all the patients. The four schedules are as follows: open boxes, D × 5; diagonal shading, D × 10; horizontal shading, D × 5 × 2; vertical shading, D × 4 × 3 × 3. A, 12% proliferating fraction. B, 20% proliferating fraction.]

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administration in our patients. Thus, the present approach may help define more advantageous topotecan schedules in future clinical trials, which would improve the tumor response while controlling the toxicity.

Acknowledgments

We thank Valerie McPherson for data management and Drs. Lisa Iacono and Burgess Freeman for their invaluable assistance in conducting this study.

References

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