ARTICLE

Relationship Between Topotecan Systemic Exposure and Tumor Response in Human Neuroblastoma Xenografts

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Background: **Topotecan is a topoisomerase I inhibitor with activity against xenografts of childhood solid tumors and established clinical activity against neuroblastoma and rhabdomyosarcoma. We have studied the relationship between systemic exposure to and the antitumor activity of topotecan lactone (the active form of the drug) in the xenograft models. Furthermore, we determined whether the responses seen in these models occur at systemic exposure levels that are tolerable in children.** ***Methods:*** **Neuroblastoma xenografts derived from the tumors of six different patients were established subcutaneously in immune-deprived mice. Topotecan was administered by intravenous bolus injection 5 days a week for 2 consecutive weeks, repeated every 21 days for three cycles. The minimum daily doses that induced complete responses (CRs) and partial responses (PRs) were determined. Topotecan lactone pharmacokinetic studies were performed in both tumor-bearing and nontumor-bearing mice.** ***Results:*** **The minimum doses associated with CRs and PRs in four of the six neuroblastoma xenografts were 0.61 and 0.36 mg/kg body weight, respectively. The topotecan lactone single-day systemic exposures associated with these doses were 88 and 52 ng · hr/mL, respectively. There was an approximately sixfold difference in topotecan lactone systemic exposure (290 ng · hr/mL versus 52 ng · hr/mL) associated with achieving CRs in the least-sensitive and most-sensitive tumors, respectively.** ***Conclusions:*** **Neuroblastoma xenografts are highly sensitive to topotecan therapy, and responses in mice are achieved at systemic exposures similar to those that are clinically effective and tolerable in children. These results support the concept of deriving preclinical data relating systemic exposure to antitumor activity in xenograft models. Such data may be valuable in making informed decisions regarding the clinical development of new agents. [J Natl Cancer Inst 1998;90:505–11]**

During the past 20 years, there has been a progressive shift from the use of syngeneic transplantable murine tumors to the use of human tumor xenografts for identification and development of new therapeutic entities. The basis for this emphasis on the use of human tumors is that such heterografts may better represent their respective histiotype in patients. Support for this notion is based on the ability of xenografts to identify agents of known clinical utility against the respective disease in patients. However, there is less compelling data obtained from xenografts of human tumors to assure the value of these models in identifying agents that ultimately prove efficacious in human cancers that the models attempt to simulate. In part, failure to rightly predict the drug response may be due to species differences between mice and humans; mice may be more or less tolerant of an agent. Thus, if the former condition applies, the model may overpredict clinical utility (e.g., the diarylsulfonylureas) *(1);* if the latter condition applies, the models may underestimate clinical utility (e.g., doxorubicin and etoposide). Unfortunately, in most instances where the clinical activity does not support the preclinical expectation, retrospective studies are not generally undertaken to determine why the preclinical data were not accurate. In the work presented, we have undertaken a study with a drug where the preclinical activity has been quite predictive of its clinical utility against two pediatric tumor types, rhabdomyosarcoma and neuroblastoma. The data suggest that for certain classes of antitumor agents, more detailed preclinical studies, of the sort presented, may be valuable in the analysis of activity in clinical trials and valuable in guiding clinical drug development.

We reported the activity of the camptothecin analogue topotecan (9-dimethylaminomethyl-10-hydroxy camptothecin) against a panel of rhabdomyosarcoma xenografts *(2),* and topo-

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tecan is currently being evaluated as a single agent in a phase II study against previously untreated patients with advanced-stage disease. Preliminary results indicate significant activity in this tumor type. Our studies also suggested activity against tumors of neural origin, such as medulloblastoma *(3,4),* and neuroblastoma *(5).* Activity has been demonstrated in patients with neuroblastoma in phase I *(6,7)* and II *(8)* trials. Presented here is the evaluation of topotecan against a panel of neuroblastoma xenografts established from biopsy material at diagnosis or at relapse. Topotecan lactone single-day systemic exposure in the mouse has been related to minimum doses that induce partial and complete responses when intravenous topotecan was administered using a protracted schedule of administration. To evaluate if the presence of tumor would alter topotecan disposition in tumor-bearing mice, topotecan pharmacokinetic studies were performed in nontumor-bearing mice and mice bearing NB-1643 and NB-1691 neuroblastoma xenografts. The results of this study may have specific use in developing topotecan treatment strategies in patients with neuroblastoma as well as a broader application to developmental cancer therapeutics.

Materials and Methods

Immune Deprivation of Mice

Female CBA/CaJ mice (The Jackson Laboratory, Bar Harbor, ME), 4 weeks of age, were immune deprived by thymectomy and whole-body irradiation (1200 cGy). Irradiated mice received marrow harvested from nonirradiated, thymectomized donors (3×10^6 nucleated bone marrow cells) within 6–8 hours of irradiation *(1,9).* Tumor pieces of approximately 3 mm^3 were implanted in the space of the dorsal lateral flanks of the mice to initiate tumor growth. Tumor-bearing mice were randomized into groups of six or seven prior to initiating therapy. Animal care was in accord with institution guidelines.

Tumor Lines

Briefly, all six neuroblastoma tumors were from young patients (1–3 years) with advanced disease. Tumor characteristics, tumor stage, site, and previous treatment received by respective patients are shown in Table 1. With the exception of xenograft NB-EB, each tumor demonstrated amplification of N-MYC (Thompson J, Houghton P: manuscript in preparation). Once established as xenografts, further transplantations were from mouse to mouse, rather than from cell culture to mice. For chemotherapy studies, all tumors were used within six passages of their engraftment in mice. Each tumor grew routinely in more than 95% of recipient mice and all retained human origin as determined by karyotype (Thompson J, Houghton P: manuscript in preparation).

Growth Inhibition Studies

All mice bearing bilateral subcutaneous tumors received the chemotherapeutic agent when tumors were approximately 0.2–1 cm in diameter. The procedures have been reported previously *(1).* Briefly, tumor diameters were measured every 7 days using Vernier callipers interfaced with a Dell microcomputer. Tumor volumes were calculated assuming tumors to be spherical using the formula $[(\pi/6)\times d^3]$, where d is the mean diameter. Tumor volumes were determined for at least 12 weeks after starting treatment.

Table 1. Characteristics of neuroblastoma xenograft derived from *in vitro* cell lines

Tumor line	Stage	Site	Previous patient therapy*
NB-1382.2	C	Retroperitoneum	VCR, VP-16, CTX, CDDP, Carbo
NB-1643	D	Retroperitoneum	None
NB-1691	D	Adrenal	AraC, Dauno, 6-TG, VP-16, 5-AzaC
NB-1771	D	Adrenal	None
NB-EB	D	Adrenal	CTX, DOX, CDDP, VM-26
NB-SD	D	Bone marrow	CTX, DOX, CDDP, VM-26

*Patient therapy prior to establishing cell lines *in vitro.* VCR = vincristine; VP-16 = etoposide; CTX = cyclophosphamide; CDDP = cisplatin; Carbo = carboplatin; AraC = cytarabine; Dauno = daunorubicin; 6TG = 6-thioguanine; 5-AzaC = 5-azacytidine; and VM-26 = teniposide.

Tumor Response

For comparison of different treatment regimens, tumor responses were analyzed for the time (weeks) individual tumors required to reach four times their volume at initiation of therapy and tumor regression. The proportion of tumors that failed to reach four times their volume at the start of treatment was estimated by the Kaplan–Meier method *(10),* where times to reach four-times the original volume were censored in mice that died for any reason. The exact logrank test was used to assess differences in times for tumors to increase fourfold in volume among treatment groups versus the control group *(11).* All P values were two-sided. No adjustments were made for multiple comparisons. For individual tumors, a partial response (PR) was defined as the regression of tumor volume by more than 50% but with measurable tumor at all times. A complete response (CR) was defined as the complete disappearance of measurable tumor mass at some point after initiating therapy. Maintained CR (MCR) was CR without tumor regrowth within the study time frame (12 weeks).

The overall minimum effective dose of topotecan achieving CR and PR for the series of human neuroblastoma xenografts evaluated was defined as the dose that achieved CR or PR in at least two thirds (67%) of the xenograft lines tested, while the minimum effective dose of topotecan achieving CR and PR for individual xenograft lines was defined as the minimum dose of topotecan producing CR and more than 50% regressions in all tumors within the treatment group, respectively.

Formulation and Administration

Topotecan was generously provided by Dr. R. K. Johnson (SmithKline Beecham Pharmaceuticals, Philadelphia, PA). Topotecan powder was dissolved in 0.9% NaCl (0.25 mg/mL) and administered intravenously (0.05 mL/10 g body weight) at dose of 0.36, 0.61, 1.0, or 2.0 mg/kg as a short injection (duration of administration was <1 minute) into lateral tail vein daily for 5 days for 2 consecutive weeks followed by a 9-day rest period, referred to as one cycle of therapy {[(d×5)2]}. Mice received three cycles {[(d×5)2]3} over a period of 8 weeks.

Drug Administration and Sample Collection

Topotecan exists in pH-dependent equilibrium between the active (lactone) and the inactive (carboxylate) forms. The pharmacokinetics of topotecan lactone were evaluated after a single dose of topotecan (0.5 mg/kg [1.7 mg/m^2], 1.25 mg/kg [4.2 mg/m^2], and 2.0 mg/kg [6.7 mg/m^2]) administered by direct injection into a lateral tail vein of immune-deprived nontumor-bearing mice. Since the systemic clearance of topotecan did not change with dose (i.e., linear disposition), we chose to conduct our pharmacokinetic studies in tumor-bearing mice after a single dose of topotecan 1.25 mg/kg (4.2 mg/m^2). Heparinized blood samples (~1 mL) were collected by cardiac puncture (three animals per time point) before drug administration and at 0.25, 1, 2, 4, and 6 hours after drug administration. All blood samples were handled and processed as previously described *(12–14).* An isocratic high-performance liquid chromatography assay with fluorescence detection was used to determine topotecan lactone plasma concentrations *(14).*

Pharmacokinetic Analysis

A two-compartment model using maximum likelihood estimation was fit to topotecan plasma concentration versus time data (ADAPT II) *(15).* Estimated two-compartment model parameters included the volume of the central compartment (V_c), elimination rate constant (k_e), and the rate constants (k_{cp}, k_{pc}) for drug transfer between central and peripheral compartments. Pharmacokinetic parameters calculated from these estimates included systemic clearance, volume of distribution at steady state (Vd_{ss}), and area under the plasma concentration–time curve (AUC) *(16).* The relationship between topotecan lactone disposition and time did not differ significantly among tumor-bearing and nontumor-bearing mice ($P = .72$). Thus, we took advantage of the linearity in topotecan clearance and extrapolated from the 1.25-mg/kg dose the topotecan AUC associated with the minimum dose achieving CRs and PRs. Compartmental pharmacokinetic parameters were determined using three mice per sample time point. The concentration–time profile was modeled by applying linear regression to the log concentration. R squared was used to examine model fitness.

Results

Topotecan Antitumor Response

Each of the neuroblastoma xenografts grew in more than 95% of the recipient mice. Tumors had consistent growth rates in untreated mice. NB-EB and NB-SD tumors demonstrated the most rapid growth rates, with volume doubling times of 4.7 and 7.4 days, respectively. NB-1771 xenografts were slowest growing with volume doubling times of approximately 18 days. At the highest dose evaluated (2 mg/kg per administration), there was a low incidence of drug-induced lethality (<4%), with weight loss being less than 10% for the 8-week duration of therapy. Responses for individual tumors in control and treated groups for representative experiments are shown in Figs. 1 and 2, and data analysis is given in Table 2. At the highest dose level (2 mg/kg per administration), CR was achieved in NB-1691, NB-1382.2, and NB-SD xenografts, and CR was maintained at week 12 (MCR) in two of these (NB-1691 and NB-1382.2) neuroblastoma lines (Fig. 1). Although NB-SD tumors were quite responsive, masses were detectable in several mice at the end of 8-week treatment period. NB-1643, NB-1771, and NB-EB xenografts were more sensitive, and CR was achieved at 1 mg/kg per administration, the highest dose level evaluated (Fig. 2).

Minimum Effective Topotecan Dose Achieving Tumor Response

To determine the minimum effective dose of topotecan that induced CR or PR in at least four (67%) of six tumor lines, the responses of all neuroblastoma xenografts were determined at topotecan doses of 1.0, 0.61, and 0.36 mg/kg administered intravenously daily for 5 days per week for 2 weeks repeated every 21 days {[(d×5)2]3} (Table 3). Of note, in highly sensitive tumors (NB-1382.2, NB-1643, NB-1771, and NB-EB), the rate at which tumors regressed remained relatively constant between groups treated with decreasing doses of topotecan; this may indicate that cell kill per dose did not increase markedly with increasing dose levels. This would be consistent with cell cycle-phase-specific activity (i.e., S-phase_killing) of topoisomerase I inhibitors. For NB-1643, NB-EB, and NB-1382.2 tumors, CRs were obtained at 0.36 mg/kg, whereas CRs were obtained at 0.61 mg/kg in NB-1771. At the 0.36-mg/kg dose, CRs were maintained at week 12 in each tumor except NB-EB. In addition, the 0.36-mg/kg dose induced PR of all NB-1691 xenografts but caused only stable disease in NB-SD tumors. Thus, the overall minimum effective dose of topotecan achieving CR and PR for the neuroblastoma xenograft series evaluated was 0.61 and 0.36 mg/kg, respectively. Furthermore, there was an approximately sixfold difference in dose intensity required to induce CR in NB-1643 (0.36 mg/kg) and NB-1691 (2 mg/kg) tumors, whereas NB-SD tumors were relatively more resistant.

Topotecan Pharmacokinetics

Topotecan disposition was evaluated at 0.5, 1.25, and 2.0 mg/kg in nontumor-bearing mice and at 1.25 mg/kg in mice bearing NB-1643 and NB-1691 xenografts. Topotecan lactone plasma concentration–time plots after a dose of 1.25 mg/kg in nontumor bearing mice and mice bearing neuroblastoma xeno-

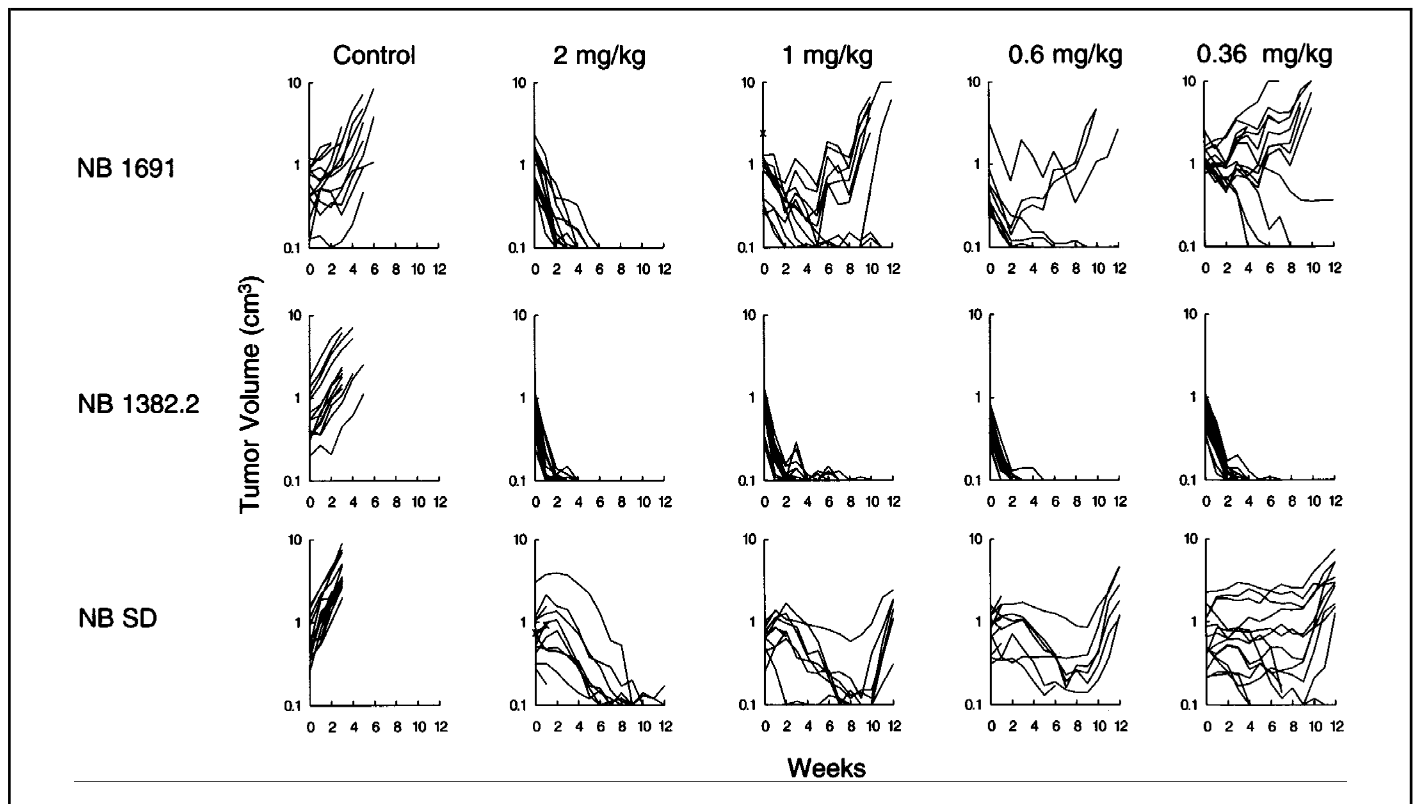

Fig. 1. Responses of neuroblastoma xenografts NB-1691, NB-1382.2, and NB-SD to topotecan treatment. Tumor-bearing mice were treated with saline (control) or topotecan at the indicated dose levels given intravenously, administered daily for 5 days per week for 2 consecutive weeks [(d×5)2] repeated every 21 days for three cycles as described in the ''Materials and Methods'' section.

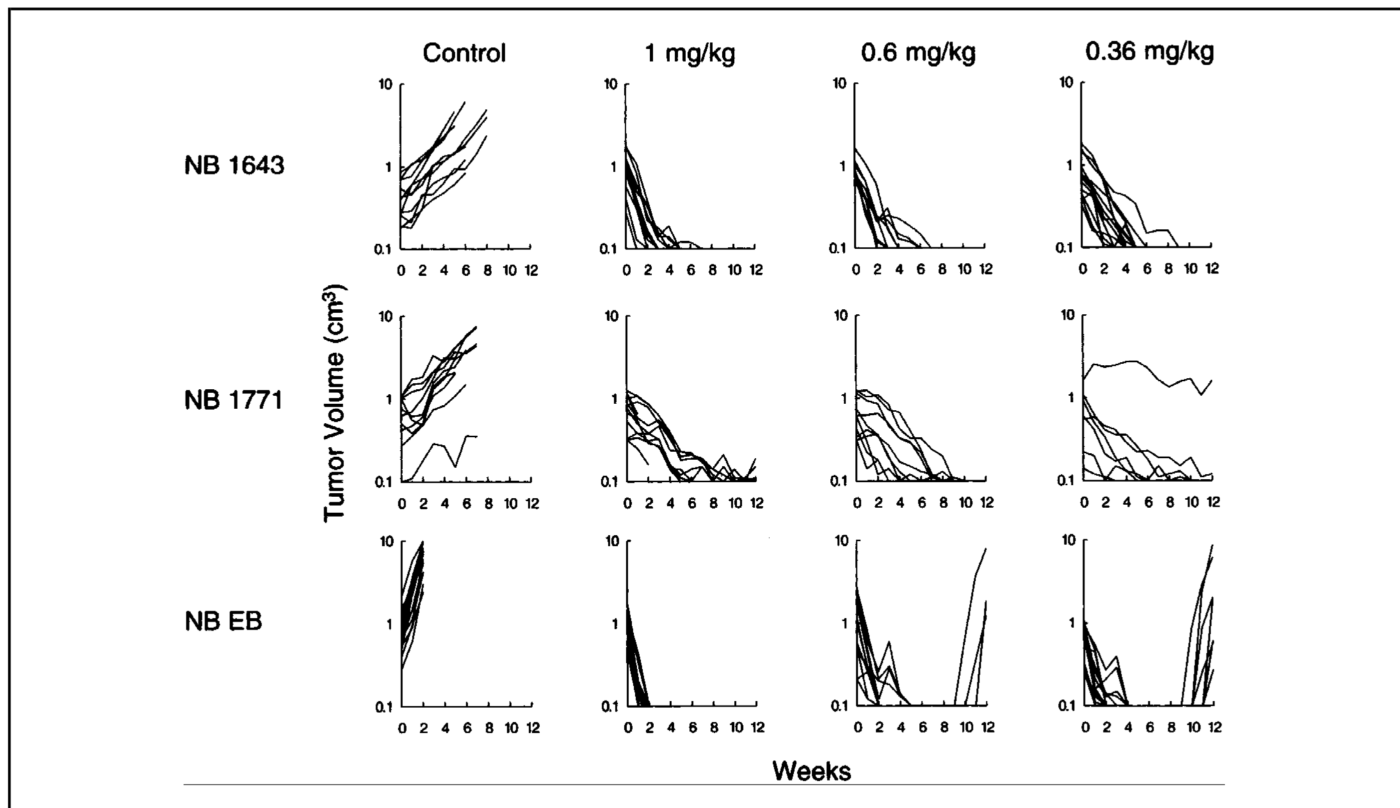

Fig. 2. Responses of neuroblastoma xenografts NB-1643, NB-1771, and NB-EB to topotecan treatment. Tumor-bearing mice were treated with saline (control) or topotecan at the indicated dose levels given intravenously, administered daily for 5 days per week for 2 consecutive weeks [(d×5)2])2] repeated every 21 days for three cycles as described in the ''Materials and Methods'' section.

grafts are shown in Fig. 3. No statistically significant difference between topotecan disposition was noted between the tumor-bearing and nontumor-bearing animals at the 1.25-mg/kg dose ($P = .72$). Moreover, topotecan systemic clearance and half-life was constant over the dosage range examined in nontumor-bearing mice, indicating linear disposition ($P = .62$; Table 4).

Topotecan Systemic Exposures Associated With Tumor Response

Topotecan AUC associated with the minimum dose achieving CRs and PRs were extrapolated from the AUC estimated after a dose of 1.25 mg/kg in mice bearing NB-1691 and NB-1643 neuroblastoma xenografts. Topotecan pharmacokinetics are linear (C_{max} $r^2 = .99$; AUC $r^2 = .99$) over the dose range examined (Table 4); therefore, we extrapolated from the data obtained at the 1.25-mg/kg dose level. From this extrapolation, the topotecan AUC estimated to be associated with the overall minimum effective dose of topotecan achieving CR (0.61 mg/kg) and PR (0.36 mg/kg) was 88 and 52 ng · hr/mL, respectively. To determine whether the intrinsic resistance of NB-1691 xenografts could be in part due to altered systemic disposition in mice bearing this xenograft, we determined the systemic exposure in mice bearing a sensitive tumor (NB-1643) compared with mice bearing a resistant tumor (NB-1691). The topotecan lactone AUC associated with the minimum effective dose of topotecan achieving CR in NB-1691 (2 mg/kg) and NB-1643 (0.36 mg/kg) xenografts were 290 and 52 ng · hr/mL, respectively. The similar systemic disposition in mice bearing these tumors suggests that intrinsic resistance of NB-1691 xenografts is not a consequence of altered systemic disposition of topotecan.

Discussion

We have previously reported the activity of topotecan against xenografts derived from several pediatric tumors *(2–4)*. Notably, topotecan was active against rhabdomyosarcoma xenografts and some neural tumors, and preliminary results indicated activity against a limited number of childhood neuroblastoma *(3–5)*. Subsequently, responses in patients with rhabdomyosarcoma and also neuroblastoma were observed in both phase I and II trials *(6–8)*. However, it is of importance to validate preclinical models where responses achieved following drug systemic exposures are achievable in patients. We therefore re-evaluated topotecan in an expanded panel of neuroblastoma xenografts to determine how accurate the response data are at systemic exposures achievable in patients. Our objectives were therefore to determine if objective responses in these models occurred at clinically achievable systemic exposures (and thus whether the models accurately paralleled the sensitivity of this histiotype) and to further use these models to gain insight into optimal scheduling that could guide future clinical trials design.

Although, the efficacy of topotecan against human tumor xenografts has been reported, this is the first study reporting topotecan systemic exposures associated with the minimum doses achieving objective responses in the xenograft model *(2–4)*. The clinical importance of these data are underscored by the use of preclinical xenograft models in identifying new chemo-

Table 2. Responses of neuroblastoma xenografts to three cycles of topotecan*

Tumor	Dose†	Censored‡	Fraction of mice that achieved 4× initial tumor volume by week 12	Average time (wks) for tumor to achieve 4× initial tumor volume (standard deviation)	*P*§	Response of uncensored mice: Fraction of PRs	Response of uncensored mice: Fraction of CRs	MCR‖
NB-1382.2	0	0	7/7	3.3 (0.5)		0	0	0
	2 [(dx5)2]3 iv	0	0/7	—	.001	0	7/7	7/7
	1.0 [(dx5)2]3 iv	0	0/7	—	.001	0	7/7	7/7
	0.61 [(dx5)2]3 iv	0	0/6	—	.001	0	6/6	6/6
	0.36 [(dx5)2]3 iv	0	0/7	—	.001	0	7/7	7/7
NB-1643	0	0	6/6	5.2 (1.5)		0	0	0
	1.0 [(dx5)2]3 iv	0	0/6	—	.002	0	6/6	6/6
	0.61 [(dx5)2]3 iv	1	0/6	—	.001	0	6/6	6/6
	0.36 [(dx5)2]3 iv	0	0/7	—	.001	0	7/7	7/7
NB-1691	0	1	4/4	5.3 (2.1)		0	0	0
	2 [(dx5)2]3 iv	0	0/5	—	.008	0	5/5	5/5
	1.0 [(dx5)2]3 iv	1	4/6	10.3 (1.3)	.019	1/6	3/6	2/3
	0.61 [(dx5)2]3 iv	1	1/4	9	.024	2/4	2/4	2/2
	0.36 [(dx5)2]3 iv	1	4/6	8.5 (1.7)	.029	2/6	1/6	1/1
NB-1771	0	0	4/4	5.5 (1.3)		0	0	0
	1.0 [(dx5)2]3 iv	2	0/3	—	.024	2/3	1/3	0/1
	0.61 [(dx5)2]3 iv	0	0/5	—	.008	0	5/5	5/5
	0.36 [(dx5)2]3 iv	0	0/4	—	.029	1/4	3/4	3/3
NB-EB	0	0	7/7	1.9 (0.4)		0	0	0
	1.0 [(dx5)2]3 iv	0	0/7	—	.001	0	7/7	7/7
	0.61 [(dx5)2]3 iv	0	1/6	12	.001	0	6/6	4/6
	0.36 [(dx5)2]3 iv	0	2/7	11.5 (0.7)	.001	0	7/7	2/7
NB-SD	0	0	7/7	2.9 (0.4)		0	0	0
	2 [(dx5)2]3 iv	3	0/4	—	.001	0	4/4	3/4
	1.0 [(dx5)2]3 iv	3	1/4	12	.001	1/4	2/4	1/2
	0.61 [(dx5)2]3 iv	4	0/3	—	.001	2/3	0/3	0
	0.36 [(dx5)2]3 iv	1	2/6	12 (0)	.001	2/6	0/6	0

*iv = intravenous administration; CR = complete response; PR = partial response; and MCR = maintained complete response.
†Administered (dx5)2 intravenously (iv) every 21 days for three cycles.
‡Number of censored mice. Censored implies the mouse died prior to week 12 and before the tumor grew to 4× initial volume.
§*P* values were obtained using exact logrank tests. *P* values compare each treatment group with the control group for time to 4× initial tumor volume.
‖Fraction of CRs maintained through week 12.

therapeutic agents for the treatment of childhood cancers. The use of preclinical translational studies in animals are fundamental to the design and interpretation of clinical trials in humans, and direct comparison of drug systemic exposures removes variability in dose–response associated with pharmacokinetic differences *(7,17–20)*. This is especially important for anticancer drugs that may have a steep exposure-response relationship and narrow therapeutic index *(13,17–21)*. Most important, preclinical drug disposition studies allow for more precise comparisons of systemic exposures and interpretation of antitumor response between preclinical models and humans.

Table 3. Topotecan minimum effective dose achieving complete response (MEDCR) and partial response (MEDPR) administered to neuroblastoma xenografts on [(dx5)2]3 schedule*

Tumor	MEDPR	MEDCR
NB1382.2		⩽0.36
NB1643		⩽0.36
NB1691	⩾0.61	⩾2.00
NB1771	⩽0.36	⩽0.61
NBEB	⩽0.36	⩽0.36
NBSD	⩾1.00	⩾2.0

*Administered (dx5)2 intravenously every 21 days for three cycles.

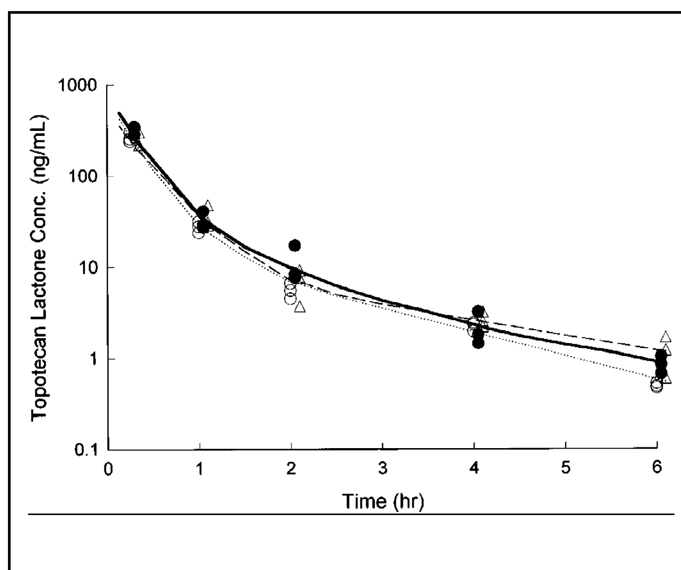

Fig. 3. Topotecan lactone concentration–time profiles after administration of 1.25 mg/kg intravenously in nontumor-bearing mice and mice bearing NB-1691 and NB-1643 human neurblastoma xenografts. Individual data points and best fit line of the data are represented for topotecan lactone in nontumor mice (—●), and mice bearing NB-1691 (—○) and NB-1643 (----△) human neuroblastoma xenografts.

Table 4. Topotecan lactone pharmacokinetic parameters

Pharmacokinetic parameters*	Nontumor-bearing mice 0.5 mg/kg	Nontumor-bearing mice 1.25 mg/kg	Nontumor-bearing mice 2.0 mg/kg	Mice bearing NB-1691, 1.25 mg/kg	Mice bearing NB-1643, 1.25 mg/kg
V_c, L/m^2	5.0	5.7	6.5	6.5	8.5
K_e, h^{-1}	3.5	2.9	2.9	3.2	2.5
$t½_\beta$, h	1.1	1.7	1.4	1.2	1.8
Vd_{ss}, L/m^2	16.5	11.9	10.7	10.5	14.8
CL, L/m^2 per h	17.6	16.5	18.6	20.6	21.1
AUC$_{0-\infty}$, ng · h/mL	81.8	227.0	324.7	181.0	181.6
C_{max}, ng/mL	158.7	498.4	711.4	427.8	358.2

*V_c = volume of the central compartment; k_e = elimination rate constant; $t½_\beta$ = elimination rate of the β phase; Vd_{ss} = volume of distribution at steady state; CL = systemic clearance; AUC = area under the plasma concentration–time curve; and C_{max} = maximum plasma concentration.

To test this approach, we initially determined the minimum effective dose of topotecan achieving objective tumor responses in mice bearing human neuroblastoma xenografts. The rank order of topotecan sensitivity for this panel of tumors, based on the dose necessary to induce CRs and PRs, was NB-1643 = NB-1382.2 > NB-EB > NB-1771 > NB-1691 ≥ NB-SD. Interestingly, although both are highly sensitive to topotecan, NB-1643 was established from the tumor of an untreated patient, whereas NB-1382.2 was established from a heavily treated patient. The topotecan doses defined as the minimum dose inducing CR (0.61 mg/kg) and the minimum dose achieving PR (0.36 mg/kg) represent less than 30% of the maximum tolerated dose in mice. This further suggests, to determine clinically relevant doses and systemic exposures that are associated with tumor response in xenograft models, the dose of chemotherapeutic agents may need to be adjusted in mice due to differential species sensitivity to the toxic effects of these chemotherapeutic agents. For topotecan [and other camptothecin analogs; *(22)*], mice appear to tolerate systemic exposures that are in excess of those tolerated in humans. Thus, to understand optimal scheduling and efficacy of combination therapies combining this class of drug, it is important that the drug dosage achieves systemic exposures that are relevant to patients.

We next determined the systemic exposure of topotecan associated with objective tumor responses in neuroblastoma xenograft models and compared it with systemic exposures tolerated and associated with response in children with neuroblastoma *(7)*. For the series of neuroblastoma xenografts evaluated, the overall topotecan lactone systemic exposures associated with the minimum dose achieving CR (0.61 mg/kg) and PR (0.36 mg/kg) were 88 and 52 ng · hr/mL, respectively. A recent Pediatric Oncology Group study *(7)* in which topotecan was administered daily for 5 consecutive days repeated every 21 days {[(d×5)1]3} reported a relationship between topotecan single-day AUC and tumor response in children with neuroblastoma, with objective tumor responses associated with topotecan lactone AUC greater than or equal to 120 ng · hr/mL. This is consistent with topotecan lactone single-day AUC associated with response in the xenograft model. Although the schedule of administration differs between the clinical trial {[(d×5)1]3} and this preclinical study {[(d×5)2]3}, cumulative topotecan systemic exposures per cycle associated with objective tumor responses are consistent between children with neuroblasotma (~600 ng · hr/mL per 21-day cycle) and mice bearing neuroblastoma xenografts (520 ng · hr/mL per 21-day cycle).

In addition, determining both differences in minimum effective dose and systemic exposures associated with response in xenograft models can be used to compare sensitivity of human neuroblastoma tumor xenografts to topotecan. Interestingly, of the six neuroblastoma models examined, CR could be achieved in four tumor lines at systemic exposures of 88 ng · hr/mL, whereas two tumors were significantly less sensitive, where CR could be achieved at greater than or equal to 290 ng · hr/mL. The fivefold to sixfold difference in minimum dose causing CR between NB-1691 (2.0 mg/kg) and NB-1643 (0.36 mg/kg) tumor xenografts is consistent with topotecan lactone single-day systemic exposures (290 and 52 ng · hr/mL, respectively) associated with response. Thus, relative inherent sensitivities between different tumor histiotypes and within a single tumor histiotype may be compared by dose and systemic exposure intensity required to achieve response. Similar topotecan pharmacokinetic parameters and AUC in non-tumor bearing mice and mice bearing NB-1643 and NB-1691 neuroblastoma xenografts would suggest that these tumors do not alter topotecan disposition.

In summary, we have demonstrated the sensitivity of a panel of neuroblastomas to topotecan. This new agent induced both CRs and PRs of advanced tumors derived from both newly diagnosed or heavily treated patients. Analysis of topotecan lactone systemic exposures associated with antitumor activity in the xenograft model are tolerable and achieved clinical responses in children with neuroblastoma. These data support the concept of determining systemic exposure associated with the minimum effective dose achieving objective responses in mice bearing human tumor xenografts. This information can then be used to make informed decisions in the development of new agents for the treatment of childhood and adult cancers.

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Notes

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