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ORIGINAL ARTICLE

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Phase I study of the combination of topotecan and irinotecan in children with refractory solid tumors

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Abstract *Purpose*: We have shown in xenograft studies that the antitumor activities of topotecan and irinotecan are highly schedule- and dose-dependent, with a high frequency of response at low, protracted dose schedules. Preclinical and clinical data suggest that topotecan and irinotecan have different antitumor activities and mechanisms of resistance, and non-overlapping toxicities, providing a rationale for their combination. Combining both agents may increase the amount of camptothecin delivered to the tumor, without additive toxicity. *Methods*: We conducted a phase I study in children with refractory solid tumors to determine the maximum tolerated dose (MTD) of irinotecan when administered with a targeted systemic exposure (TSE) of topotecan and to define the dose-limiting toxicity (DLT) of this combination. Irinotecan was administered IV over 60 min followed by topotecan over 30 min daily for 5 days for two consecutive weeks. We initially fixed the topotecan-TSE to 80 ± 10 ng*h/ml and investigated the ability to escalate irinotecan (starting dose 16 mg/m^2/d). Topotecan and irinotecan pharmacokinetics were determined. *Results*: Eleven patients (median age 10 years) were enrolled. Owing to DLT, irinotecan was de-escalated to 12 (level -1; $n=3$) and 9 (level -2; $n=3$) mg/m^2/day, and topotecan-TSE was reduced to 60 ± 10 ng*h/ml (level -3; $n=2$). DLTs were neutropenia ($n=8$), typhlitis ($n=5$), and skin rash ($n=1$). MTD could not be reached. Median (range) irinotecan and topotecan lactone systemic clearances were 50.3 (16.6–76.2) l/h/m^2 and 27.6 (14.7–55.9) l/h/m^2, respectively. The pharmacokinetics profile of each agent was similar to that seen in previous single agent studies. One patient with neuroblastoma and one with rhabdomyosarcoma had a partial and a complete response, respectively. *Conclusion*: Despite promising antitumor activity, the combination of topotecan and irinotecan given on a protracted schedule does not warrant further development in children due to unacceptable toxicity.

Keywords Topotecan · Irinotecan · Camptothecin analogues · Topoisomerase-I · Children

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Introduction

The antitumor activity of 20(S)-camptothecin, an alkaloid isolated from the plant *Camptotheca acuminata* has been recognized for over three decades [52], although its marked toxicity precluded its clinical development [14, 33]. The interest in camptothecin has been rekindled in recent years by the identification of the DNA topoisomerase-I as its cellular target [23], and several camptothecin analogs with better toxicity profiles have been developed. Two camptothecin analogs, topotecan and irinotecan, have proven to be among the most effective compounds for treating human cancer.

Xenograft models have been useful for defining the optimal clinical use of camptothecin agents. Studies in mice bearing human solid tumor xenografts have shown that topotecan and irinotecan are among the most active of the anticancer drugs [7, 16, 20, 22, 24, 39, 48, 55, 56]. Moreover, studies in these xenograft models have shown that the antitumor activity of this class of agents is highly dependent on dose and schedule. Responses are better when the agents are given at low doses daily, for protracted periods of time. Among all the schedules investigated, the schedule of daily administration for five consecutive days for two consecutive weeks [(qd $\times$ 5) $\times$ 2] has shown to be the most effective one[22, 48, 55]. In some of these studies, this schedule of prolonged administration induced responses in xenografted tumors that had been unresponsive to intermittent administration of the agents at high doses [22]. These schedules of protracted administration probably produce greater antitumor activity by maximizing the number of covalent topoisomerase I-DNA complexes that are formed.

Topotecan and irinotecan are being investigated in pediatric cancers, and both appear to be very promising. Several phase I and phase II studies of topotecan have been performed in children, and various schedules have been investigated: continuous infusion for 72 h [3, 40] or 120 h, [12] daily for 5 days [29, 35, 38, 50] and daily for 5 days for two consecutive weeks [43]. The best responses have been obtained using the protracted schedules of administration. The antitumor effect of topotecan given at 2 mg/m^2/d for five consecutive days has been evaluated in different phase II studies in children with advanced untreated malignancies. Responses have been obtained in 66% of children with neuroblastoma [29] and in 46% of children with rhabdomyosarcoma [38]. Because a more protracted administration is more effective than a shorter exposure in the xenograft model of neuroblastoma [48, 49,56], our institution has explored the [(qd $\times$ 5) $\times$ 2] schedule in previously untreated patients with metastatic neuroblastoma. By adjusting the individual patient's systemic exposure, responses have been obtained in approximately 70% of children (VM Santana, personal communication). The pharmacokinetic analysis in some of these studies have documented a tight relation between the topotecan systemic exposure and the clinical effect [12, 40, 45, 50]. On the basis of these findings, we have successfully adjusted individual topotecan systemic exposure to reduce interpatient variability in drug exposure, and responses have been observed in 33% of children with refractory solid tumors [43]. Using this schedule, the dose-limiting toxicity (DLT) is neutropenia.

In contrast to topotecan, the use of irinotecan has been less widely studied in children. Three phase I studies, all using fractionated schedules, have been reported [4, 13, 34]. Blaney et al. reported a phase I study of irinotecan in 35 children with refractory solid tumors [4]. Using the daily $\times$ 5 schedule, the DLT in heavily pretreated patients was myelosuppression, and the maximum tolerated dose (MTD) was 39 mg/m^2/d. For less-heavily pretreated patients, the DLT was diarrhea, and the MTD was 50 mg/m^2/d. Responses were observed in two patients, for a total response rate of 6%. In a phase I study in children with refractory solid tumors using a fractionated schedule over three days, Muguishima et al. reported that DLT were diarrhea and myelosuppression, and the MTD was 160–180 mg/m^2 [34]. Using this schedule, responses were obtained in 4 of 28 patients, for a response rate of 14%. On the basis of data derived from the xenograft model, we have explored the feasibility of the protracted schedule of administration [(qd $\times$ 5) $\times$ 2] [13]. The phase I study showed that this protracted schedule is well tolerated. The MTD was 20 mg/m^2/d. In a population of heavily pretreated children with recurrent solid tumors, partial responses were obtained in 22% of the children, and prolonged disease stabilization was observed in 70% of the cases. Of note, three of four patients with recurrent rhabdomyosarcoma had partial responses. DLT was diarrhea and abdominal cramps, and more importantly, myelosuppression was minimal with this schedule.

The data derived from xenograft models and from phase I studies that used the [(qd $\times$ 5) $\times$ 2] schedule have shown that the plasma systemic exposures of either topotecan or irinotecan alone required for optimal antitumoral effects in mice bearing human tumor xenografts are higher than those achievable in children without significant toxicity [13, 48]. Additional studies performed in the xenograft model have shown that lower topotecan systemic exposures are still associated with antitumor effect . However, in these preclinical models, the systemic exposure–antitumor effect curve is very steep, and minimal decreases in the systemic exposure result in a significant decrease of the antitumor effect. Therefore, approaches to enhance the exposure of tumor cells to topoisomerase I inhibitors should be investigated. In this phase I study, we hypothesized that combining these two camptothecin analogues with non-overlapping toxicities would potentially result in greater interaction with topoisomerase-I, thus increasing the antitumor activity with acceptable toxicity. Thus, the objective of this phase I clinical trial was to evalute the safety of the combination of fixed IRN dosages with targeted systemic exposures (TSE) of topotecan. We desired to define the

MTD for IRN and MTSE for topotecan as well as the DLT for the combination.

Patients and methods

Patient eligibility

Patients 21 years old or younger with recurrent solid tumors unresponsive to conventional treatment, or with newly diagnosed solid tumors for which no conventional treatment was available, were eligible for this protocol. Other eligibility criteria included a life expectancy of at least 8 weeks; Eastern Cooperative Oncology Group performance status ≤ 2; recovery from the toxic effects of prior chemotherapy; hemoglobin level greater than 8 g/dl, absolute neutrophil count greater than 1,000/cu.mm, and platelet count greater than 75,000/cu.mm (unless bone marrow was infiltrated by tumor); adequate liver function (bilirubin level $\leq$ three times normal, ALT $\leq$ three times normal); adequate renal function (serum creatinine concentration $\leq$ three times normal for age); and normal metabolic parameters (serum electrolytes, glucose, calcium, and phosphorus); absence of prior craniospinal irradiation, total body irradiation, or irradiation to $>20\%$ of bone marrow volume with >25 Gy. Patients with recurring tumors after stem cell transplant (SCT) were also eligible for the study. Patients were ineligible for study entry if they had an active infection, or if they were pregnant or lactating. The study was approved by the institutional review board, and informed written consent was obtained from patients, parents, or guardians as appropriate, according to institutional guidelines.

Drug administration and study design

Irinotecan (CPT-11 or Camptosar [Pharmacia & Upjohn, Kalamazoo, MI]) was supplied as a sterile, clear aqueous solution, which was diluted with 5% dextrose injection (USP), or 0.9% sodium chloride injection (USP), before intravenous administration (over 60 min). Topotecan (Hycamtin [SmithKline Beecham, Philadelphia, PA]) was reconstituted with 2 ml of sterile water for injection (USP), and further dilutions were made in 50 ml of 5% dextrose in water, and it was administered intravenously over 30 min.

Irininotecan and topotecan were administered as separate intravenous infusions daily for five consecutive days, followed by a 2-day rest, and an additional five consecutive day course [(qd $\times$ 5) $\times$ 2]. Patients received granulocyte-colony stimulating factor (5 mcg/kg), beginning 24 h after the last dose of chemotherapy, daily, subcutaneously, until the absolute neutrophil count was $>1{,}000$/cu.mm after the expected nadir. The study design included an initially fixed systemic exposure of topotecan, with escalation of the irinotecan dosage. The initial systemic exposure of topotecan selected was 80 ± 10 ng*h/ml, which represents approximately 80% of the maximum tolerated exposure in our phase I study of topotecan using the same [(qd $\times$ 5) $\times$ 2] schedule [43]. The initial dose of topotecan was 2.5 mg/m^2/d, and subsequent doses were adjusted to attain the fixed systemic exposure, as previously described [43]. The starting irinotecan dosage selected was 16 mg/m^2/d, which corresponds to approximately 80% of the MTD in our phase I study of irinotecan using the [(qd $\times$ 5) $\times$ 2] schedule [13]. Treatment courses were repeated at 28-day intervals in the absence of DLT, and in the absence of disease progression.

In the absence of DLT, the study called for dose escalation of irinotecan in 20% increments (*level 2*: 20 mg/m^2/d; *level 3*: 24 mg/m^2/d; and *level 4*: 29 mg/m^2/d). In the presence of diarrhea as only DLT, the study was designed to investigate the role of selective gastrointestinal decontamination and called for the addition of oral cefixime, first to ameliorate diarrhea at the same level, and then attempt to escalate. In the presence of other DLT, the study design included dose de-escalation of irinotecan (*level* -1: 12 mg/m^2/d; and *level* -2: 9 mg/m^2/d). Because DLT was encountered at level -2, the protocol was amended for de-escalation of the topotecan systemic exposure (*level* -3: topotecan systemic exposure 60 ± 10 ng*h/ml, irinotecan 9 mg/m^2/d). A minimum of three patients were treated and evaluated at each dose level. If one of the first three patients entered at any given dose level experienced DLT, three additional patients were entered at the same level.

Because diarrhea was the DLT in the phase I study of irinotecan [13], each patient was given antidiarrheal medication (loperamide) and instructed to begin treatment at the first episode of poorly formed or loose stools or at the earliest onset of bowel movements more frequent than normally expected for the patient.

Toxicity was graded and monitored according to the NCI Common Toxicity Criteria version 2.0, with the following modifications: (1) Unacceptable or dose-limiting hematologic toxicity was defined as grade 4 neutropenia or thrombocytopenia lasting >10 days from the completion of chemotherapy administration in two of a cohort of three to six patients; and (2) Non-hematologic DLT was defined as grade 3 or 4 toxicity with the specific exclusion of grade 3 nausea or vomiting, grade 3 diarrhea lasting <72 h, grade 3 or 4 stomatitis lasting <72 h, grade 3 fever, and grade 3 hepatic toxicity resolving prior to the next course of chemotherapy in two of a cohort of three to six patients. The maximum tolerated dose was defined as the dose level just below the dose at which two or more patients developed DLT.

Patient evaluation

Before admission to the study, each patient underwent a complete history and physical examination. Imaging and bone marrow studies were obtained prior to starting treatment and repeated prior to each course as appropriate. Laboratory studies included complete blood cell

counts, urinalysis, and assays of serum blood urea nitrogen, creatinine, uric acid, bilirubin, AST, ALT, lactate dehydrogenase, alkaline phosphatase, glucose, sodium, potassium, chloride, CO_2, magnesium, calcium, albumin, and phosphorus. These studies were performed before treatment, at 3- to 4- week intervals, and at the end of treatment. Blood urea nitrogen, creatinine, AST, and alkaline phosphatase were assayed weekly, and complete blood cell counts were obtained at least twice weekly.

A complete response was defined as complete regression of all apparent tumor masses, including lesions noted on imaging, and clearing of the bone marrow of tumor cells, persisting at least 4 weeks. A partial response was defined as greater than 50% and less than 100% regression of all tumor masses in the absence of any new lesions, or a decrease ≥50% in the bone marrow blast tumor cell count. A mixed response was defined as greater than 50% reduction in the size of one or more masses, with no progression of other lesions. Stable disease was defined as the absence of complete response, partial response, and progressive disease. Progressive disease was defined as greater than 25% increase in the size of all measurable tumor areas, or the appearance of any new lesions. Responses were classified as such only if they were present on two or more evaluations separated by at least 4 weeks.

Pharmacokinetic studies

Plasma samples were collected for topotecan pharmacokinetics before and 0.25, 1, and 6 h after completion of the topotecan infusion on day 1 of each course and at the same times on subsequent days of course 1 as needed to assess the targeted systemic exposure. Likewise, plasma samples were collected for irinotecan pharmacokinetic assessment before, at the end of the irinotecan infusion, and 0.25, 0.5, 1, 2, 4, and 6 h after the end of the irinotecan infusion. The pharmacokinetics of irinotecan and its metabolites SN-38, and SN-38 glucuronide (SN-38G) were evaluated after the first irinotecan dose of the first course and, in a subset of patients, after the last dose of the first course. Topotecan and irinotecan samples were collected and processed immediately to isolate the lactone form of each. At each time point, 3 ml of whole blood were collected from an i.v. site contralateral to the infusion site and placed in a heparinized tube. Plasma was immediately separated from whole blood, and 200 µl of plasma were added to 800 µl of cold (−30°C) methanol. The methanolic mixture was vortex mixed for 10 s and then centrifuged for 2 min at 5,500 × *g*. The supernatant was decanted into a screw top tube and analyzed by HPLC with fluorescence detection. Topotecan was detected with a fluorescence detector with excitation at 370 nm and emission at 530 nm as previously described [45, 57]. Irinotecan and metabolites were detected with a fluorescence detector with excitation at 380 nm and and emission varying from 460 nm to 530 nm as previously described [37].

Pharmacokinetic analysis

A two-compartment model was fit to the topotecan lactone plasma concentrations, using a Bayesian approach via maximum a posteriori probability (MAP) algorithm as implemented in ADAPT II (Biomedical Simulations Resource, Los Angeles, CA, USA) [11]. Estimated model parameters included the volume of the central compartment, elimination rate constant, and the intercompartment rate constants. Calculated pharmacokinetic parameters included the area under the concentration-time curve (AUC) from zero to infinity, systemic clearance, beta half-life, and steady-state volume of distribution.

A multi-compartment model (one-compartment for each component with the exception of SN-38 lactone where two-compartments were used) was simultaneously fit to irinotecan lactone and carboxylate, SN-38 lactone and carboxylate, and SN-38G lactone and carboxylate plasma concentrations using a MAP algorithm as implemented in ADAPT II. Estimated model parameters included the volume of the central compartment for irinotecan lactone, irinotecan lactone elimination rate constant, and the intercompartment rate constants. Calculated pharmacokinetic parameters included the AUC from 0 h to 7 h for each of the components, irinotecan lactone clearance, and apparent clearance of the remaining components.

Results

Patients

Eleven patients were enrolled onto this phase I study between December 1998 and December 1999. The characteristics of the patients are listed in Table 1. Predominant diagnoses were neuroblastoma ($n = 5$), and osteosarcoma ($n = 2$). All but one patient (with a diagnosis of gliomatosis cerebri) had been extensively pretreated, with a median of 2 prior regimens (range, 0–6). Six patients had previously received high-dose chemotherapy and autologous SCT, a median of 24 months (range, 11–67 months) prior to enrollment. Thirteen courses were administered at four different dosage levels. All patients were assessable for toxicity. Three patients could not complete the first course due to toxicity (typhlitis, $n = 3$; myelosuppression, $n = 2$;, and septic shock, $n = 1$), and thus were not considered evaluable fo response.

Toxicity

The combination of irinotecan and topotecan resulted in significant toxicity in this group of 11 heavily pretreated children. Toxicities are listed in Table 2. Because of DLT, de-escalation of the dose of irinotecan (levels −1 and −2), and de-escalation of the systemic

Table 1 Patient characteristics, toxicity and response to therapy

Level	Patient	Diagnosis	ECOG	Number of prior regimens	DLT	Best response	Number of courses
1	7 yo WM	Neuroblastoma	0	2[a]	No	SD	2
1	15 yo WM	Gliomatosis cerebri	0	None	Skin, neutropenia	SD	1
1	12 yo BM	Nasopharyngeal carcinoma	0	1	Mucositis, infection, neutropenia	SD	1
−1	19 yo WM	Germ cell tumor	2	3[a]	Diarrhea, typhlitis, neutropenia	PD	1
−1	8 yo BM	Neuroblastoma	0	3	Neutropenia, typhlitis	SD	1
−1	9 yo WM	Neuroblastoma	0	5[a]	Neutropenia	SD	1
−2	3 yo WF	Neuroblastoma	2	1[a]	No	PR	2
−2	13 yo WF	Osteosarcoma	1	1	Neutropenia	PD	1
−2	10 yo WM	Osteosarcoma	0	2	Typhlitis, infection	SD	1
−3	10 yo WF	Neuroblastoma	0	2[a]	Typhlitis, neutropenia	SD	1
−3	12 yo WF	Rhabdomyosarcoma	0	2[a]	Typhlitis, neutropenia	PR	1

[a]Prior high-dose chemotherapy and SCT

Table 2 Summary of toxicities

Toxicity		Grade			
		1	2	3	4
Hematologic[a]	Neutropenia				11[a]
	Thrombocytopenia			7	4
	Anemia		1	8	1
Gastrointestinal	Anorexia	2	2		
	Nausea	2	2	1	
	Emesis		5	1	
	Abdominal pain		2	4	
	Diarrhea	1	5	5	
	Melena			3	
	Typhlitis			5	
Others	Febrile neutropenia			5	
	Rash		5	1	
	Infection			7	
	Headache	2			
	Stomatitis				1

[a]Duration >10 days in eight patients

exposure of topotecan (level −3) were required. Despite the use of G-CSF, all patients developed grade 4 neutropenia (median duration, 11 days; range, 8–27 days). In 8 patients, neutropenia lasted > 10 days (DLT). Five patients developed typhlitis, which was confirmed by imaging (ultrasound and/or computed tomography); in three of them, the chemotherapy could not be completed due to this gastrointestinal toxicity. No cases of intestinal perforation were encountered. Ten patients developed late-onset diarrhea (grade 1, one patient; grade 2, seven patients; grade 3, four patients), but diarrhea met the definition of DLT in only one patient, and therefore selective gastrointestinal decontamination with oral cefixime was not used. One patient had an episode of septic shock during the neutropenic period, from which he recovered uneventfully. Grade 2 skin toxicity was observed in 5 patients as a mild self-limited erythematous rash, and one patient developed a generalized self-limited pruritic skin rash (grade 3). An additional patient with recurrent nasopharyngeal carcinoma, who had received irradiation to the nasopharynx, developed grade 4 mucositis in the previously irradiated area. All in all, DLTs were myelosuppression in eight patients, typhlitis in five patients, infection in two patients and skin toxicity in one patient. One of the patients with typhlitis had a recurrent germ cell tumor and had previously received radiation therapy to the abdomen (42.6 Gy). Only two patients did not develop any DLT. Because DLT was encountered at all dose levels, the MTD could not be achieved.

Irinotecan pharmacokinetics

Irinotecan pharmacokinetic data were obtained from all 11 patients on day 1, and from seven patients on day 12. Three patients did not receive a full 12-day course of therapy, and one patient refused day 12 pharmacokinetic studies. The day 1 irinotecan pharmacokinetic parameters are summarized by dose level in Table 3. The median (range) irinotecan lactone clearance for all pharmacokinetic studies was 52.7 l/h/m^2 (16.6–77.0 l/h/m^2). In the seven patients with paired day 1 and day 12 pharmacokinetic studies, irinotecan clearance did not differ by study day ($P = 0.15$).

Table 3 Irinotecan pharmacokinetic parameters according to dosage level

Parameter	Median (range)		
	9 mg/m^2/d ($n=6$)	12 mg/m^2/d ($n=3$)	16 mg/m^2/d ($n=3$)
Irinotecan lactone clearance (l/h/m^2)	44.4(27.0–76.2)	56.1(16.6–57.0)	50.3(41.8–55.2)
Irinotecan lactone AUC (ng*h/ml)	136.9(121.7–195.3)	176.3(50.2–204.7)	227.0(91.7–292.3)
SN-38 lactone AUC (ng*h/ml)	24.4(17.0–29.2)	19.7(11.0–34.9)	40.2(34.1–86.8)
SN-38G lactone AUC (ng*h/ml)	41.9(20.9–52.2)	63.6(20.5–147.4)	53.2(46.9–73.0)

Table 4 Summary of the topotecan lactone pharmacokinetic parameters ($n=35$ observations)

Parameters	Mean	Median	Range
V_c (l/m^2)	36.0	34.0	22.5–69.2
K_{el} (h^{-1})	0.81	0.80	0.51–1.10
Beta (h^{-1})	0.23	0.22	0.11–0.44
$t_{1/2beta}$ (h)	3.27	3.08	1.57–6.15
CL (l/h/m^2)	29.1	27.6	14.7–55.9
Vd_{ss} (l/m^2)	99.7	95.9	55.5–157.6

Topotecan pharmacokinetics

Table 4 summarizes the topotecan pharmacokinetic parameters derived from all patients enrolled in the study. Insufficient patients were enrolled at each topotecan systemic exposure (80 vs. 60 ng*h/ml) to make any comparisons between exposure levels. We used our previously published approach to evaluate the success in targeting the desired topotecan systemic exposure. We performed a total of 35 pharmacokinetic studies in 12 children, and 23 AUC determinations were inside the target range while 12 were outside. Of the 12 values that were outside the range, seven were from the fixed initial dosage, and ranged from 75% to 165% of target value. The remaining five values that were outside the target were evenly spread above and below the target with a range of 76%–126%. We observed that of the 23 AUC determinations that were in the target range, four were solely based upon the initial dosage, or were considered a ''dosing success.'' We were very encouraged to note that our overall pharmacokinetic targeting success rate was 83% (i.e., of the 23 topotecan pharmacokinetic determinations, 19 were within target).

Tumor responses

Two patients had documented responses. One patient with metastatic neuroblastoma to the bone marrow had a partial response after the first course of chemotherapy, with more than 50% reduction in the bone marrow disease, but evaluation after the second course of therapy showed progressive disease. One patient with a local recurrence of an alveolar rhabdomyosarcoma of her calf had a complete response. However, because of DLT, no further courses of the combination were given, and treatment was consolidated with radiation therapy. Seven patients (4 neuroblastoma, 1 gliomatosis cerebri, 1 nasopharyngeal carcinoma, and 1 osteosarcoma) had stable disease after one course, although due to DLT, only one patient was eligible to receive a second course. One patient with a recurrent germ-cell tumor had progression of his disease during the first course of therapy.

Discussion

In this phase I study we explored the novel approach of combining two camptothecin analogues (topotecan and irinotecan) with different limiting toxicities, with the anticipation that cumulative ''camptothecin lactone'' exposures might be achieved in excess of those achieved in patients using single agents at their MTDs. A similar approach has been explored by Lokich [30], and by Holcombe et al. [18] in two phase I studies. Lockich administered irinotecan (50–125 mg/m^2) and topotecan (1–1.5 mg/m^2) on a weekly schedule for four consecutive weeks to 21 adult patients with refractory solid tumors. In that study, DLT was neutropenia, and the MTD was irinotecan 125 mg/m^2/week and topotecan 1.5 mg/m^2/week. Responses were observed in three patients [30]. In a phase I/II on patients with metastatic colon cancer, topotecan was administered as continuous infusion for 2 weeks at a dose of 0.2–0.25 mg/m^2/d, and irinotecan was given at a dose of 62 mg/m^2 weekly for three consecutive weeks. Using this schedule, myelosuppression was mild, and DLT was diarrhea. No responses were observed [18].

Compelling evidence derived from preclinical and clinical data support the rationale for this combination. First, we hypothesized that this combination would result in synergistic activity. Since the formation of a covalent cleavable complex is a requisite for cytotoxicity, with increasing formation of complexes, the probability of generating a double DNA strand break would increase. One concern would be that combination of topotecan with SN-38 would saturate potential topoisomerase complex formation. However, this seems unlikely as complex formation in vitro remains linear with drug concentrations 100- to 1,000-fold above the concentrations achieved in patients. Further, cytotoxicity of camptothecin analogues relates not only to the levels of covalent cleavable complexes formed, but also to the location of the complex within DNA (i.e., qualitative differences in cleavage sites). Assays of in vitro cleavage of pKCAK-3–1 plasmid DNA by yeast or human topoisomerase I have shown both quantitative and quali-

tative differences in cleavage patterns in the presence of different camptothecin analogues [28]. Although not dramatic, there appear to be differences in cleavage patterns induced by topotecan and SN-38 [28]. These agents might potentially complement in extending the DNA sites where complexes are formed, providing another theoretical advantage to the combination of topotecan and irinotecan.

Another piece of evidence that would support this combination is the accumulating data from preclinical and clinical studies that show that topotecan and irinotecan have different spectra of antitumor activity in various models of human cancer. For example, when they are administered at the MTD using the optimal schedule in preclinical studies, irinotecan consistently causes complete regressions of colon adenocarcinoma xenografts from adults or from adolescent children. In contrast, these tumors have intrinsic resistance to topotecan [21]. Clinical data support the fact that these agents may have different spectra of activity in that irinotecan has demonstrated activity against colorectal carcinoma in adult trials [10], whereas topotecan does not have significant activity against this disease [8]. Furthermore, the differences in antitumor activities may also reflect different mechanisms of resistance. Resistance to camptothecin analogues can result from reduction of cellular topoisomerase I activity [42], structural mutation of DNA topoisomerase I [28, 53], or altered cellular accumulation of the topoisomerase I inhibitor [17, 25, 31], including active efflux mechanisms [2, 5, 6, 19]. However, there may be specific mechanisms of resistance to each drug, and resistance to one drug may be associated with sensitivity to the other. Xenograft studies have shown that tumors selected in vivo for resistance to topotecan retain sensitivity to irinotecan [21]. These data suggest qualitatively different mechanisms of acquired resistance to topoisomerase I interactive agents in several tumor models. Results of clinical trials in children are inadequate to determine if they will corroborate the results of the xenograft studies. However, in our recent phase I study of irinotecan, four patients who had developed progressive disease with topotecan had objective evidence of response to irinotecan [13].

Based on the toxicity profiles of topotecan and irinotecan when given in the protracted [(qd $\times$ 5) $\times$ 2] schedule, we anticipated that the combination of both agents would result in tolerable toxicity. However, this combination resulted in more hematologic and gastrointestinal toxicity than expected, and as a result, an MTD could not be reached. Of particular concern is the high incidence of neutropenic enterocolitis, even at the lowest doses of the combination, which suggest a synergistic effect on the colonic mucosa. When irinotecan is administered as a single infusion, myelosuppression is usually the DLT [32]. In contrast, protracted schedules of administration result in severe gastrointestinal toxicity [13, 32, 36]. Phase I studies of irinotecan performed in children consistently show gastrointestinal toxicity as the DLT [4, 13], although heavily pretreated patients are at a higher risk of developing myelosuppression [4]. The mechanism of the late-onset diarrhea is unknown. However, there is evidence that suggests that it may be related in part to the excretion of SN-38, which normally undergoes glucuronidation in the liver to form a noncytotoxic derivative. The resulting SN-38 glucuronide, which is excreted in the bile, may be converted back to cytotoxic SN-38 in the gut by bacterial β-glucuronidase, a conversion that would increase damage to the gastrointestinal mucosa [15]. Animal studies have shown that β-glucuronidase activity is highest in the cecal and colonic contents [46]. These results suggest that the late-onset diarrhea may be caused by exposure of the cecal and colonic mucosa to increased concentrations of SN-38 as the result of bacterial deglucuronidation of SN-38 glucuronide.

For intravenous topotecan, phase I and phase II studies have shown that myelosuppression is the DLT regardless of the schedule of administration [3, 35, 40, 43, 50]. However, when topotecan is given as protracted schedule, 10–20% of patients develop diarrhea [43, 50] towards the end of the cycle, suggesting that mucosal damage also takes place. This is not surprising, as gastrointestinal toxicity was common in the early studies of the parental compound 20(S)-camptothecin [14, 33], suggesting that all camptothecin analogues have the potential to induce some degree of colonic damage.

Oral administration of antibiotics, which inhibits the β-glucuronidase activity and reduces the accumulation of SN-38 in the large intestine in an animal model, may prevent the development of irinotecan-induced diarrhea [46, 47]. Clinical studies have shown that it is possible to ameliorate the diarrhea with the concurrent administration of oral antibiotics [27]. The design of this phase I study had included the use of selective gastrointestinal decontamination, should diarrhea be the DLT. However, we were unable to explore this objective since myelosuppression was the main DLT. Thus, the combined administration of topotecan and irinotecan appeared to have a synergistic toxic effect resulting in severe myelosuppression and typhlitis. The degree of toxicity was higher than we had anticipated. Our population was heavily pretreated, and six patients had previously undergone high-dose chemotherapy with autologous stem cell rescue. However, the type and degree of toxicities did not seem to correlate with the intensity of prior treatments. In the phase I study by Lokich, there was a high incidence of hematologic toxicity when irinotecan and topotecan were combined on a weekly schedule, but the incidence of diarrhea was not higher than with the administration of irinotecan alone [30].

We observed no pharmacokinetic interaction between the two agents. To minimize the interpatient variability in topotecan systemic exposure, we individualized the topotecan dosage, and our overall pharmacokinetic targeting success rate was 83% (i.e., of the 23 topotecan pharmacokinetic determinations, 19 were

within target). Topotecan and irinotecan clearance values were both comparable to those observed in children obtained from single agent studies [9, 43]. In addition, neither topotecan nor irinotecan systemic exposures were individually greater than those observed in previous phase I studies in children. Topotecan systemic exposures were targeted at 60–80% of the MTSE of single agent regimens and irinotecan and metabolite systemic exposures were not greater than those observed in children at the single agent MTD of 20 mg/m^2/d on this protracted schedule. Irinotecan clearance values and systemic exposures demonstrated the large interpatient variability that has been previously reported in children [9, 51].

Despite the severe toxicity of the combination of topotecan and irinotecan, responses were observed in one patient with neuroblastoma and one patient with rhabdomyosarcoma, who had a complete response at the lowest dose level. These results confirm the preclinical and clinical studies that have shown that camptothecin analogues are very effective against pediatric malignancies, in particular, rhabdomyosarcoma [13,38] and neuroblastoma [29,56]. The use of topoisomerase I inhibitors in combination with other anticancer agents needs thus to be investigated further. Several phase I and phase II studies of multiagent chemotherapy that include topotecan or irinotecan have been conducted [1,41,44,51,54]. However, as shown in our study, the design of such studies needs to take into consideration that the efficacy and toxicity of these drug combinations depend on the schedule of administration as well as on the choice of drugs; camptothecin analogs may produce an antagonistic rather than a synergistic effect when combined with certain drugs [5,26].

In conclusion, the combination of topotecan and irinotecan in a protracted schedule resulted in more toxicity that would be expected from previous studies of single agent topotecan or irinotecan at higher systemic exposures, and further investigation of this combination is not warranted.

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