

*Featured Article***Phase I Trial of Temozolomide and Protracted Irinotecan in Pediatric Patients with Refractory Solid Tumors**

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**Abstract**

**Purpose:** The purpose is to estimate the maximum-tolerated dose (MTD) of temozolomide and irinotecan given on a protracted schedule in 28-day courses to pediatric patients with refractory solid tumors.

**Experimental Design:** Twelve heavily pretreated patients received 56 courses of oral temozolomide at 100 mg/m<sup>2</sup>/day for 5 days combined with i.v. irinotecan given daily for 5 days for 2 consecutive weeks at either 10 mg/m<sup>2</sup>/day (*n* = 6) or 15 mg/m<sup>2</sup>/day (*n* = 6). We assessed toxicity, the pharmacokinetics of temozolomide and irinotecan, and the DNA repair phenotype in tumor samples.

**Results:** Two patients experienced dose-limiting toxicity (DLT) at the higher dose level; one had grade 4 diarrhea, whereas the other had bacteremia with grade 2 neutropenia. In contrast, no patient receiving temozolomide and 10 mg/m<sup>2</sup>/day irinotecan experienced DLT. Myelosuppression was minimal and noncumulative. No pharmacokinetic interaction was observed. Drug metabolite exposures at the MTD were similar to exposures previously associated with single-agent antitumor activity. One complete response, two partial responses, and one minor response were observed in Ewing's sarcoma and neuroblastoma patients previously treated with stem cell transplant. Responding patients had low or absent O<sup>6</sup>-methylguanine-DNA methyltransferase expression in tumor tissue.

**Conclusions:** The MTD using this schedule was temozolomide (100 mg/m<sup>2</sup>/day) and irinotecan (10 mg/m<sup>2</sup>/day),

with DLT being diarrhea and infection. Drug clearance was similar to single-agent values, and clinically relevant SN-38 lactone and MTIC exposures were achieved at the MTD. As predicted by xenograft models, this combination and schedule appears to be tolerable and active in pediatric solid tumors. Evaluation of a 21-day schedule is planned.

**Introduction**

Camptothecins have emerged as an important class of chemotherapeutic agents for both adult and pediatric malignancies. Irinotecan is a camptothecin prodrug that is converted by carboxylesterases to SN-38, a potent topoisomerase I poison that stabilizes the enzyme-DNA covalent complex and causes S-phase-specific cytotoxicity after collision with the advancing replication fork (1). Irinotecan has shown a wide range of activity against adult solid tumors, including gastrointestinal (2–4), lung (5), brain (6), and gynecologic cancers (7). Substantial antitumor effects have also been demonstrated against pediatric tumors in preclinical experiments (8, 9) and Phase I trials (10–12). These encouraging results, particularly for patients with rhabdomyosarcoma and neuroblastoma, have stimulated additional investigation in the pediatric Phase II setting (13, 14). The broad spectrum of activity and favorable toxicity profile of irinotecan provide compelling reasons to find strategies to additionally increase its effectiveness.

Irinotecan has routinely been administered as a single i.v. dose of 125–350 mg/m<sup>2</sup> every 1–3 weeks (reviewed in Ref. 15). However, preclinical experiments using mouse models of pediatric tumors have shown superior efficacy with protracted low-dose administration (9) such as the [(dx)2] schedule in which drug is given for 5 consecutive days for 2 weeks in a row. Protracted scheduling of irinotecan optimizes the systemic exposure of this S-phase-specific drug and changes the dose-limiting toxicity (DLT) from myelosuppression (11, 12) to diarrhea (10), potentially allowing for the addition of myelosuppressive agents without cumulative toxicity. The established pediatric single-agent maximum-tolerated dose (MTD) of irinotecan on a [(dx)2] schedule is 20 mg/m<sup>2</sup>/day (10).

Temozolomide is an imidazotetrazine prodrug that undergoes spontaneous hydrolysis at physiological pH to the active metabolite monomethyl triazenoimidazole carboxamide (MTIC), which mediates cytotoxicity primarily by methylating DNA at the O<sup>6</sup> position of guanine (16). Clinical activity has been demonstrated against high-grade glioma (17, 18) and melanoma (19), and modest antitumor effects have also been seen against mouse models of pediatric solid tumors (20, 21). Because of excellent oral bioavailability, the drug has routinely been given p.o. daily for 5 days every 28 days, with the DLT being myelosuppression. The single-agent MTD of temozolomide for pediatric patients on this schedule is 200–215 mg/m<sup>2</sup>/day (22, 23).

In addition to the single-agent activity and nonoverlapping toxicity profiles described above, this combination is attractive because of significant therapeutic synergy demonstrated by

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Houghton *et al.* (21) in preclinical experiments. They showed that the combination of noncurative doses of each drug resulted in complete responses (CRs) in xenograft models of neuroblastoma, rhabdomyosarcoma, and glioblastoma multiforme. This therapeutic synergy is greatest when temozolomide is given before irinotecan, suggesting that temozolomide potentiates the cytotoxic effects of irinotecan (24).

On the basis of these preclinical observations, we performed a Phase I trial to estimate the MTD of this combination and characterize the toxicity profile in pediatric patients with relapsed solid tumors. We also evaluated the pharmacokinetic profile of each agent to define drug clearance and assess for an interaction. Finally, because previous studies suggested certain DNA repair phenotypes correlate with sensitivity to temozolomide (20) and irinotecan (25), we assessed the status of *O*<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) and mismatch repair protein (MMR) in available tumor tissue.

## Materials and Methods

**Eligibility.** Patients being treated at Primary Children's Medical Center (Salt Lake City, UT) for recurrent solid tumors or brain tumors for which conventional therapies had failed were eligible for this protocol. Eligibility requirements included a life expectancy of at least 8 weeks, Eastern Cooperative Oncology Group performance status of 0–2, recovery from the toxic effects of prior therapy, and adequate bone marrow function as determined by a hemoglobin level > 8 g/dl, absolute neutrophil count > 1000/ $\mu$ l, and a platelet count > 75,000/ $\mu$ l. In addition, patients were required to have adequate liver function (bilirubin < 1.5 $\times$  normal and alanine aminotransferase < 2.5 $\times$  normal) and renal function (serum creatinine < 3 $\times$  normal for age). Patients were ineligible if they had prior treatment with temozolomide or had active diarrhea or other gastrointestinal conditions that could affect drug absorption or confound observation of drug toxicity. Patients were also ineligible if they had prior craniospinal irradiation because previous Phase I trials had identified a separate, lower MTD for temozolomide for such patients (22). Because enzyme-inducing anticonvulsants have been documented to affect irinotecan clearance (26), patients were ineligible if they were taking these medications. The study was approved by the Institutional Review Board, and informed consent was obtained from parents and patients, as appropriate, according to institutional guidelines.

**Drug Formulation and Administration.** Irinotecan (Camptostar; Pharmacia & Upjohn, Kalamazoo, MI) was supplied as single-dose vials of 100 mg of irinotecan hydrochloride. Irinotecan was diluted in 5% dextrose solution to a final concentration of 0.12–1.1 mg/ml before i.v. administration. Temozolomide (Temodar; Schering Corporation, Kenilworth, NJ) was supplied in capsules of 5-, 20-, or 100-mg strength, and doses were rounded off to the nearest 5 mg.

Patients were given temozolomide orally on an empty stomach once daily for the first 5 days of the study. One h after each dose of temozolomide, irinotecan was infused i.v. over 1 h. After a 2-day rest, irinotecan was administered as a single agent for 5 more consecutive days. The starting doses were exactly half of each drug's previously reported pediatric single-agent MTD. At dose level 2, irinotecan was increased from 10 to 15

mg/m<sup>2</sup>/day, whereas temozolomide was held constant at 100 mg/m<sup>2</sup>/day. Treatment courses were every 28 days based on previous schedules for temozolomide (22, 23). An additional 7 days were allowed for patients to recover from toxicity, but patients who still had grade 3 or 4 toxicity at 35 days were not eligible to receive additional therapy. Dose assignments were based on the traditional three-in-a-cohort Phase I study design, and there were no inpatient dose escalations. Patients were eligible to receive subsequent courses of therapy if they were clinically well and recovered from nonhematological toxicity, did not have evidence of progressive disease (PD), and had an absolute neutrophil count > 750/ $\mu$ l and platelet count > 75,000/ $\mu$ l.

The potential for developing diarrhea and abdominal pain was discussed in advance with all patients and their caretakers, and instructions were given to start loperamide immediately if these symptoms occurred, as has been previously done when administering protracted irinotecan (10).

**Patient Evaluation and Assessment of Toxicity and Response.** Before enrollment, each patient underwent a complete history and physical examination, and appropriate laboratory investigations (complete blood count and comprehensive metabolic panel) were performed to determine eligibility. Measurable disease, if present, was documented by appropriate imaging studies, as well as bone marrow aspirates and biopsies when marrow involvement was suspected. Imaging studies were repeated after the first course for all patients and then every 1–3 courses as clinically indicated. While on study, complete blood counts were obtained twice weekly, whereas complete metabolic profiles were obtained once weekly.

Toxicity was assessed by the National Cancer Institute Common Toxicity Criteria version 2.0 and followed throughout all courses of chemotherapy. Estimation of the MTD, however, was based only on observations during course 1. Hematological DLT was defined as the following: grade 4 neutropenia lasting >10 days; grade 3–4 thrombocytopenia lasting >35 days after the first dose of chemotherapy; or thrombocytopenia requiring two or more platelet transfusions at least 48 h apart given because of grade 4 thrombocytopenia or clinical bleeding. Non-hematological DLT was defined as any grade 3 or 4 toxicity with the specific exclusion of the following: grade 3 nausea or vomiting; grade 3 diarrhea lasting <72 h; grade 3 stomatitis lasting <72 h; grade 3 fever; or grade 3 hepatic toxicity resolving before the next course of chemotherapy.

Responses were based on changes in tumor volume using three-dimensional imaging measurements and the formula: volume = 0.52 (anteroposterior  $\times$  trans  $\times$  long). A CR was defined as complete regression of all apparent tumor masses, including lesions noted on imaging and/or clearing of the bone marrow of tumor cells, persisting at least 4 weeks. A partial response (PR) was defined as >50% and <100% regression of all tumor masses in the absence of any new lesions. A minor response was defined as a reduction of 25–50% of tumor size, with no new tumor progression noted. PD was defined as >25% increase in the size of all measurable tumor areas, or the appearance of new sites of disease. Stable disease was defined as the absence of CR, PR, minor response, or PD.

**Pharmacokinetics.** We performed pharmacokinetic studies of the combination of temozolomide and irinotecan to

determine whether a pharmacokinetic interaction existed on day 1 of the first course. For patients weighing >20 kg, samples were also collected on day 5. To measure the disposition of temozolomide and its active metabolite MTIC, 3 ml of whole blood was collected in a lithium heparin tube before temozolomide administration and again at 0.25, 0.5, 1, and 4 h after the dose of temozolomide. Samples were processed and evaluated by isocratic high-performance liquid chromatography as described previously (20).

The pharmacokinetics of irinotecan and its metabolites SN-38 and SN-38 glucuronide (SN-38G) were evaluated after administration of the temozolomide dose followed 1 h later by the irinotecan dose. Heparinized blood samples from a peripheral catheter contralateral to the site of irinotecan infusion were obtained before the infusion and at 0.25, 0.5, 1, 2, 4, and 6 h after the end of the infusion. Samples were processed, and plasma concentrations of the lactone and carboxylate forms of irinotecan, SN-38, and SN-38 glucuronide were assessed by high-performance liquid chromatography with fluorescence detection as described previously (27).

Temozolomide and MTIC plasma concentration-time data were modeled using maximum *a posteriori* Bayesian estimation as implemented in ADAPT II (28). The prior parameter estimates were derived from a pediatric population reported previously (29). A first-order absorption, one-compartment linear model, which included first-order MTIC formation and elimination, was used to simultaneously describe temozolomide and MTIC disposition (30). The area under the curve (AUC) was calculated from the model parameters. These estimates allowed for calculation of the apparent systemic clearance. For irinotecan and metabolites, a multicompartment 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-38 glucuronide lactone and carboxylate plasma concentrations using a maximum *a posteriori* Bayesian estimation as implemented in ADAPT II. The irinotecan lactone clearance was estimated and the AUC (0–7 h) was estimated for each of the components.

To investigate the effects of varying irinotecan dose and concurrent use of temozolomide on irinotecan disposition, we included irinotecan dose and temozolomide administration as covariates in a linear-mixed effects model using S-plus (S-plus version 6.1; Insightful Corporation, Seattle, WA). To aid in this comparison, we included the pharmacokinetics obtained from historical controls of children receiving irinotecan as a single agent at i.v. doses of 15–45 mg/m<sup>2</sup> as Phase I or II evaluations without concurrent dosing of temozolomide (31, 32).

**Assessment of MGMT Expression.** Immunohistochemistry staining for MGMT (dilution 1:400, mouse clone MT23.2, donated by Dr. Tom Brent) was performed on paraffin sections using an indirect biotin avidin detection system (Ventana IVIEW DAB; Ventana, Tucson, AZ) with an automated immunostainer (Ventana). Pretreatment included blocking of endogenous biotin using Ventana AB Block and heat-induced epitope retrieval via steam cooking in Dako Target Retrieval solution (Dako, Carpinteria, CA) at 99°C for 30 min, followed by a 30-min cool-down period. Hematoxylin was used as a counterstain.

Multiple cell lines with previously established expression (CEM, A693) or lack of expression (TK6) of MGMT were used as positive and negative controls, respectively. Grading of the level of MGMT expression was as follows: 3+ = intense dark nuclear staining; 2+ = moderate nuclear staining; 1+ = weak nuclear staining; and 0 = no staining. The aforementioned cell lines showed consistently reproducible staining (CEM-3+, A693-2+, and TK6-0), and all patient tumor samples were graded for MGMT expression against these controls by a single pathologist (C. F.). Additionally, endothelial cells within patient samples acted as a positive internal control, typically showing 1 to 2+ staining intensity.

**Assessment of MMR Protein Status.** MMR status was assessed indirectly by PCR for microsatellite instability on blood from each patient as a control. Briefly, for paraffin-embedded tissues, three 20- $\mu$ m paraffin sections were dissolved in xylene, microfuged, and then washed three times with 100% ethanol and vacuumed dry. A modified antigen retrieval method followed by dissolving the pellet in 1.0 ml 1 $\times$  citrate buffer (pH 6.0) and boiling for 30 min. EX-WAX DNA Extraction kit for Paraffin-Embedded Tissue (Intergen, Purchase, NY) was then used as per kit instructions to extract the DNA. For blood samples, the DNA Extraction kit (Stratagene, La Jolla, CA) was used to extract DNA from 5 ml of heparinized whole blood as per kit instructions.

Microsatellite analysis was done by measurement of PCR amplified products of the following mono- and dinucleotide markers: BAT25, D1S102, D2S123, D2S390, D4S174, D5S346, D6S253, D10S212, D11S935, D14S48, D14S49, D15S118, D16S422, D17S250, and D19S246. Homologous primer sequences were used as reported in the National Center for Biotechnology Information UniSTS database (NCBI 5/1/2002)<sup>7</sup> with the exception of BAT25, which is described in the Genome DataBase (GDB 12/13/2001).<sup>8</sup> All forward primers were synthesized with a fluorescent dye, FAM or HEX, on the 5'-end for fluorescence detection of the amplified fragments. Reverse primers contained nonhomologous GTTCT sequence at the 5'-end to encourage the consistent nontemplated addition of adenosine that is common with TaqDNA polymerase (33). Amplification of the microsatellites was done using True Allele PCR PreMix (Applied Biosystems, Foster City, CA). Each 10- $\mu$ l reaction contained 5  $\mu$ M of each forward and reverse primer and 50 ng of template DNA. Thermal cycling conditions were as follows: initial denaturation at 95°C for 10 min followed by 30 cycles of 1 min at 95°C; 1 min at 60°C; and 1 min at 72°C. A final extension was done at 72°C for 10 min. Capillary electrophoresis of amplified products was performed on an Applied Biosystems 3100 Genetic Analyzer. Fragment size analysis and allele calling were performed using Applied Biosystems GeneScan 3.7 and Genotyper 3.7 software packages, respectively.

<sup>7</sup> Internet address: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists>.

<sup>8</sup> Internet address: <http://www.gdb.org>.

## Results

**Patients.** Twelve patients were enrolled on this Phase I study between January 2002 and February 2003. All 12 patients were evaluable for toxicity, and 11 were evaluable for response. One patient with recurrent ependymoma had no measurable disease at time of enrollment and thus response could not be evaluated.

Patient characteristics are listed in Table 1. Fifty-six courses have been administered to date, with a median of 4 courses/patient. All patients received temozolomide (100 mg/m<sup>2</sup>/day × 5) combined with irinotecan [10 mg/m<sup>2</sup>/day (*n* = 6) or [15 mg/m<sup>2</sup>/day (*n* = 6)], given on the [(dx5)2] schedule. Predominant diagnoses were Ewing's sarcoma (*n* = 7) and neuroblastoma (*n* = 2). Patients had received a median of three prior multiagent chemotherapy regimens (range, 1–6), and many had been heavily pretreated. In fact, 8 (67%) of the 12 patients had received high-dose chemotherapy with autologous stem cell transplant (ASCT), including 5 (42%) who received tandem transplants. The median time from last stem cell transplant to study enrollment was 15.5 months (range, 1–59 months). Four patients had prior treatment with irinotecan (median, 2.5 courses, range, 2–6), with no significant responses noted. No patients had been previously treated with temozolomide.

**Hematological Toxicity.** The toxicity of this regimen is summarized in Table 2. Despite the enrollment of heavily pretreated patients, hematological toxicity was minimal. Grade 4 neutropenia occurred in only 1 of 56 courses and lasted <5 days. The median nadir absolute neutrophil count during the first course was 2000/μl and occurred at a median of 13 days into the course. There was no grade 4 thrombocytopenia during the entire study, and no patients required platelet transfusions. Three patients received packed RBC transfusions for anemia during the first course, but none received transfusions with later courses. No hematopoietic growth factors were used during this study. There was no evidence of cumulative hematological toxicity in patients receiving up to 17 courses.

Table 1 Patient Characteristics (*n* = 12)

Male/female	10/2
Age (years)	
Median	12.5
Range	1–23
Diagnoses	
Ewing's sarcoma	7
Neuroblastoma	2
Wilms tumor	1
Ependymoma	1
Low-grade glioma	1
Number of prior multiagent chemotherapy regimens	
Median	3
Range	1–6
Prior high-dose therapy/stem cell transplant	8
Single transplant	3
Tandem transplant	5
Number of courses	56
Number of courses/patient	
Median	4
Range	1–17

Table 2 Grade 3–4 toxicity summary for first course and entire study<sup>a</sup>

Grade 3–4 toxicity	IRN <sup>b</sup> (10 mg/m <sup>2</sup> /day) TEM (100 mg/m <sup>2</sup> /day) 6 patients 37 courses		IRN (15 mg/m <sup>2</sup> /day) TEM (100 mg/m <sup>2</sup> /day) 6 patients 19 courses	
	Course 1	Other courses	Course 1	Other courses
Neutropenia <sup>c</sup>	1	2	1	0
Thrombocytopenia	0	2	0	0
Diarrhea	0	0	1 <sup>d</sup>	2
Vomiting	0	0	1	1
Fever	0	1	0	0
Infection	0	0	1 <sup>d</sup>	0

<sup>a</sup> Toxicities noted during first course/entire study.

<sup>b</sup> IRN, irinotecan; TEM, temozolomide.

<sup>c</sup> All resolving within 5 days and not considered dose limiting.

<sup>d</sup> Defined as dose-limiting toxicity.

**Nonhematological Toxicity.** Grade 1–2 diarrhea starting several days after initiating therapy was the most common toxicity encountered, occurring at least once in all 12 patients and overall in 18 (32%) of 56 total courses administered. Mild diarrhea was readily controlled with early initiation of loperamide. Grade 3–4 diarrhea occurred only in patients receiving the higher dose of irinotecan, complicating 16% of these courses. Delayed-onset diarrhea was defined as dose limiting for 1 patient at this dose level. Early-onset diarrhea characterized by the cholinergic syndrome (15) was not observed. A second patient also had DLT at the same dose level, consisting of *Enterococcus* bacteremia with an absolute neutrophil count of 900/μl. Grade 1–2 fever was noted in 7 courses, but only in the above patient was fever accompanied by neutropenia or a documented infection. Nausea and vomiting was typically mild and easily controlled with 5-HT<sub>3</sub> antagonists. Grade 2 transaminase elevation occurred in 2 courses but resolved by the start of the next course. No other significant nonhematological toxicity was noted.

**Delivery of Therapy.** Three patients were already hospitalized for supportive care when they received their first course of treatment; all other courses were given in the outpatient setting. In fact, >90% of the total doses of irinotecan were administered at home using home-health infusion services. All courses were started at 28-day intervals with the exception of 1 patient whose fifth course was delayed 1 week because of a platelet count of 72,000/μl on day 28. Only 2 patients required hospital admission for treatment complications: 1 patient developed nonthrombocytopenic hematochezia attributed to constipation and was observed for 18 h, and the second patient was admitted for fever and bacteremia as noted above.

**Pharmacokinetics.** Evaluable day 1 pharmacokinetic studies were performed for irinotecan (*n* = 10) and temozolomide (*n* = 9). One patient did not have evaluable temozolomide samples because of logistical reasons, and 2 patients did not have testing performed because of parental refusal. Evaluable pharmacokinetic studies were also performed on the fifth day of the first course in patients weighing >20 kg for irinotecan (*n* = 5) and temozolomide (*n* = 4). Day 5 studies were not performed in other patients because of parental refusal, logistical reasons,

**Table 3** Summary of temozolomide pharmacokinetic parameters in patients with pharmacokinetic studies on day 1 ( $n = 9$ )

Parameter (units)	Mean	Median	Range
TEM <sup>a</sup> AUC ( $\mu\text{g/ml}\cdot\text{h}$ )	28.6	20.4	(13.5–74.2)
TEM clearance ( $\text{liter/h/m}^2$ )	4.7	4.9	(1.3–7.4)
MTIC AUC ( $\mu\text{g/mL}\cdot\text{h}$ )	0.85	0.50	(0.39–1.82)
Ka ( $\text{hr}^{-1}$ )	9.3	2.7	(0.3–49)
Tau (hr)	0.09	0.03	(0.01–0.35)
TEM $t_{1/2}$ (h)	2.5	2.1	(0.5–5.9)

<sup>a</sup> TEM, temozolomide; AUC, area under the curve; Cl, clearance; MTIC, 5-(3-methyltriazene-1-yl)imidazole-4-carboxamide.

or weight < 20 kg. Table 3 summarizes the temozolomide pharmacokinetic parameters. In the group of patients with paired day 1 and 5 pharmacokinetic studies, we did not observe qualitative changes in temozolomide or MTIC disposition. For example, the median temozolomide clearance determined on these days was 6.1 and 6.4 liter/h/m<sup>2</sup>, respectively. Although only 4 patients were studied on both days 1 and 5, these results suggest that irinotecan administration did not alter temozolomide disposition in these patients.

The irinotecan pharmacokinetic parameters are summarized by dose level in Table 4. Because we observed SN-38 AUCs in the same range as AUCs associated with higher irinotecan doses in previous single-agent studies (31, 32), we investigated the effects of irinotecan dose and concomitant temozolomide administration on the disposition of irinotecan. The median irinotecan lactone clearance was significantly decreased with decreasing irinotecan dose ( $P = 0.02$ ). When temozolomide administration was added to the model, we observed no additional difference in clearance, indicating no interaction between the two agents.

**Antitumor Activity.** The responses to treatment are summarized in Table 5 and lasted a median of 6 months. A CR was noted in an 18-year-old with metastatic Ewing's sarcoma previously treated with high-dose chemotherapy and tandem ASCT. After the second course of therapy, there was complete resolution of a solitary pleural-based nodule previously proven on needle biopsy to be recurrent disease (Fig. 1). The patient electively discontinued therapy in complete remission after 6 courses of treatment.

Two patients had confirmed partial responses. The first was a 14-year-old who was treated for relapsed Ewing's sarcoma

with high-dose chemotherapy and ASCT and then sustained a second relapse 9 months later. A 63% reduction in multifocal skull disease was noted after 3 courses of temozolomide and irinotecan, and the patient received a total of 6 courses before disease progression. The second patient was a 7-year-old who had developed sino-orbital relapse of high-risk neuroblastoma 13 months after treatment with high-dose chemotherapy and tandem ASCT. A partial response was achieved after 3 courses, and the patient received 5 courses before disease progression. This patient was the only 1 of the 4 responders who was treated at the higher irinotecan dose level.

One patient with relapsed Ewing's sarcoma also achieved a minor response, consisting of 44% reduction in extensive pelvic disease noted after the third course. Similar to the other responding patients, this patient also had been previously treated with high-dose chemotherapy and ASCT, as well as pelvic irradiation. Disease progression occurred after 7 courses of therapy. Finally, 1 patient with stage 4 neuroblastoma has had stable,

**Table 5** Correlation of response with MGMT<sup>a</sup> and MMR protein status

	Disease	Dose level			MMR	Response	No. of courses
		MGMT	MGMT	MGMT			
<b>Responders</b>							
1	ES	1 <sup>b</sup>	0	Not done	CR	6 <sup>c</sup>	
2	ES	1	0	Proficient	PR	6	
3	NB	2 <sup>d</sup>	1+	Proficient	PR	5	
4	ES	1	0	Proficient	MR	7	
5	NB	1	1+	Deficient	SD	17	
<b>Nonresponders</b>							
1	ES	2	0	Proficient	PD	2	
2	ES	2	2+	Proficient	PD	3	
3	ES	2	3+	Proficient	PD	2	
4	WT	2	2+	Proficient	PD	1	
5	LGG	1	0	Proficient	PD	1	
6	ES	1	3+	Not done	PD	1	
7	EP	1	1+	Not done	NE	5	

<sup>a</sup> MGMT, O<sup>6</sup>-methylguanine-DNA methyltransferase; MMR, mismatch repair phenotype; ES, Ewing's sarcoma; NB, neuroblastoma; WT, Wilms tumor; LGG, low-grade glioma; EP, ependymoma; NE, not evaluable.

<sup>b</sup> Dose level 1 = Irinotecan 10 mg/m<sup>2</sup>/day; temozolomide 100 mg/m<sup>2</sup>/day.

<sup>c</sup> Patient electively withdrew himself from study after 6 courses.

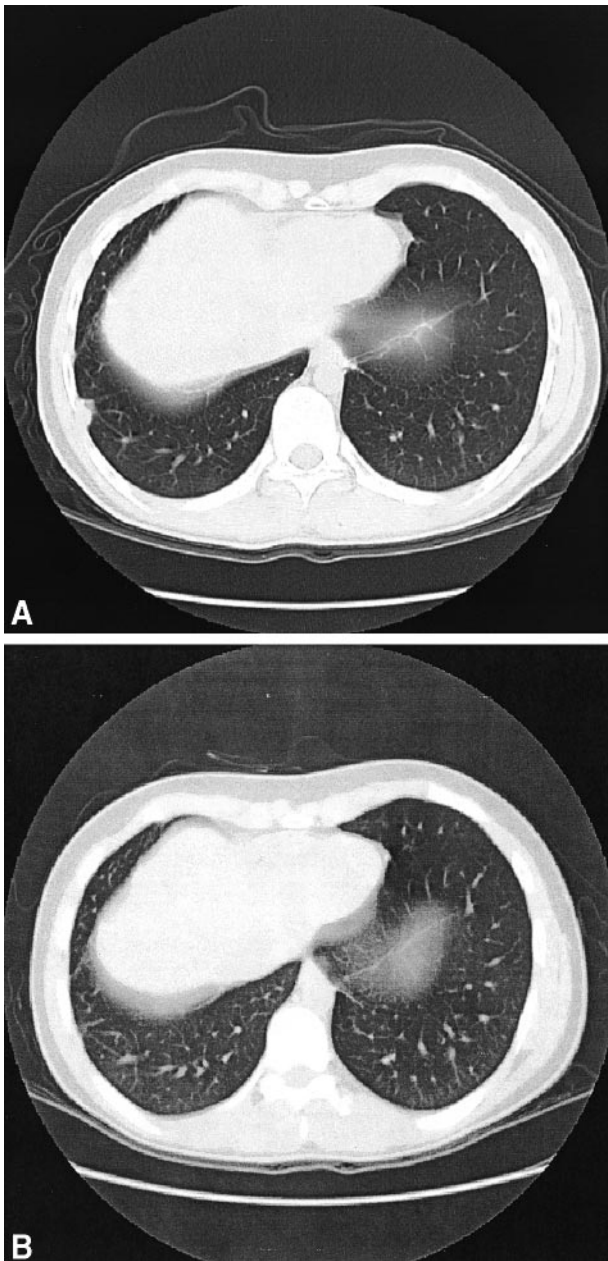
<sup>d</sup> Dose level 2 = Irinotecan 15 mg/m<sup>2</sup>/day; temozolomide 100 mg/m<sup>2</sup>/day.

**Table 4** Summary of irinotecan pharmacokinetic parameters by dose in patients receiving concomitant temozolomide and in historical controls who received irinotecan at a dose of 20 mg/m<sup>2</sup>/day  $\times$  5 days for 2 weeks

Parameter (units)	With temozolomide		Without temozolomide
	Irinotecan 10 mg/m <sup>2</sup> 6 patients 10 study days	Irinotecan 15 mg/m <sup>2</sup> 4 patients 5 study days	Irinotecan 20 mg/m <sup>2</sup> 35 patients 57 study days
Irinotecan lactone AUC ( $\text{ng/ml}\cdot\text{h}$ )	222.0 (84.9–462.5) <sup>a</sup>	375.3 (170.7–478.0)	267.7 (152.0–744.8)
Irinotecan lactone clearance ( $\text{liter/h/m}^2$ )	38.1 (13.4–108.2)	36.8 (27.2–80.6)	60.0 (15.1–120.4)
SN-38 lactone AUC ( $\text{ng/ml}\cdot\text{h}$ )	31.6 (12.3–49.9)	38.0 (24.6–48.4)	18.5 (4.5–70.1)
SN-38G lactone AUC ( $\text{ng/ml}\cdot\text{h}$ )	69.2 (42.8–236.2)	102.6 (69.8–130.3)	80.1 (37.8–371.0)

<sup>a</sup> Median values listed, with ranges in parentheses.

<sup>b</sup> AUC, area under the curve.



**Fig. 1** A, pretreatment computed tomography image demonstrating right pleural-based tumor nodule confirmed by needle biopsy to be recurrent Ewing's sarcoma. B, corresponding images done after 2 months of temozolomide and irinotecan show resolution of nodule and minimal pleural irregularity from previous biopsy.

low-level bone marrow involvement throughout 17 courses of therapy and continues on study.

**DNA Repair Phenotype Analyses.** All 12 patients had paraffin-embedded archival tumor samples analyzed by immunohistochemistry for expression of MGMT. In addition, 10 of the 12 samples were analyzed for microsatellite instability as an indirect assessment of MMR. Results of these assays, paired with the patient's response, are listed in Table 5. MGMT ex-

pression loosely correlated with best imaging response. All 4 patients with imaging responses had low (1+) or absent MGMT expression. In contrast, the 4 patients with MGMT scores of 2–3+ all had PD. However, we noted that 2 patients with MGMT scores of 0 still had PD, implicating mechanisms of resistance other than MGMT in some tumors. Furthermore, only 1 patient's tumor had MMR deficiency, which would suggest resistance to temozolomide; however, this patient had stable disease for 17 months. Therefore, specific conclusions are difficult to make from this small heterogeneous group of heavily pretreated patients.

## Discussion

This Phase I trial was designed as a direct translation of preclinical experiments showing that irinotecan activity can be improved by protracted administration (9) and pretreatment with temozolomide (21, 24). Although it is unclear exactly how the cytotoxicity of irinotecan is potentiated by temozolomide, it may be related to temozolomide-induced methylation of DNA causing localization and enhancement of topoisomerase I cleavage complexes (34), allowing irinotecan to more effectively stabilize the DNA-enzyme complex and cause cytotoxicity after collision with the advancing replication fork.

Our results validate many of the key findings of the preclinical experiments. No pharmacokinetic interaction was observed between the agents, toxicity was manageable, and we achieved clinically relevant exposures of SN-38 and MTIC at the MTD, which translated into objective imaging responses. The temozolomide and MTIC AUC values were similar to those observed when temozolomide showed activity as a single agent against mouse models of pediatric tumors (20, 21). Therefore, although the temozolomide dosage administered in this study was relatively lower than those used in the murine studies, the systemic exposures were closer than would be predicted by a strict linear dose:exposure relationship. Likewise, this absence of a linear dose:exposure relationship was also observed with SN-38; the irinotecan lactone clearance decreased with decreasing dose. For patients in our study, this dose-dependent clearance resulted in similar exposures to the active metabolite SN-38 lactone at the MTD of 10 mg/m<sup>2</sup>/day as those achieved in other studies with irinotecan administered at 20 mg/m<sup>2</sup>/day (31, 32), the single-agent MTD for this protracted schedule.

Although many adult trials have examined the combination of irinotecan with other cytotoxics, this is the first reported combination study using a protracted dosing schedule of irinotecan. This schedule was chosen because multiple investigators have demonstrated the superior preclinical efficacy of irinotecan when given in smaller divided doses compared with large single infusions (9, 35–38). In addition, this schedule was used in the xenograft studies of this drug combination (21, 24) and has been shown to be feasible, safe, and active in the Phase I (10) and II (13) settings. In fact, protracted irinotecan administration at the single-agent MTD results in greater cumulative SN-38 lactone exposures compared with large intermittent bolus doses (10, 39). Because of the availability of home-health services at our institution, we were able to administer >90% of the irinotecan doses at the patient's home, providing an additional benefit in terms of quality-of-life and time away from the hospital. In

contrast to our dosing schedule, adult Phase I trials of this combination have administered temozolomide for 5–14 consecutive days combined with single-dose irinotecan every 1–3 weeks (40–43). Whether a more abbreviated irinotecan schedule is as effective is unknown because direct comparisons of single-dose *versus* protracted irinotecan administration in the Phase II setting have not been reported.

Overall, toxicity was quite manageable in this cohort of heavily pretreated patients. As expected, grade 1–2 diarrhea occurred in all patients but only in one-third of total courses. This late-onset diarrhea was usually well controlled with aggressive use of loperamide. More severe grade 3–4 diarrhea occurred in 3 courses and only at the higher irinotecan dose. Hematological toxicity was quite limited and did not appear to be cumulative in patients receiving up to 17 courses. Only 2 patients required hospital admission for complications of therapy, and there was only one documented infection during the study.

The antitumor activity demonstrated in this Phase I study is very encouraging, especially considering the extensive pretreatment of the patients. Four (36%) of 11 assessable patients had significant reduction in tumor size on imaging, including one CR, two partial responses, and one minor response. A fifth patient has had stable disease throughout 17 courses and still remains on study. Our study included a relatively large proportion of patients with Ewing's sarcoma, and activity was demonstrated in 3 (43%) of these 7 patients. This activity against Ewing's sarcoma is interesting, given that no responses were reported in previous trials in which 19 Ewing's sarcoma patients were treated with either single-agent irinotecan (11–13) or temozolomide (23, 44).

The fact that 3 of the 4 responses occurred at doses that are half of each drug's single-agent MTD replicates the results seen in the mouse xenograft studies in which relatively low, noncurative doses of each agent resulted in significant responses when the drugs were given in combination (21). Because myelosuppression in our heavily pretreated group of patients was minimal and noncumulative, it is possible that this combination can be given more frequently than every 28 days, allowing for increased dose intensity through interval compression. On the basis of toxicity, only 2 (4%) of 56 courses administered in this study would not have been able to be delivered on day 21 instead of day 28. Whether increasing dose intensity in this manner will still be well tolerated is the focus of an upcoming clinical trial.

Preclinical studies have linked the DNA repair phenotype in tumors to sensitivity to temozolomide (20) and more recently irinotecan (25). Although the mouse xenograft studies of this combination conducted by Houghton *et al.* (21) showed responses independent of the MGMT or MMR status, we observed a correlation in that all 4 responding patients, as predicted, had functional MMR and low or absent expression of MGMT. This raises the possibility that administering the suicide protein *O*<sup>6</sup>-benzylguanine, which reverses the effects of MGMT, in conjunction with temozolomide and irinotecan may help overcome chemotherapy resistance, as has been suggested in preclinical studies (45). However, 2 patients with functional MMR and absent MGMT expression had PD, suggesting that other mechanisms may also be affecting resistance to chemo-

therapy. Clearly, conclusions from this small number of heterogeneous patients are difficult to make, and the relationship between DNA repair phenotype and response would be more completely investigated in the Phase II setting.

In summary, we found the combination of temozolomide and irinotecan given on a low-dose, protracted schedule to be well tolerated and active for heavily pretreated pediatric patients with relapsed solid tumors. The MTD on our study was temozolomide (100 mg/m<sup>2</sup>/day) given dx5 combined with irinotecan (10 mg/m<sup>2</sup>/day) given [(dx5)2] given in 28-day cycles, although interval compression may ultimately allow for greater dose intensity. Despite these relatively low doses, clinically relevant SN-38 and MTIC exposures were achieved at the MTD, and objective imaging responses were seen in over one-third of evaluable patients in this Phase I trial. These results validate the use of preclinical experiments to predict pharmacokinetic and clinical activity and to provide solid rationale for clinical trials (46). The safety and activity demonstrated in this study supports additional clinical investigation of this combination, particularly for patients with Ewing's sarcoma and neuroblastoma.

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