

## Phase I Trial of Poly-L-Glutamate Camptothecin (CT-2106) Administered Weekly in Patients with Advanced Solid Malignancies

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**Abstract Purpose:** CT-2106 is a 20(S)-camptothecin poly-L-glutamate conjugate. This linkage stabilizes the active lactone form of camptothecin and enhances aqueous solubility. In addition, poly-L-glutamate is postulated to increase tumor delivery of the active compound through enhanced permeability and retention effect in tumor. We studied a weekly schedule of CT-2106 in patients with refractory solid tumor malignancies.

**Experimental Design:** CT-2106 was infused (10 min i.v. infusion) on days 1, 8, and 15 of each 28-day cycle. Plasma and urine were analyzed for total and unconjugated camptothecin by high-performance liquid chromatography equipped with a fluorescence detector. Toxicity and response assessments were done with Common Toxicity Criteria for Adverse Events version 3 and Response Evaluation Criteria in Solid Tumors, respectively.

**Results:** Twenty-six patients were enrolled. Median age was 58 years (range, 36-83) and median number of doses was 6 (range, 1-9). The most frequent tumor type (50%) was melanoma. Dose limiting toxicities were thrombocytopenia and fatigue. A weekly dose of 25 mg/m<sup>2</sup> given every 3 of 4 weeks was the maximum tolerated dose. The majority of grade 3 and 4 toxicities were hematologic. The pharmacokinetic profile of conjugated and unconjugated camptothecin showed a polyexponential decline with similar terminal half life ( $t_{1/2}$  range was 44-63 and 31-48 h for conjugated and unconjugated, respectively). Pharmacokinetics of conjugated and unconjugated camptothecin were dose and time independent in the tested dose range. Urinary excretion of conjugated and unconjugated camptothecin accounted for about 30% and 4% of the administered dose, respectively.

**Conclusions:** CT-2106 has a more manageable toxicity profile compared with unconjugated camptothecin. The maximum tolerated dose is 25 mg/m<sup>2</sup> weekly given 3 of 4 weeks. This compound results in prolonged release of unconjugated camptothecin.

Numerous derivatives of the camptothecin molecule are currently in clinical use or in trial; these compounds are topoisomerase I inhibitors (1). The parent camptothecin molecule is compromised in its clinical utility by several factors: it is poorly water soluble (2), and it undergoes conversion at physiologic pH to the inactive carboxylate form (3) of the drug, which binds avidly to human serum albumin (3-5). Several camptothecin derivatives have been explored in

clinical trials. The sodium salt of camptothecin was relatively inactive with significant toxicity in clinical trials (6, 7). CT-2106, a poly-L-glutamic acid-glycine-camptothecin conjugate, has the potential to deliver higher active camptothecin doses to tumor tissue compared with normal tissues, thereby reducing toxicity due to the enhanced permeability and retention effect of the poly-glutamate backbone. Covalent binding through the hydroxyl group of camptothecin to poly-glutamate (a biodegradable polymer of glutamic acid) via a glycine linker enhances the aqueous solubility of camptothecin and prevents opening of the lactone ring and subsequent camptothecin binding to albumin with decrease in free camptothecin fraction and inactivation (8, 9). In preclinical models, CT-2106 has shown antitumor activity in multiple human tumor cell lines including lung and colon lines. In patients with solid tumors, every-3-week dosing of CT-2106 was convenient and well tolerated (10). The maximum tolerated dose was 75 mg/m<sup>2</sup> and significant activity was reported. The present phase I study was designed to determine the maximum tolerated dose, toxicity, pharmacokinetics, and response rate of CT-2106 when administered weekly for 3 consecutive weeks of every 4-week cycle.

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Received 11/29/06; revised 4/1/07; accepted 6/22/07.

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**Note:** Presented at the 2006 American Society of Clinical Oncology Annual Meeting, Atlanta, Georgia.

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doi:10.1158/1078-0432.CCR-06-2821

**Table 1.** Patient characteristics, dose escalation, and response

Patient no.	Age/gender	Tumor type	No. prior regimens	No. cycles (doses)	Best response
25 mg/m <sup>2</sup>					
1	52/M	Melanoma	3	1 (3)	PD
2	48/M	Melanoma	3	3 (9)	SD
3	65/F	Pancreas	3	2 (6)	PD
4	59/F	Histiocytoma	3	3 (9)	SD
5	50/M	Melanoma	3	2 (6)	PD
6	50/F	Melanoma	1	2 (6)	PD
30 mg/m <sup>2</sup>					
7	50/F	Breast	3	<1 (1)	PD
8	36/M	Osteosarcoma	2	2 (6)	PD
9	66/F	Breast	5*	2 (6)	PD
10	83/F	Melanoma	1	2 (6)	PD
20	63/M	Melanoma	5†	2 (6)	PD
21	37/F	Breast	3	<2 (4)	SD
22	67/M	Melanoma	2	1 (3)	NA
23	47/F	Lung	3	<2 (5)	PD
24	66/M	Squamous cell	3	2 (6)	PD
25	56/M	Melanoma	2	<2 (5)	PD
26	37/F	Breast	3	1 (3)	PD
35 mg/m <sup>2</sup>					
11	78/F	Melanoma	3	<1 (2)	PD
12	72/M	Pancreas	2	1 (3)	PD
13	56/M	Melanoma	3	<1 (1)	PD
14	78/M	Lung	1	2 (6)	PD
15	61/M	Melanoma	2	2 (6)	PD
16	60/M	Lung	3	2 (6)	PD
17	68/M	Melanoma	3	2 (6)	PD
18	57/F	Colon	3	2 (6)	PD
19	49/F	Melanoma	1	2 (6)	PD

Abbreviations: PD, progression of disease; SD, stable disease.

\*2 regimens were hormonal therapy.

† 2 regimens were immunotherapy.

## Materials and Methods

**Patients.** Adults with advanced solid tumors refractory to three or fewer cytotoxic chemotherapy regimens, with an Eastern Cooperative Oncology Group performance status of 0 to 2, were eligible for inclusion. Adequate bone marrow and renal and hepatic functions were required. Patients with a history of hemorrhagic cystitis, microscopic hematuria (>5 RBC/high-power field or greater than the laboratory reference range), prior pelvic radiation treatment, or progressive brain metastases were excluded. Patients with treated brain metastases needed to have documented stable disease (as assessed by computed tomography or magnetic resonance imaging) for at least 8 weeks after completion of definitive treatment and be neurologically intact. Patients with significant medical conditions or infectious diseases and those completing radiotherapy, major surgery, or any myelosuppressive, biological, or hormonal agents within 4 weeks before the start of CT-2106 dosing were also excluded. The University of South Florida Institutional Review Board approved the study protocol and all patients signed voluntary written informed consent.

**Trial design.** An open-label, dose escalation study of CT-2106 administered weekly for 3 of 4 weeks was conducted. The dose-escalation schema started with a dose of 25 mg/m<sup>2</sup> and the planned escalation was in 5 mg/m<sup>2</sup> increments. Each cohort had a minimum of three patients with an increase in the size of the cohort to explore any dose limiting toxicities (DLT). If no DLT was encountered during the first cycle (4 weeks), the dose was escalated to the next level for the next cohort of patients. If no DLT was observed in the first cycle at a given dose level but unusual and/or cumulative toxicity was observed in subsequent cycles, the accrual at that dose level was expanded.

Treatment was continued until unacceptable toxicity or disease progression occurred. DLT was defined as any grade 4 neutropenia lasting >5 days (without growth factor), febrile neutropenia of unknown origin without documented infection when absolute neutrophil count is  $<0.5 \times 10^9/L$ , thrombocytopenia with nadir  $<25 \times 10^9/L$  or any bleeding requiring platelet transfusion, treatment-related grade 3 or 4 nonhematologic toxicity (excluding manageable diarrhea, nausea, and vomiting of all grades), treatment delay of >2 weeks due to drug-related toxicity, and drug-related death. The maximum-tolerated dose was defined as one dose level below the dose at which at least two patients (of six) experienced a DLT.

**Study drug.** CT-2106 for injection was supplied lyophilized in 20-mL glass single-use vials. The drug product was reconstituted and diluted by adding sterile water; the resultant solution contained 80 mg/10 mL of conjugated camptothecin. The required volume of reconstituted drug product was transferred to a low-adsorption plastic i.v. bag and immediately diluted to a total volume of 50 mL with 5% dextrose for injection. Diluted CT-2106 solutions were stable for 4 h at room temperature or for 24 h refrigerated at 2°C to 8°C. The diluted drug product was administered with a pump as a 10-min infusion into a peripheral vein or central line. A low-adsorption administration set, without an inline filter, was used.

**Dosing schedule and study assessments.** CT-2106 was administered at doses of 25, 30, or 35 mg/m<sup>2</sup> infused i.v. over 10 min on days 1, 8, and 15 of each 28-day cycle. The starting dose was selected based on results of nonclinical studies in mice and rats where CT-2106 doses providing 97 or 115 mg/m<sup>2</sup> conjugated camptothecin (given every 3 weeks) were below the maximum tolerated dose and on the basis of the Q3 week phase 1 study that determined that a dose of 75 mg/m<sup>2</sup> given every 3 weeks was well tolerated and accumulation did not occur.

**Table 2.** Dose limiting toxicities

Dose (mg/m <sup>2</sup> )	DLT/evaluable patients	DLT
25	1/6	Grade 3 fatigue
30	2/9	Grade 3 and 4 thrombocytopenia; large volume hematuria
35	2/9	Grade 3 fatigue; grade 3 and 4 thrombocytopenia; grade 4 neutropenia

In the case of unresolved toxicity, up to a 2-week delay in treatment was permissible (4 weeks for hematuria). Cystoscopy was done if grade  $\geq 2$  hematuria occurred, and the dose of CT-2106 was reduced at the next treatment to the previous dose level. Up to two dose reductions were permitted. Toxicities were assessed each visit using National Cancer Institute Common Toxicity Criteria version 3.

Clinical chemistry, hematology, coagulation profile, urinalysis, and clinical evaluations were done at baseline, during the study, and at the end of treatment visit. A 12-lead electrocardiogram; computed tomography scan of the chest, abdomen, and pelvis; and a full-body bone scan when indicated were required before study entry. Disease response was assessed every two cycles according to Response Evaluation Criteria in Solid Tumors criteria.

**Pharmacokinetics.** Blood samples were collected in tubes containing sodium heparin in cycles 1 and 2 at the following times: before treatment and at 10 min, 30 min, and 1, 2, 10, 24, 48, 72, 96, and 120 h after the start of infusion. In addition, at both cycles, blood samples were collected before and at the end of infusion at days 8 and 15. Urine samples were collected in the first and second cycles during the following periods: 0 to 4, 4 to 10, 10 to 24, 24 to 48, 48 to 72, 72 to 96, and 96 to 120 h.

After collection, blood samples were centrifuged at 2,500 rpm for 5 min at 4°C to separate plasma that was stored at -70°C until analysis. After volume measurement and recording, urine samples were stored at -70°C until analysis. The concentrations of total camptothecin (unconjugated plus conjugated to the polymer) and of unconjugated camptothecin released from polymer were assayed both in plasma and urine.

After addition of 10-hydroxy camptothecin as internal standard, the assessment of total camptothecin in plasma and urine included a basic hydrolysis using 0.5 N NaOH to release the conjugated camptothecin from the poly-L-glutamic acid. Afterward, the camptothecin released and the preexisting unconjugated camptothecin were extracted with methanol.

The concentrations of unconjugated camptothecin were measured after methanolic extraction directly from plasma and urine. After centrifugation, the methanolic fractions for the assay of total and unconjugated camptothecin were acidified with 1 N HCl to convert all the camptothecin into the lactone form and were analyzed by high-performance liquid chromatography with fluorescence detection set at 360/550 and 360/440 nm for the internal standard and camptothecin, respectively.

Chromatographic separation was achieved through an Eclipse, XDB-C18, 5- $\mu$ m analytic column under isocratic conditions [acetonitrile: 0.1 ammonium acetate (pH 4), 30:70 (v/v), 1 mL/min]. This analytic method allowed the quantitation of both total and unconjugated camptothecin down to 5 ng/mL both in plasma and urine. Plasma and urine concentrations of conjugated camptothecin were obtained by mathematical subtraction of the unconjugated fraction from the total camptothecin concentration. Pharmacokinetic calculations in plasma and urine were done using a noncompartmental approach [linear trapezoidal rule for area under the curve (AUC) calculation and linear regression of natural log-transformed plasma concentration versus time data] using WinNonLin Enterprise software version 4.1 (Pharsight Corp.).

## Results

**Patient characteristics.** A total of 26 patients were enrolled (Table 1). Fourteen (54%) were male. Melanoma was the most common malignancy (50%). Median age was 58 years (range, 36-83 years). Patients received a median of 6 doses (range, 1-9). Median number of prior regimen was 3 (range, 1-3).

**Dose escalation and toxicity profile.** All patients were assessable for toxicity; however, only those patients who received three doses of study drug or experienced a DLT were evaluable for maximum tolerated dose determination (Table 2). One DLT (grade 3 fatigue) occurred in the first three patients in the 25 mg/m<sup>2</sup> dose cohort and the cohort was expanded to include three more patients. No more DLTs occurred at 25 mg/m<sup>2</sup> and four patients were enrolled (three were evaluable) in the 30 mg/m<sup>2</sup> cohort. No DLTs occurred in these four patients. Eleven patients were enrolled (nine were evaluable) in the 35 mg/m<sup>2</sup> cohort. DLTs were observed in three patients (grade 3 and 4 thrombocytopenia, grade 4 neutropenia, and grade 3 fatigue); therefore, the 30 mg/m<sup>2</sup> cohort was expanded to include seven additional patients (six were evaluable). Two patients experienced DLTs in the

**Table 3.** Number of patients with study drug-related adverse events ( $n = 26$ )

	Common Toxicity Criteria toxicity grade		
	Grade 2	Grade 3	Grade 4
Nonhematologic, $n$ (%)			
Nausea	5 (19)	0	0
Fatigue	2 (8)	2 (8)	0
Hematuria	4 (15)	0	0
Vomiting	4 (15)	0	0
Diarrhea	4 (15)	0	0
Urinary frequency	2 (8)	0	0
Retching	1 (4)	1 (4)	0
Hematologic, $n$ (%)			
Anemia	8 (31)	5 (19)	0
Neutropenia	0	2 (8)	3 (12)
Thrombocytopenia	1 (4)	0	4 (15)
Lymphopenia	1 (4)	0	1 (4)

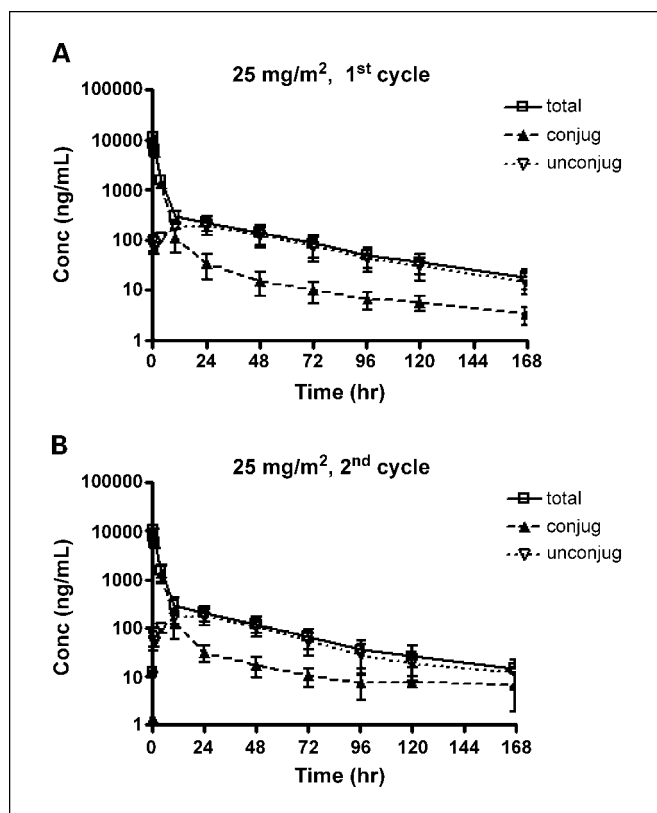


Fig. 1. Mean plasma concentration-time profiles for total, conjugated, and unconjugated camptothecin (CPT) at the recommended dose of 25 mg/m<sup>2</sup>. A, cycle 1. B, cycle 2.

expanded cohort. One patient had grade 4 thrombocytopenia and one patient had grade 3 thrombocytopenia and large volume hematuria. The patient who experienced large volume hematuria had previously received three cycles of high-dose ifosfamide for sarcoma and this was thought to be a contributing factor. Based on these findings, it was concluded that the maximum tolerated dose is 25 mg/m<sup>2</sup>.

The majority of grade 3 and 4 toxicities were hematologic (Table 3). Grade 4 thrombocytopenia, neutropenia, and lymphopenia were observed. Grade 3 anemia, neutropenia, fatigue, and retching were also documented. Only 15% had grade 2 hematuria. Only one case of grade 2 alopecia was reported on this trial. Diarrhea was reported but it was mild (six patients had grade 1, five with grade 2, one with grade 3, and none with grade 4).

Three patients did not complete a full cycle of treatments (three-weekly treatments). One patient on the 30 mg/m<sup>2</sup> cohort had brain metastasis discovered on day 10; a patient on the 35 mg/m<sup>2</sup> had brain metastasis with intracerebral hemorrhage discovered on day 10; and another patient on the 35 mg/m<sup>2</sup> cohort withdrew consent on day 7. She had had nausea/vomiting 5 h after infusion on day 1, which had resolved with lorazepam and granisetron, and had an episode of supraventricular tachycardia that was thought to be unrelated to drug on day 4; she withdrew consent on day 7.

**Pharmacokinetic variables.** After the administration of CT-2106, plasma concentrations of total, conjugated, and unconjugated camptothecin declined polyexponentially with similar

terminal half-life. At the first cycle after the administration of the recommended dose of 25 mg/m<sup>2</sup>, conjugated and unconjugated camptothecin declined with a  $t_{1/2}$  of 63 ± 18 and 36 ± 4 h (mean ± SD), respectively.

Conjugated camptothecin accounted for the majority of total camptothecin up to about 20 h, whereas, from this time on, total camptothecin was mainly represented by the unconjugated form (Fig. 1). At the recommended dose of 25 mg/m<sup>2</sup> on the first cycle, conjugated camptothecin peak plasma concentrations and AUC were 11.0 ± 1.4 mg/L and 27 ± 4.9 mg h/L, respectively. At the same dose level,  $C_{max}$  and AUC of unconjugated camptothecin were 0.21 ± 0.05 mg/L and 14.0 ± 5.2 mg h/L, respectively (Fig. 2).

The conjugated camptothecin was characterized by a low volume of distribution at steady state ( $V_{ss}$ ), a larger volume of distribution during the terminal phase ( $V_z$ ), and a low systemic clearance.

The low value of  $V_{ss}$  suggests that the conjugated polymer is distributed in plasma (1.72 L/m<sup>2</sup>) and in extracellular body fluids (9.2 L/m<sup>2</sup>), as expected for a polymeric, high molecular weight compound. The volume of distribution of the conjugated camptothecin in the post-distribution phase  $V_z$  was much larger than the total body water (24 L/m<sup>2</sup>) and accounted for ~10 times the volume of distribution at steady state ( $V_{ss}$ ; Table 4). The difference between the estimates of  $V_{ss}$  and  $V_z$  indicates that the distribution process is fairly slow.

Elimination efficiency of conjugated compound is rather low, with renal excretion accounting for 30% of total plasma

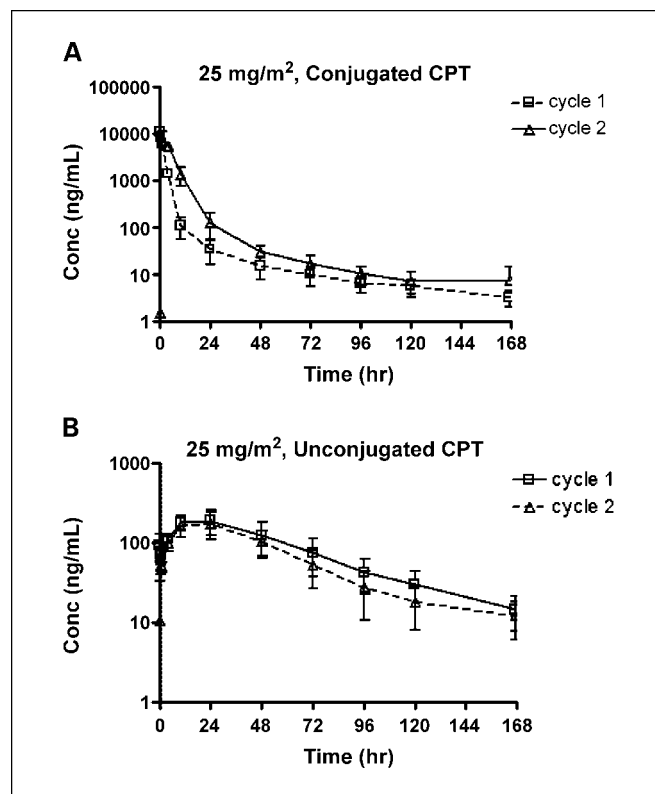


Fig. 2. Mean plasma concentration-time profiles of conjugated (A) and unconjugated (B) camptothecin (cycles 1 and 2) after administration of CT-2106 at 25 mg/m<sup>2</sup>.

**Table 4.** Summary of the main pharmacokinetic variables (mean  $\pm$  SD) of conjugated and unconjugated camptothecin during the first and second cycles of treatment with CT-2106

Conjugated camptothecin											
Dose (mg/m <sup>2</sup> )	Cycle no.	Subject no.	C <sub>max</sub> (mg/L)	C <sub>max</sub> /dose (mg/L/mg/m <sup>2</sup> )	t <sub>1/2,z</sub> (h)	AUC (mg·h/L)	AUC/dose (mg·h/L/mg/m <sup>2</sup> )	CL (L/h/m <sup>2</sup> )	V <sub>ss</sub> (L/m <sup>2</sup> )	V <sub>z</sub> (L/m <sup>2</sup> )	A <sub>e</sub> (% dose)
25	1	6	11 $\pm$ 1.4	0.44 $\pm$ 0.06	63 $\pm$ 18	27 $\pm$ 4.9	1.07 $\pm$ 0.20	0.96 $\pm$ 0.17	7.6 $\pm$ 1.04	85 $\pm$ 16	34 $\pm$ 6.4
	2	6	10 $\pm$ 1.9	0.41 $\pm$ 0.08	50 $\pm$ 19	25 $\pm$ 7.7	1.03 $\pm$ 0.31	NA	NA	NA	28 $\pm$ 5.4
30	1	11	14 $\pm$ 2.0	0.47 $\pm$ 0.07	51 $\pm$ 34	36 $\pm$ 7.4	1.19 $\pm$ 0.25	0.87 $\pm$ 0.16	8.2 $\pm$ 7.5	60 $\pm$ 30	32 $\pm$ 11
	2	9	13 $\pm$ 1.8	0.44 $\pm$ 0.06	44 $\pm$ 16	36 $\pm$ 8.6	1.19 $\pm$ 0.29	NA	NA	NA	25 $\pm$ 11
35	1	9	16 $\pm$ 1.9	0.44 $\pm$ 0.06	47 $\pm$ 18	42 $\pm$ 7.5	1.19 $\pm$ 0.21	0.86 $\pm$ 0.15	7.7 $\pm$ 5.3	56 $\pm$ 17	35 $\pm$ 41
	2	4	15 $\pm$ 1.7	0.43 $\pm$ 0.05	54 $\pm$ 16	40 $\pm$ 2.3	1.13 $\pm$ 0.07	NA	NA	NA	31 $\pm$ 8.0

Unconjugated camptothecin										
Dose (mg/m <sup>2</sup> )	Cycle no.	Subject no.	C <sub>max</sub> (mg/L)	C <sub>max</sub> /dose (mg/L/mg/m <sup>2</sup> )	t <sub>1/2,z</sub> (h)	AUC (mg·h/L)	AUC/dose (mg·h/L/mg/m <sup>2</sup> )	T <sub>max</sub> (h)	A <sub>e</sub> (% dose)	
25	1	6	0.21 $\pm$ 0.05	0.008 $\pm$ 0.002	36 $\pm$ 4.4	14 $\pm$ 5.2	0.57 $\pm$ 0.21	17 $\pm$ 7.7	4.38 $\pm$ 1.73	
	2	6	0.18 $\pm$ 0.06	0.007 $\pm$ 0.002	31 $\pm$ 6.1	12 $\pm$ 4.8	0.48 $\pm$ 0.19	19 $\pm$ 7.3	3.73 $\pm$ 0.73	
30	1	11	0.39 $\pm$ 0.31	0.013 $\pm$ 0.010	37 $\pm$ 9.3	31 $\pm$ 31	1.03 $\pm$ 1.03	18 $\pm$ 8.7	4.61 $\pm$ 1.84	
	2	9	0.35 $\pm$ 0.13	0.012 $\pm$ 0.004	48 $\pm$ 26	28 $\pm$ 17	0.93 $\pm$ 0.55	19 $\pm$ 7.0	5.58 $\pm$ 5.45	
35	1	9	0.33 $\pm$ 0.15	0.009 $\pm$ 0.004	36 $\pm$ 13	28 $\pm$ 22	0.80 $\pm$ 0.63	19 $\pm$ 7.1	2.91 $\pm$ 2.17	
	2	4	0.33 $\pm$ 0.23	0.009 $\pm$ 0.006	47 $\pm$ 36	40 $\pm$ 56	1.14 $\pm$ 1.59	19 $\pm$ 19	4.60 $\pm$ 2.21	

Abbreviations: C<sub>max</sub>, maximum plasma concentration; C<sub>max</sub>/dose, C<sub>max</sub> normalized to the dose of 1 mg/m<sup>2</sup>; t<sub>1/2,z</sub>, terminal half-life; AUC, area under the plasma concentration-time curve from 0 to 8; AUC/dose, AUC normalized to the dose of 1 mg/m<sup>2</sup>; CL, total plasma clearance; V<sub>ss</sub>, steady-state volume of distribution; V<sub>z</sub>, volume of distribution during the terminal phase; A<sub>e</sub>, amount excreted in urine; T<sub>max</sub>, time to reach the maximal plasma concentration; NA, not applicable.

clearance and systemic plasma clearance below the liver plasma flow in humans (26 L/h/m<sup>2</sup>; Table 4).

Conjugated camptothecin progressively released the unconjugated form that reached the maximal plasma concentration 17 to 20 h post-dosing and then declined with similar terminal half-life as conjugated camptothecin (31-48 h on average). This observation suggests that the disposition of unconjugated camptothecin is formation rate limited (i.e., the kinetics of unconjugated camptothecin depends on the release rate from the CT-2106 polymeric backbone).

Plasma concentrations of both conjugated and unconjugated camptothecin increased in direct proportion with the dose, and no accumulation was observed between the two treatment cycles.

**Tumor responses.** Twenty-five patients were assessable for response. No objective responses (complete or partial) were noted. Three patients had stable disease; two received 25 mg/m<sup>2</sup> and one received 30 mg/m<sup>2</sup> (Table 1). One patient had breast cancer, one histiocytoma, and one melanoma.

## Discussion

Camptothecin analogues have become mainstays of chemotherapy for numerous malignancies (11). The mechanisms of action and resistance to camptothecins have extensively been investigated (12-14). The parent camptothecin molecule has several disadvantages: it is highly insoluble, and it is in the inactive carboxylate form at physiologic pH and, additionally, the inactive carboxylate form binds preferentially to human serum albumin, further shifting the equilibrium to this form (15, 16).

Clinical trials with the parent camptothecin (NSC 10880) in solid tumors, melanoma, and gastrointestinal malignancies

(6, 7, 17, 18) were conducted by the National Cancer Institute more than 30 years ago. This compound was handicapped by dose limiting hemorrhagic cystitis (occurring in 5 of 15 patients) and severe bone marrow suppression (7, 18). Further investigation showed that a large fraction of camptothecin was excreted in urine where an acidic environment favors closing of the lactone and a reactivation of anti-topoisomerase I activity and toxicity resulting in hemorrhagic cystitis. Subsequently, many analogues of camptothecin have been synthesized to overcome some of these shortcomings (19). Irinotecan and topotecan, which are approved for the treatment of variety of malignancies, are two of these analogues (20). Both these agents, although extremely useful, have specific shortcomings (2, 19, 21-23).

To overcome some of these issues with camptothecin analogues, attachment of camptothecin to polymeric carrier molecules has been tested preclinically and in clinical trials (24-38). Polymeric backbones can increase the water solubility of camptothecin and protect the lactone form, as well as offer controlled release and high tissue concentrations depending on the polymer chosen. The compound used in the current trial, CT-2106, has camptothecin covalently conjugated to a poly-L-glutamate through a glycine linker, which stabilizes the lactone ring and reduces binding of camptothecin to albumin. This conjugate slowly degrades to release active camptothecin in tumor tissue, in the circulation, and in metabolizing organs. This theoretically could lead to decreased systemic concentrations of free camptothecin, resulting to reduced toxicity, especially hemorrhagic cystitis.

The conjugation to polyglutamate is also believed to enhance drug distribution to the tumor because the increased permeability observed in tumor vasculature and the lack of lymphatic drainage away from the disease tissue prevent large molecules

from being easily removed from the tumor space (39, 40). This effect, referred to as the enhanced permeability and retention effect, is thought to confer a benefit on macromolecules and liposomally packaged drugs in terms of increased tumor delivery of drug.

A phase I study of CT-2106 in patients with advanced malignancies who have received no more than three prior chemotherapy regimens was presented recently (10). This trial described the same agent given every 3 weeks. The maximum tolerated dose was 75 mg/m<sup>2</sup>. Patients were treated on day 1 of each 21-day cycle. DLTs observed were grade 3 or 4 neutropenia and concomitant grade 3 or 4 thrombocytopenia in the three patients treated at 105 mg/m<sup>2</sup> and grade 2 hematuria in a patient treated at 25 mg/m<sup>2</sup>. Due to confounding patient conditions, it was unclear if the hematuria was drug related and the cohort was expanded to six patients without further observation of hematuria. Other serious drug-related toxicities were grade 4 hypersensitivity in a patient with a history of hypersensitivity to irinotecan and grade 3 anemia in one patient. Remaining drug-related adverse events (mild or moderate) include anemia (14), fatigue (10), nausea (8), diarrhea (7), vomiting (5), alopecia (5), and hematuria (1). Four patients exhibited stable disease for  $\geq 9$  weeks; two lung cancer patients dosed at 25 mg/m<sup>2</sup> exhibited stable disease for  $>36$  weeks (12 cycles). Plasma C<sub>max</sub> and AUC values for conjugated and unconjugated camptothecin indicated CT-2106 pharmacokinetic linearity from 12 to 75 mg/m<sup>2</sup>. At the maximum tolerated dose (75 mg/m<sup>2</sup>), the apparent terminal t<sub>1/2</sub> of unconjugated camptothecin was  $\sim 40$  h. Five days after the first administration (cycle 1), the excretion in urine of conjugated and unconjugated camptothecin accounted on average for 22.2% and 4.0% of the administered dose (75 mg/m<sup>2</sup>), respectively. The excretion pattern did not change during cycle 2.

In the current phase I trial, CT-2106 was well tolerated without the toxicities normally associated with camptothecin, specifically severe diarrhea. Our results are consistent with what has been reported in patients with advanced solid tumors treated with CT-2106 every 3 weeks. The absence of objective responses (complete or partial) was not surprising in this heavily treated population; the tumor histology was skewed toward melanoma.

The pharmacokinetics of conjugated camptothecin are characterized by a low V<sub>ssr</sub>, a high V<sub>z</sub>, and a limited systemic clearance. By comparing V<sub>ss</sub> and V<sub>z</sub>, we can speculate that after the initial limited distribution, conjugated camptothecin is slowly and progressively taken up by the tissues. Accumulation in tumor tissue and in tissues of the reticulodendothelial

system with slow release of free camptothecin was observed in preclinical studies.<sup>3</sup> The increasing concentrations of unconjugated camptothecin in plasma, reaching a maximum  $\sim 20$  h after the start of the infusion and then exceeding that of the conjugated fraction, is likely due to release from tissue stores into the systemic circulation after release from the polymeric backbone.

The drug was mainly excreted in the conjugated form. Indeed, the fraction of conjugated and unconjugated camptothecin excreted into urine accounted for about 30% and 4% of the administered dose, respectively. Moreover, the reduced renal excretion of unconjugated camptothecin compared with other camptothecins could explain the limited side effect of camptothecins on the urinary tract.

Plasma concentrations of both conjugated and unconjugated camptothecin increased in direct proportion with the dose and no accumulation was observed between the two treatment cycles.

Toxicity with this compound consisted largely of myelosuppression. Grade 3 and 4 thrombocytopenia was seen in 4 (15%) patients whereas grade 3 and 4 neutropenia was seen in 5 (20%) of patients. Mild to moderate anemia was frequently observed; 12 (46%) patients had grade 2 or 3 anemia. The major nonhematologic toxicity seen was fatigue, with grade 2 or 3 fatigue seen in 4 (16%) patients. Whereas hematuria was a major concern, based on past experience with camptothecin, in this trial, only grade 2 hematuria was seen in 4 (15%) of patients. The first patient experienced hematuria in cycle 3 at the 25 mg/m<sup>2</sup> dose; she had previously had ifosfamide treatment for sarcoma. The other patients who had cycle 2 hematuria were in the 30, 30, and 35 mg/m<sup>2</sup> dose cohorts. An additional patient experienced hematuria (grade 1) in the context of grade 4 thrombocytopenia at cycle 1.

Based on this trial and a companion CT-2106 every-3-week trial (10), it seems that good delivery of camptothecin is possible with an acceptable toxicity profile. It seems that either schedule is reasonable for efficacy assessment. Because the 3-weekly schedule is more convenient and weekly scheduling does not permit higher dose intensity, the 3-weekly schedule may be reasonably pursued. Currently, further testing is under way with this (and with other macromolecular camptothecin compounds) in disease-specific settings guided by preclinical data suggesting enhanced activity of CT-2106 compared with irinotecan and topotecan in models of ovarian, gastrointestinal, and non-small-cell lung cancers.

<sup>3</sup> S. Stromatt, C. Allievi, P. Angiuli, and A. Eisenfeld, unpublished data.

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