Phase I and Pharmacokinetic Study of Gimatecan Given Orally Once a Week for 3 of 4 Weeks in Patients with Advanced Solid Tumors

Andrew X. Zhu,[1] Neal Ready,[2] Jeffrey W. Clark,[1] Howard Safran,[2] Antonino Amato,[3] Nadia Salem,[3] Silvia Pace,[4] Xiaoying He,[1] Nela Zvereva,[1] Thomas J. Lynch,[1] David P. Ryan,[1] and Jeffrey G. Supko[1]

Abstract

Purpose: A phase I study was conducted to determine the dose-limiting toxicities (DLT) and maximum tolerated dose (MTD) of gimatecan, a lipophilic camptothecin analogue, administered orally once a week for 3 weeks.

Experimental Design: Adult patients with advanced solid tumors with good performance status and adequate hematologic, hepatic, and renal function were eligible for the study. The plasma pharmacokinetics of the drug was characterized during the initial 28-day cycle.

Results: A total of 33 patients were evaluated at 7 dose levels ranging from 0.27 to 3.20 mg/m^2/wk. Anemia, fatigue, neutropenia, nausea, and vomiting were the principal toxicities. DLTs experienced by 3 of 7 patients in dose level 7 (3.20 mg/m^2) were grade 2 hyperbilirubinemia and grade 3 to 4 fatigue. DLT (anorexia and nausea) occurred in only 1 of 11 patients evaluated at the MTD of 2.40 mg/m^2. There were no objective responses, although disease stabilization was observed in 4 patients. Gimatecan has a very long apparent biological half-life (mean $\pm$ SD, 77 $\pm$ 37 h) and exists in plasma almost entirely as the pharmacologically active intact lactone form. At the MTD, mean peak concentrations of the drug in plasma ranged from 67 to 82 ng/mL for the 3 weekly doses and the mean concentration 7 days after dosing was 15 $\pm$ 18 ng/mL.

Conclusions: Administration of gimatecan orally once a week at doses that are well tolerated provides continuous exposure to potentially effective plasma concentrations of the biologically active form of the drug. This regimen deserves further evaluation to define its antitumor activity in specific tumor types either alone or in combination with other agents.

Camptothecins are topoisomerase I inhibitors that have proven to be among the most active classes of antineoplastic agents against many types of malignancies (1). Two compounds in this class, topotecan and irinotecan, are approved for clinical use in the United States. Topotecan is presently indicated as a second-line therapy for advanced ovarian cancer and small cell lung cancer (2, 3). Irinotecan is approved for treating advanced colorectal cancer both as first-line therapy in combination with 5-fluorouracil and as salvage treatment in 5-fluorouracil refractory disease (4–6). The development of more efficacious and orally bioavailable camptothecins has since received considerable interest for several reasons. First, despite the early success of topotecan and irinotecan, their clinical activity has been limited to a few tumor types in contrast to the broad-spectrum antitumor activity exhibited in preclinical tumor models. Second, both drugs are subject to transport efflux mediated by several multidrug efflux proteins, which contributes to the resistance of cancer cells to camptothecins. Lastly, both compounds were developed for intravenous administration, and oral dosage forms, which would be advantageous for prolonged treatment schedules, have not been approved.

Gimatecan (7-[(*E*)-*tert*-butoxyiminomethyl]camptothecin) is an investigational orally bioavailable camptothecin analogue that exerts stronger and more persistent DNA cleavage than other members of the camptothecin family (7, 8). This is likely related to the presence of a highly lipophilic *O*-alkyl oxime substituent at position 7 of the camptothecin molecule. Gimatecan is 10 times more potent than topotecan against a variety of human tumor cell lines *in vitro*. It is also more active and has a wider therapeutic index than topotecan against human tumor xenografts in mice, including non-small cell lung cancer, colon, prostate, glioblastoma, and ovarian cancer when given orally (9, 10). Moreover, in contrast to other camptothecins, gimatecan showed activity against tumors expressing multidrug resistance mechanisms mediated by transport systems such as P-glycoprotein, breast cancer resistance protein, and multidrug resistance-associated protein (8, 11, 12).

Authors' Affiliations: [1]Massachusetts General Hospital, Dana-Farber/Harvard Cancer Center, Harvard Medical School, Boston, Massachusetts; [2]Rhode Island Hospital, Brown Medical School, Providence, Rhode Island; [3]Sigma-Tau Research, Inc., Gaithersburg, Maryland; and [4]Sigma-Tau Spa, Pomezia, Italy

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Note: A.X. Zhu and J.G. Supko contributed equally to this study.

Requests for reprints: Andrew X. Zhu, Massachusetts General Hospital, Dana-Farber/Harvard Cancer Center, Harvard Medical School, 55 Fruit Street, LH/POB 232, Boston, MA 02114. Phone: 617-724-0786; Fax: 617-724-3166; E-mail: azhu@partners.org.

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Translational Relevance

Camptothecins are topoisomerase I inhibitors that have proven to be among the most active classes of antineoplastic agents. Gimatecan is an investigational camptothecin analogue with several pharmacologic properties that are superior to those exhibited by either topotecan or irinotecan, the only camptothecins currently approved for clinical use. We performed a phase I study to determine the toxicities, MTD, and pharmacokinetics of gimatecan given orally, once a week for 3 weeks, in patients with advanced solid tumors. In notable contrast to other camptothecins, the pharmacokinetic behavior of gimatecan was actually found to be more favorable in humans than mice. Disease stabilization persisting for 4 to 6 months in 4 of 33 patients represented preliminary evidence of antitumor activity. In consideration of its enhanced lactone stability in plasma, oral bioavailability, and good tolerability, gimatecan warrants further evaluation, either alone or in combination with other agents, to assess its efficacy against solid tumors.

In general, the antitumor activity of the camptothecins in preclinical models is highly schedule dependent, with prolonged intermittent administration of relatively low doses consistently eliciting superior efficacy than shorter-term treatment with higher doses (13). Gimatecan exhibits analogous behavior, and schedules selected for evaluation in the initial phase I trials, repeated daily and weekly administration, were based on the two most effective dosing schedules identified in preclinical efficacy studies (8, 10, 11, 14). In particular, oral treatment with gimatecan at a relatively low dose (0.25 mg/kg) once daily for 5 days on 5 consecutive weeks or a higher dose (5 mg/kg) given 10 times at an interval of 8 to 10 days between doses achieved comparable therapeutic effects based on the rate of long-term disease-free mice bearing subcutaneously implanted xenografts of human lung and ovarian carcinoma cell lines (11). Therefore, although the initial phase I study of gimatecan done in Europe concurrently evaluated oral dosing on a daily times five schedule for 1, 2, or 3 weeks every 28 days (15), we undertook a phase I trial with an alternative schedule to administer the drug orally once a week for 3 of 4 weeks in patients with refractory solid tumors. The primary objectives of the study were to determine the maximum tolerated dose (MTD), identify the dose-limiting toxicities (DLT), and characterize the plasma pharmacokinetics of gimatecan when given according to this schedule.

Patients and Methods

Patient selection. The study was restricted to patients with a histologically confirmed solid tumor that was either refractory to conventional therapy or for which no standard treatment existed. Patients had to be at least ages 18 years with a minimum life expectancy of 3 months. The mandated time between prior treatment and entry into the study was ≥2 weeks for radiotherapy and ≥4 weeks for major surgery and chemotherapy, with the exception of chloroethylnitrosoureas and mitomycin C for which the minimum time interval was 6 weeks. Complete recovery from the effects of any earlier intervention was required. Minimum eligibility requirements of the protocol included the following: Eastern Cooperative Oncology Group performance status ≤2; absolute neutrophil count ≥2,000/μL; platelet count ≥100,000/μL; serum creatinine <1.5 times upper normal limit; total bilirubin <1.0 mg/dL, alkaline phosphatase ≤2.5 times upper normal limit, and aspartate aminotransferase and alanine aminotransferase ≤3 times upper normal limit; or, in case of liver metastases, total bilirubin <1.0 mg/dL, alkaline phosphatase ≤5 times upper normal limit, and aspartate aminotransferase and alanine aminotransferase ≤5 times the upper normal limit. Patients with evidence of symptomatic brain or leptomeningeal disease, occurrence of a myocardial infarction within 12 months, congestive heart failure or uncontrolled hypertension, uncontrolled infection, and pregnancy or breast-feeding were excluded. Patients with gastrointestinal dysfunction that could alter absorption or motility, such as an active peptic ulcer, inflammatory bowel disease, lactose intolerance, malabsorption syndromes, or gastrointestinal surgical procedure that could affect drug absorption, were excluded. Concurrent use of antibiotics in the previous 72 h, corticosteroids, and H_2 antagonists, antiacids, or proton pump inhibitors on treatment days was not permitted.

Drug administration and toxicity assessments. The protocol was approved by the Scientific Review Committee and Human Protection Committee of Dana-Farber/Harvard Cancer Care and Rhode Island Hospital. A signed written informed consent document satisfying all federal and institutional requirements was obtained as a condition of registration. Patients underwent a history, physical examination and performance status determination, an electrocardiogram and chest X-ray, a complete blood count with platelet and differential counts, a serum chemistry profile, and urinalysis within 14 days of initiating therapy.

Gimatecan was supplied by Sigma-Tau Research as capsules containing 0.1, 0.25, 0.5, and 1.0 mg drug. It was taken orally in the morning, either 1 h before or after eating, once every 7 days for 3 consecutive weeks. The dose was escalated from an initial level of 0.27 mg/m^2/wk using a modified Fibonacci method. Treatment was delivered on an outpatient basis whenever possible. Concurrent supportive care, including narcotics and antiemetics, was permitted as needed, although antiemetics were not given routinely until patients began to experience nausea and vomiting. Additional cycles of therapy were administered at intervals of 28 days to patients who continued to satisfy all pretreatment eligibility criteria. Treatment was discontinued on the occurrence of DLT or tumor progression.

A physical examination and determination of hematologic and serum chemistry variables were done every week and 1 month after the last dose of drug was received by patients removed from the study. Drug-related toxicities were evaluated during each 28-day cycle of therapy and graded according to the National Cancer Institute Common Toxicity Criteria version 2. DLT was defined as any of the following events attributable to the study drug that occurred during the first cycle of treatment: drug-related death; febrile neutropenia; grade 4 neutropenia persisting for at least 7 days; grade 4 thrombocytopenia; grade 3 to 4 nausea or vomiting despite antiemetic treatment; grade 3 to 4 diarrhea despite antidiarrhea management; grade 2 cardiac, liver, or renal toxicity; any other grade ≥3 nonhematologic toxicities; and failure to recover from toxicity after a 1-week delay in starting a subsequent cycle.

Cohorts of 3 patients were scheduled for entry into each dose level. The first patient had to be observed for ≥1 week before treating additional patients, who could be entered simultaneously. Escalation of the dose to the next higher level proceeded after all 3 patients had received the first cycle of therapy with the preceding dose and each was observed for at least 28 days without evidence of a DLT. Three additional patients were entered into a given dose level if a single patient experienced a DLT during cycle 1, with dose escalation proceeding in the absence of DLT in these patients. The occurrence of a DLT in 2 patients from any cohort of 3 to 6 during cycle 1 established the preceding dose level as the MTD. When the MTD was tentatively defined, this dose level was expanded to evaluate a total of 10 patients to better define the toxicity profile and confirm tolerance. Intrapatient

dose escalation was permitted for patients experiencing no adverse events grade ≥1 provided that evaluation of the next higher dose level had been satisfactorily completed.

Evaluation of response. A baseline assessment of all measurable disease using any appropriate radiologic technique was done within 21 days before starting therapy. Biochemical markers of disease, such as carcinoembryonic antigen, prostate-specific antigen, CA-125, or CA-19-9, were also determined whenever applicable and repeated after every second cycle. Reevaluation of malignant disease that was initially measured by physical examination or plain radiographs was done after each cycle. Tumors measured by computed tomography or magnetic resonance imaging were similarly evaluated after every 2 cycles of treatment. Response and progression were evaluated as recommended by the Response Evaluation Criteria in Solid Tumors Committee (16).

Pharmacokinetic studies. Study samples and solutions of the drug were protected from direct exposure to light because gimatecan is subject to photolytic degradation. Blood specimens (5 mL) were drawn from an arm vein into tubes containing freeze-dried sodium heparin before dosing and at the following times after taking the drug on weeks 1, 2, and 3 of cycle 1: 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 24, 48, 72, and 168 h. An aliquot of blood (1.0 mL) was removed from the collection tube for immediate centrifugation (10,000 × *g*, 1 min). Two 100 μL aliquots of plasma were pipetted into separate microcentrifuge tubes to which 20 μL internal standard (IS) working solution (20-*O*-butyryl-camptothecin, 200 ng/mL in acetonitrile) and 400 μL acetonitrile chilled to -70°C were promptly added. The tubes were vigorously mixed by vortexing and centrifuged (10,000 × *g*, 2 min), after which 400 μL supernatant was pipetted into another tube, standing in crushed dry ice, and stored at or below -70°C until assayed for the intact lactone form of gimatecan. The remainder of the blood sample, which had been allowed to stand in wet ice, was then centrifuged (1,300 × *g*, 4°C, 10 min) and the plasma was stored at or below -70°C until assayed for total gimatecan.

Gimatecan lactone and total gimatecan (intact lactone plus opened-ring carboxylate forms of the compound) were determined in plasma samples by high-performance liquid chromatography with fluorescence detection. Procedures comprising the assay were based on analytical methods that had been previously developed for pharmacokinetic studies of camptothecin and several of its analogues (17–19). Analytical reference samples of gimatecan and the IS were provided by Sigma-Tau. Working solutions of gimatecan were made weekly by quantitatively diluting the primary stock solution (0.1 mg/mL in acetonitrile) to 20 μg/mL with *N*,*N*-dimethylformamide/20 mmol/L aqueous trifluoroacetic acid (1:1, v/v). Calibration standards were made by serially diluting the working solution with human donor plasma to 8 concentrations ranging from 1 to 100 ng/mL. Quality-control solutions were similarly prepared with concentrations of 3, 15, 45, and 90 ng/mL.

Acetonitrile extracts of the plasma samples that had been stored for subsequent determination of gimatecan lactone were removed from the freezer, one at a time, and diluted with 100 μL of 100 mmol/L ammonium acetate buffer (pH 5.0). After vortexing, 100 μL of the solution were immediately injected into the high-performance liquid chromatography system. Another sample was removed from the freezer and diluted for analysis several minutes before the chromatographic run ended. Calibration standards and quality-control solutions were prepared for analysis according to the same procedure as described in the above for the study samples, except that 400 μL protein-free supernatant was diluted with 100 μL of 100 mmol/L trifluoroacetic acid and loaded into the autosampler for overnight analysis. Samples were prepared for determining total gimatecan by vigorously mixing 200 μL plasma, 20 μL IS working solution, and 800 μL acetonitrile in a microcentrifuge tube. After centrifugation (10,000 × *g*, 2 min), 800 μL supernatant was pipetted into another microcentrifuge tube and evaporated using a vacuum concentration system with a sample compartment temperature of 45°C. The extract was reconstituted for high-performance liquid chromatography by sequentially adding 75 μL *N*,*N*-dimethylformamide and 75 μL of 100 mmol/L trifluoroacetic acid and vortexing. The solution was transferred into an autosampler vial for batch analysis (injection volume, 100 μL).

Analyses were done using an Agilent 1100 series modular high-performance liquid chromatography system (Agilent Technologies) consisting of an isocratic pump, autosampler, and a column thermostat. Chromatography was done at 40°C on a Luna phenyl-hexyl high-performance liquid chromatography column (5 μm, 15 cm × 4.6 mm) preceded by a guard column (Phenomenex). The mobile phase was acetonitrile/1.0 mol/L ammonium acetate buffer (pH 5.0)/water (10:1:9, v/v/v) delivered at 1.0 mL/min. The eluent was monitored with a Hewlett-Packard model 1046 fluorescence detector with a pulsed xenon flash lamp and 305 nm cutoff filter. Operating variables of the detector were excitation wavelength, 248 nm; emission wavelength, 486 nm; lamp frequency, 220 Hz; photomultiplier gain, 13; and response time, 2 s. Chromatograms were integrated to provide peak areas.

Each study sample was assayed in duplicate, on different days, together with a complete set of calibration standards and quality-control samples. Standard curves were constructed by plotting the drug/IS chromatographic peak area ratio against the known drug concentration in each calibration standard. Linear least-squares regression was done with weighting in proportion to the reciprocal of the drug concentration normalized to the number of calibration standards. Values of the slope and γ intercept for the best-fit line of the calibration curve were used to calculate the analyte concentration in study samples. Specimens with an estimated drug concentration exceeding the upper range of the standard curve were reassayed on dilution with drug-free human plasma. The average of the two determinations of each study sample was calculated. Samples were also reassayed in cases where the individual determinations differed from their average by >10%.

The analytical method was thoroughly validated according to current recommendations (20). Gimatecan and the IS eluted as sharp, symmetrical peaks with retention times (mean ± SD) of 9.42 ± 0.16 and 7.71 ± 0.11 min, respectively. The retention time of the peak arising from photodegradation of the drug was 6.61 ± 0.07 min. Peaks that interfered with detection of gimatecan or the IS were not evident in chromatograms of drug-free plasma from several anonymous donors and plasma samples obtained shortly before dosing in subjects participating in this clinical investigation. Calibration curves exhibited excellent linearity with correlation coefficients >0.99. The lowest concentration of drug in the total gimatecan calibration curves, 1.0 ng/mL, was assayed with an accuracy of 92.4% and 9.7% precision. At all other concentrations, the between-day accuracy and precision ranged from 97.7% to 101.2% and from 1.6% to 4.7%, respectively. The lowest concentration of gimatecan lactone in the calibration curves, 5.0 ng/mL, was assayed with an accuracy of 97.9% and a precision of 5.7%. At all other concentrations, the between-day accuracy and precision ranged from 98.5% to 100.7% and from 3.0% to 5.1%, respectively.

Actual sample times were calculated relative to the beginning of drug ingestion to the sample collection time. Individual patient plasma concentration-time data for each weekly dose of gimatecan were analyzed by noncompartmental methods using WinNonlin Professional version 4.0.1 (Pharsight; ref. 21). The slope of the terminal disposition phase ($-\lambda_z$) was determined by logarithmic-linear regression and used to calculate the apparent biological half-life ($t_{1/2,z}$) as $0.693/\lambda_z$. Area under the plasma concentration-time curve (AUC) was estimated with the logarithmic-linear trapezoidal algorithm to the last time point with a measurable drug concentration before administration of the subsequent weekly dose (C_{last}) and extrapolating to infinity by addition of the quantity C_{last}/λ_z. Apparent oral clearance of the drug, CL/F, where CL is the total body clearance and F is the bioavailable fraction on oral administration, was calculated as the dose given to each patient divided by the AUC. In the event that the drug concentration was measurable in the sample obtained immediately before administration of the second or third weekly doses (C_0), CL/F was calculated after correcting the AUC to eliminate the contribution attributable to the prior dose by subtracting C_0 divided by λ_z for the preceding dose.

The accumulation factor for repeated dosing, X, was calculated by dividing the uncorrected AUC for the second and third doses by that for the first dose. Pharmacokinetic variables are reported as the geometric mean ± SD of values for individual patients at each dose level (22–24). Parametric statistical tests of pharmacokinetic variables were done using log-transformed values with $P < 0.05$ (two-tailed) considered to be significantly different.

Results

Patient characteristics. Characteristics of the 33 patients evaluable for toxicity assessments are listed in Table 1. There were 19 males and 14 females, with a median age of 61 years (range, 36-79 years), 29 of whom had a performance status of 0 or 1. The most common tumor types included colorectal cancer (14 patients; 42%), pancreatic cancer (5 patients; 15%), gastroesophageal cancer (4 patients; 12%), and lung cancer (4 patients; 12%). Other tumor types included cholangiocarcinoma (2 patients) and 1 patient each with melanoma, nasopharyngeal carcinoma, thymic carcinoma, and parotid gland carcinoma. All 33 patients had been previously treated, with 28 (85%) patients having received ≥3 prior regimens of chemotherapy and 15 (46%) patients had radiotherapy.

DLTs and determination of the MTD. The weekly dose of gimatecan was escalated from 0.27 to 3.20 mg/m^2 through 5 intermediate dose levels (Table 2). As noted earlier, the DLT definition in this study included grade 2 hepatic, cardiac, and renal toxicity in addition to the other typical hematologic and nonhematologic toxicities. There were no DLTs in patients evaluated in the initial 5 dose levels (≤1.87 mg/m^2). DLTs were experienced by 1 of 11 (9%) patients in dose level 6 (2.40 mg/m^2) and 3 of 7 (43%) patients in dose level 7 (3.20 mg/m^2), establishing 2.40 mg/m^2 as the MTD for the weekly administration schedule of gimatecan. DLTs in the single patient in dose level 6 were grade 4 anorexia, grade 3 nausea, and dehydration despite the use of antiemetics during cycle 1. The DLTs experienced by patients in dose level 7 were grade 2 hyperbilirubinemia and grade 3 to 4 fatigue.

Table 1. Patient characteristics

No. patients	33
Age (y), median (range)	61 (36-79)
Gender	
Male	19 (58)*
Female	14 (42)
Eastern Cooperative Oncology Group performance status	
0	5 (15)
1	26 (79)
2	2 (6)
Primary tumor site	
Colorectal	14 (42)
Pancreatic	5 (15)
Lung, non-small cell	4 (12)
Gastroesophageal	4 (12)
Cholangiocarcinoma	2 (6)
Other	4 (12)
Prior chemotherapy regimens	
0-2	7 (21)
≥3	26 (79)
Prior radiotherapy	15 (46)

*Values are *n* (percentage of total) patients unless otherwise defined.

Table 2. Summary of toxicities for cycle 1 of therapy

	Grade 2/3/4 toxicity at each dose level						
Dose level	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Weekly dose (mg/m^2)	0.27	0.53	0.88	1.33	1.87	2.40	3.20
No. patients evaluated	3	3	3	3	3	11	7
Hematologic toxicity							
Anemia	1/0/0			2/0/0		4/1/0	1/1/0
Neutropenia						3/0/0	1/0/1
Lymphopenia		1/0/0				1/1/0	0/1/0
Thrombocytopenia	0/1/0					1/0/0	0/1/0
Nonhematologic toxicity							
Nausea		1/0/0				3/1/0	1/0/0
Vomiting						3/0/0	1/0/0
Fatigue						2/0/0	0/1/1
Anorexia						1/0/1	
Dehydration						0/1/0	1/0/0
Hyperbilirubinemia							1/0/0
Hyperglycemia				1/0/0			

NOTE: Entries were not made for dose levels where the indicated toxicity was not observed.

Toxicities. Clinically significant toxicities observed during cycle 1 at each dose level categorized as being at least possibly related to treatment are summarized in Table 2. The frequency and severity of the toxicities increased as the gimatecan dose was escalated. Table 3 summarizes all grade 2 to 4 toxicities considered to be at least possibly treatment related that occurred during all 60 cycles of therapy. A total of 32 (97%) patients experienced at least one adverse event. Eighteen (55%) patients experienced at least one adverse event of grade ≥2 and grade 3 to 4 toxicities occurred in 14 (42%) patients. The most common treatment-related toxicities were hematologic, gastrointestinal, and fatigue. Hematologic toxicities included neutropenia, lymphopenia, anemia, and thrombocytopenia. The gastrointestinal toxicities included nausea and vomiting but no grade ≥2 diarrhea. One patient died during treatment due to a perforated bowel that was considered to be unrelated to the drug.

Antitumor activity. None of the 26 patients evaluable for efficacy assessments achieved a complete or partial response. Four (15%) patients had stable disease: 1 patient with melanoma had 4 cycles of treatment at the 0.27 mg/m^2 dose level, 1 patient with colon cancer had 6 cycles of 0.88 mg/m^2, 1 patient with cholangiocarcinoma had 4 cycles of 1.33 mg/m^2, and 1 patient with pancreatic cancer had 4 cycles of 2.40 mg/m^2. Twenty-two (85%) patients had progressive disease.

Pharmacokinetics. Mean values of the pharmacokinetic variables for total gimatecan in the cohorts evaluated at each dose level are presented in Table 4 for each weekly dose. The mean plasma concentration-time profile for total gimatecan in 11 patients treated with the MTD of 2.40 mg/m^2 is shown in Fig. 1. Following administration of the first weekly dose to

Table 3. Treatment-related toxicities for all cycles of therapy

Adverse event	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
Anemia	8 (24)	5 (15)	
Fatigue	6 (18)	1 (3)	1 (3)
Neutropenia	5 (15)		2 (6)
Nausea	5 (15)	1 (3)	
Vomiting	5 (15)		
Leukopenia	4 (12)	1 (3)	1 (3)
Anorexia	3 (9)		1 (3)
Lymphopenia	2 (6)	3 (9)	
Dehydration	2 (6)	1 (3)	
Thrombocytopenia	1 (3)	2 (6)	
Prolonged prothrombin time	1 (3)		
Hyperbilirubinemia	1 (3)		
Dyspnea	1 (3)		
Hyperglycemia	1 (3)		

NOTE: The toxicities recorded represent the maximum grade toxicity observed for a given patient for the entire course of therapy.

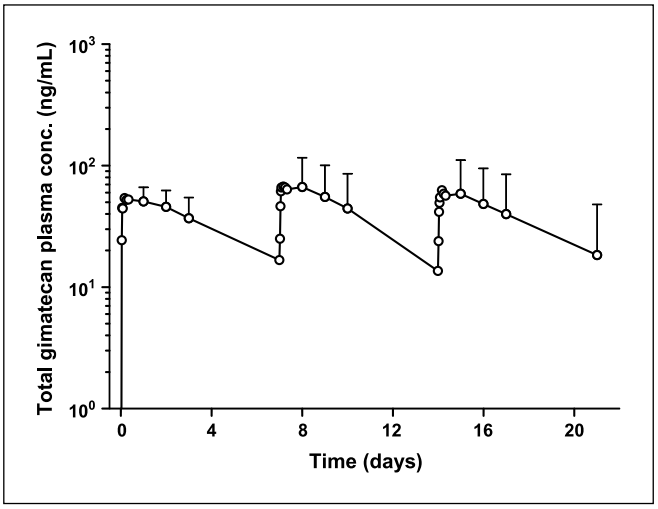

Fig. 1. Mean plasma concentration-time profile for total gimatecan in the group of 11 patients treated at the MTD of 2.40 mg/m^2. *Points,* mean concentrations of the drug in plasma at each sample time (*open circles*), connected by line segments; *bars,* SD.

patients enrolled at all 7 dose levels, measurable levels of total gimatecan (>1.0 ng/mL) were observed in the first plasma sample obtained ~30 min after dosing in 66% (21 of 32) of the patients, in the second sample obtained at 1 h in 28% of the patients (9 of 32), and at a later time (8 h) in only 1 patient. The median time of the observed maximum concentration of drug in plasma (t_{max}) was 2.0 h after dosing, with values ranging from 0.6 to 47.8 h, for the 94 doses for which pharmacokinetic data were obtained. Gimatecan was eliminated from plasma very slowly, with an apparent terminal phase half-life of 76.5 ± 37.3 h for the first dose; thus, the drug remained in plasma at significant concentrations for >7 days after dosing. As shown in Fig. 2, the observed C_{max} and AUC of total gimatecan for the first weekly dose were significantly correlated with the administered dose. Moreover, the absence of a significant correlation between CL/F and dose is indicative of linear pharmacokinetic behavior for the drug at the range of doses evaluated (Fig. 2C). In addition, there were no significant differences between overall mean values of the t_{max}, $t_{1/2,z}$, or CL/F for the first, second, and

Table 4. Mean pharmacokinetic variables for total gimatecan

Dose (mg/m^2)	Dose no.	No. patients	C_0 (ng/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2,z}$ (h)	AUC (ng h/mL)	CL/F (L/h/m^2)	X
0.27	1	3	<1.0	13.3 ± 9.3	8.9 ± 12.7	51.2 ± 8.3	910 ± 590	0.29 ± 0.23	
	2	3	<1.0	12.8 ± 6.6	4.5 ± 3.3	58.3 ± 14.6	1,039 ± 492	0.26 ± 0.12	1.14 ± 0.25
	3	3	2.1 ± 0.5	9.6 ± 7.3	2.4 ± 1.6	70.0 ± 45.0	904 ± 888	0.32 ± 0.23	0.99 ± 0.62
0.53	1	3	<1.0	28.8 ± 9.2	0.9 ± 0.3	113.8 ± 27.6	2,212 ± 413	0.24 ± 0.05	
	2	3	5.3 ± 1.7	29.9 ± 12.8	1.2 ± 0.5	109.7 ± 6.9	3,279 ± 169	0.22 ± 0.06	1.48 ± 0.16
	3	3	6.4 ± 0.7	27.9 ± 5.0	1.2 ± 0.3	110.8 ± 56.8	3,499 ± 795	0.22 ± 0.08	1.58 ± 0.15
0.88	1	3	<1.0	27.6 ± 14.5	9.0 ± 13.4	88.3 ± 38.0	3,327 ± 2,116	0.26 ± 0.16	
	2	3	7.3 ± 5.0	34.9 ± 8.1	9.8 ± 12.7	114.3 ± 21.8	4,800 ± 4,035	0.23 ± 0.15	1.44 ± 0.39
	3	3	10.4 ± 9.1	44.9 ± 7.9	1.5 ± 0.5	123.0 ± 76.9	6,726 ± 6,477	0.18 ± 0.13	2.02 ± 0.71
1.33	1	3	<1.0	46.4 ± 34.2	18.0 ± 25.9	84.3 ± 57.7	5,992 ± 9,114	0.21 ± 0.41	
	2	3	10.0 ± 19.1	56.0 ± 37.2	9.0 ± 12.9	99.7 ± 61.9	7,892 ± 12,437	0.23 ± 0.24	1.32 ± 0.37
	3	3	14.8 ± 27.9	59.0 ± 43.1	1.5 ± 0.0	100.8 ± 64.9	8,539 ± 14,372	0.23 ± 0.22	1.43 ± 0.68
1.87	1	3	<1.0	36.4 ± 17.9	2.0 ± 1.7	65.2 ± 14.6	3,078 ± 1,726	0.62 ± 0.37	
	2	3	8.6 ± 5.5	40.7 ± 12.8	10.8 ± 11.5	52.8 ± 27.2	3,733 ± 2,577	0.57 ± 0.40	1.22 ± 0.17
	3	3	8.0 ± 6.3	59.6 ± 3.3	4.6 ± 3.1	59.6 ± 35.2	5,270 ± 2,031	0.41 ± 0.11	1.71 ± 0.45
2.40	1	11	<1.0	66.9 ± 12.3	20.6 ± 49.5	80.6 ± 39.4	7,757 ± 4,861	0.31 ± 0.19	
	2	11	16.7 ± 17.0	82.0 ± 33.4	9.0 ± 9.7	71.5 ± 34.1	9,115 ± 7,930	0.37 ± 0.29	1.09 ± 0.41
	3	10	13.6 ± 18.8	75.4 ± 41.6	13.7 ± 15.7	79.8 ± 39.0	8,568 ± 8,826	0.38 ± 0.33	1.06 ± 0.59
3.20	1	6	<1.0	66.3 ± 36.7	9.0 ± 7.6	67.3 ± 44.4	7,364 ± 5,609	0.43 ± 0.37	
	2	6	9.6 ± 11.2	86.5 ± 38.7	10.3 ± 11.1	71.5 ± 35.9	9,212 ± 6,689	0.42 ± 0.28	1.30 ± 0.20
	3	6	16.4 ± 15.3	86.1 ± 40.8	10.2 ± 11.1	88.5 ± 40.7	10,825 ± 7,933	0.38 ± 0.30	1.54 ± 0.40
All	1	32			3.5 ± 4.2	76.5 ± 37.3		0.33 ± 0.24	
	2	32			4.4 ± 5.1	76.5 ± 36.5		0.33 ± 0.23	1.25 ± 0.33
	3	31			3.3 ± 4.0	86.0 ± 44.5		0.32 ± 0.24	1.35 ± 0.64

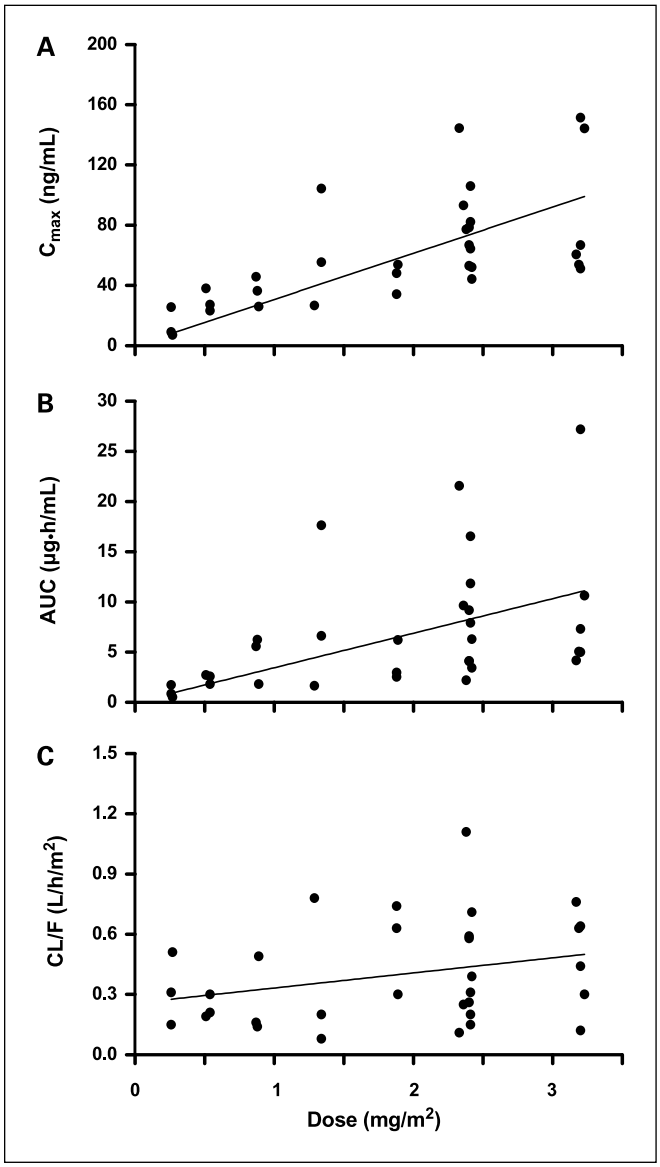

Fig. 2. Plots showing the relationship between the maximum plasma concentration (C_{max}; *A*), AUC from zero to infinity (*B*), and apparent oral clearance (CL/F; *C*) of gimatecan in individual patients for the initial dose. *Points,* observed values in individual patients; *solid lines,* generated from linear regression analysis of the data.

third weekly doses. The magnitude of interpatient variability in systemic drug exposure was high, as the overall mean CL/F values for the 3 weekly doses exhibited coefficients of variation ranging from 68.4% to 73.0%. In contrast, variability in the CL/F for each weekly dose in individual patients was relatively low, with a median value of only 20.9% (range, 1.5-83.2%). The average accumulation factor for once weekly dosing was 1.25 ± 0.33 for the second dose and 1.35 ± 0.64 for the third dose. As illustrated in Fig. 3, essentially 100% of the drug remained in the intact lactone form in all plasma samples that were assayed for both total drug and intact lactone. Accordingly, pharmacokinetic variables were not estimated from the intact lactone data, as the results would be the same as the analysis of the total drug data.

Discussion

Anticancer activity of the camptothecins is dependent on the intrinsic chemical reactivity of an α-hydroxy-δ-lactone ring (1). The lactone is also susceptible to reversible hydrolysis under physiologic conditions and the resulting opened-ring carboxylate product is biologically inactive. The equilibrium position between the active lactone and inactive carboxylate forms of the molecule in whole blood is influenced by their relative affinities for binding to plasma proteins and the extent to which the lactone form partitions into the lipid bilayer of erythrocyte cellular membranes (25, 26). Alterations in protein binding affinity and erythrocyte partitioning imparted by the presence of substituent groups on the quinoline subunit, a region of the molecule distal to the lactone ring, can dramatically affect the lactone-carboxylate equilibrium position.

Topotecan and irinotecan are both semisynthetic camptothecin analogues designed to facilitate administration of the intact lactone form in conventional parenteral vehicles by introducing a basic functional group on the quinoline moiety to enhance water solubility. Whereas topotecan is intrinsically cytotoxic, irinotecan is a prodrug that is inefficiently converted by human carboxylesterases, predominantly in the liver, to the biologically active compound, 7-ethyl-10-hydroxycamptothecin (27). The percentage of these compounds present in the active lactone form at equilibrium in human whole blood *in vitro* is only 12% for topotecan, 21% for irinotecan, and 20% for 7-ethyl-10-hydroxycamptothecin (28). Consequently, the inactive carboxylate form accounts for a relatively high fraction of the total concentrations of these compounds in plasma following administration to humans, ranging from 60% to 70% for topotecan to 25% to 49% for 7-ethyl-10-hydroxycamptothecin (1). Investigations undertaken to improve these pharmacologically disadvantageous properties revealed that the lactone-carboxylate equilibrium position can be shifted in favor of the lactone form in human blood by the presence of a

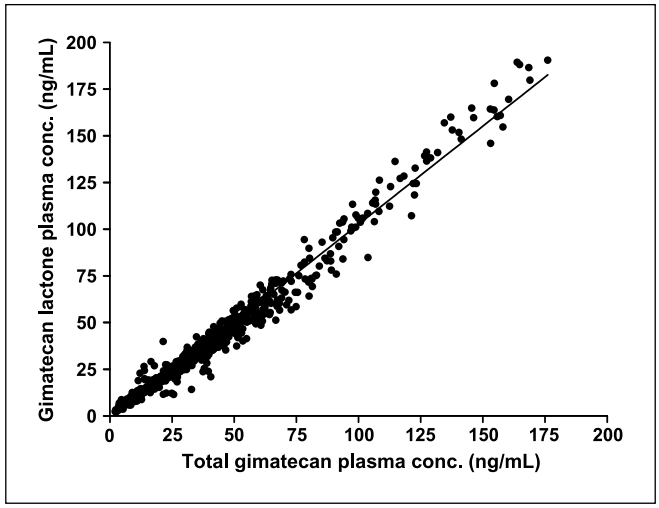

Fig. 3. Correlation between the concentrations of gimatecan lactone and total gimatecan in all 580 plasma samples obtained during the course of the clinical trial that were assayed for both forms of the drug. As indicated by the slope of the regression line (r = 0.988; slope = 1.05), 100% of the drug remained in the intact lactone form in plasma, with no discernable change over time.

lipophilic functional group at the 7-position of the camptothecin ring system (25, 26). In addition to greater lactone stability in blood, the potential therapeutic effectiveness of these highly lipophilic camptothecin derivatives may be augmented by greater tissue penetration and improved oral bioavailability (29, 30).

Karenitecin is the first compound of this type to be introduced into clinical trials (31). It is a camptothecin derivative with an alkylsilane substituent in the 7-position that that exists predominantly as the lactone form in plasma (30, 32). Clinical studies have only evaluated intravenous administration of the compound, although it exhibits excellent antitumor activity and good bioavailability when given orally to laboratory animals. Gimatecan differs from the alkylsilane camptothecins in that the lipophilic substituent in position-7 is a *tert*-butoxyiminomethyl group (7). Initial phase I clinical trials of gimatecan were designed to evaluate its administration by the oral route. This was considered to be advantageous for an anticancer agent that may require prolonged treatment for optimal therapeutic effectiveness because it minimizes the considerable expense and inconvenience to patients associated with frequent visits to an infusion clinic for receiving an intravenous therapy.

In this phase I study, the MTD for administering gimatecan orally once a week for 3 consecutive weeks during a 4-week treatment cycle was found to be 2.40 mg/m^2. The major toxicities of the drug, which included anemia, neutropenia, leucopenia, nausea, vomiting, and fatigue, were predominantly associated with the gastrointestinal tract and hemolymphopoietic system. In contrast to other camptothecins, patients treated with gimatecan did not experience significant diarrhea or alopecia. The frequency and severity of hematologic toxicities appeared to increase with escalation of the dose. Although the DLT for weekly gimatecan was not hematologic, the toxicity profile was generally similar to that reported in the phase I study evaluating the repeated daily administration of the drug, for which the main toxicity was myelosuppression with dose-limiting thrombocytopenia (15). It is entirely conceivable that the two schedules for delivering this drug, which have been evaluated, 3 of 4 weeks in this study and daily times 5 in the other phase I trial, could produce different DLTs, although the total drug administered (and hence AUC) at the MTDs were similar due to schedule-dependent differences in the pattern of systemic drug exposure (shorter exposure to higher drug levels versus longer exposure to lower drug levels).

There were no objective responses, although 4 patients exhibited disease stabilization in this study, which included patients with a variety of different malignancies who had failed multiple prior treatment regimens. In the phase I trial of the repeated daily schedules of gimatecan, 6 partial responses (endometrial cancer, non-small cell lung cancer, cervical cancer, and breast cancer) were observed in 97 evaluable patients (15). Early evidence of antitumor activity was also observed in patients with breast and ovarian cancers (33, 34). Disease-specific phase II/III studies will need to be undertaken to determine whether this agent is active either alone or in combination with other agents.

Gimatecan exhibited apparent linear pharmacokinetics following oral administration at the range of doses evaluated in this study. It is absorbed rapidly as evidenced by the occurrence of peak concentrations of the drug in plasma within 4 h for the majority (64%) of doses given. The absolute bioavailability of the compound could not be determined because a parenteral formulation suitable for intravenous administration to humans has not been developed. The magnitude of interpatient variability in systemic drug exposure was relatively large as indicated by the coefficient of variation of the mean CL/F, which ranged from 70% to 76% for the 3 weekly doses among the entire cohort of patients. In contrast, intrapatient variability was relatively low, with a median coefficient of variation of only 21% for the mean CL/F in individual patients (range, 2-83%). The very long biological half-life (77 $\pm$ 37 h) and prevalence of the intact lactone form of the compound in plasma are the most distinctive and therapeutically advantageous pharmacokinetic characteristics of gimatecan. The drug was found to exist in plasma almost entirely in the lactone form without evidence of significant conversion to the opened-ring carboxylate species in samples obtained up to 7 days after dosing. These findings are in excellent agreement with the pharmacokinetic data presented in the report of the phase I trial of the daily administration schedules of gimatecan that were done in Europe (15).

Pharmacokinetic studies in mice, in which the total concentration of gimatecan in plasma was measured, revealed that an orally administered dose of 5 mg/kg provided a mean C_{max} of 307 $\pm$ 70 g/mL and an AUC of 2,459 ng h/mL (11). In comparison, the mean C_{max} of the drug in cancer patients treated with the 2.40 mg/m^2 MTD for the weekly schedule of the drug was $\sim$5 times lower (67 $\pm$ 12 ng/mL). However, overall systemic exposure as indicated by the AUC was >3 times greater in humans (7,757 $\pm$ 4,861 ng h/mL) than mice. This is undoubtedly attributable to the much longer biological half-life of the drug in humans than in mice (12 h). Camptothecin analogues such as topotecan, irinotecan, 9-aminocamptothecin, and 9-nitrocamptothecin all exhibit impressive activity against experimental tumor models, whereas their anticancer activity in patients is modest at best. This has been attributed to the ability of mice to tolerate doses that provide a substantially greater systemic exposure to the active lactone form of these compounds than can be achieved in humans (35). Therefore, in notable contrast to other camptothecin analogues, the pharmacokinetic behavior of gimatecan actually appears to be more favorable in humans than mice.

The total dose delivered during a 4-week cycle of therapy was similar at the MTDs of the weekly times 3 (7.2 mg/m^2) and daily times 5/weekly times 3 weeks schedules (6.4 mg/m^2). Therefore, total system exposure to the drug is very similar at the MTD of both administration schedules as a consequence of the linear pharmacokinetic behavior of the drug. The primary difference is that drug accumulation on repeated dosing is greater for the daily schedule (4.2 times) than for dosing once a week (1.4 times). The C_{max} increases continually throughout the 3 weeks of repeated daily treatment, from a mean of 22 $\pm$ 9 ng/mL for the first 0.45 mg/m^2 dose to 70 $\pm$ 35 ng/mL for the 15th and final dose of the first cycle of therapy (15). It is notable that the mean C_{max} values for each of the 3 weekly doses of 2.4 mg/m^2, which ranged from 67 to 82 ng/mL, are similar to the mean C_{max} achieved with the final dose of the daily schedule. Therefore, the weekly dosing regimen provides more prolonged systemic exposure to higher concentrations of the drug than the daily schedule. In addition, plasma concentrations of the drug remained within an unusually

narrow range for a weekly oral dosing regimen, with a mean peak-to-trough ratio of only 4.8. As a consequence of its long biological half-life, administering gimatecan more frequently than once a week confers no pharmacologic advantages based on systemic drug exposure.

In conclusion, the recommended dose of oral gimatecan for future studies in adults is 2.40 mg/m^2 once a week for 3 consecutive weeks, with additional cycles of therapy given every 4 weeks. Oral gimatecan is well tolerated and has favorable pharmacokinetic properties. The drug remains almost entirely in the active, intact lactone form in plasma, and the long biological half-life supports once weekly dosing. In consideration of its broad and superior preclinical antitumor activity along with enhanced lactone stability and good tolerability, compared with other camptothecin analogues, this agent warrants further clinical evaluation, either alone or in combination with other agents, in a range of solid tumor types.

Disclosure of Potential Conflicts of Interest

A. Amato, N. Salem, and S. Pace are employed by SigmaTau.

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