A Phase I and Pharmacokinetic Study of Pemetrexed Plus Irinotecan in Patients with Advanced Solid Malignancies

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Abstract

Purpose: The main objectives of this phase I and pharmacokinetic, open-label study were to characterize the principal toxicities and determine the maximum tolerated dose of the multitargeted antifolate pemetrexed administered in combination with irinotecan. The study also sought to detect major pharmacokinetic drug-drug interactions between these agents and preliminary evidence of antitumor activity in patients with advanced solid malignancies.

Experimental Design: Pemetrexed was administered as a 10-min i.v. infusion followed by irinotecan given i.v. over 90 min every 3 weeks to patients with advanced solid malignancies. The study objectives were first pursued in heavily pretreated patients and then in lightly pretreated patients who also received vitamin supplementation.

Results: Twenty-three heavily pretreated patients enrolled in the first stage of the study, and the maximum tolerated dose level of pemetrexed/irinotecan without vitamin supplementation was 400/250 mg/m^2; further dose escalation was precluded by severe neutropenia that was protracted and/or associated with fever. In the second stage of the study, 28 lightly pretreated patients were administered pemetrexed/irinotecan with vitamin supplementation; these patients tolerated pemetrexed/irinotecan at a dose level of 500/350 mg/m^2, which reflected clinically relevant single-agent doses of both agents. No major pharmacokinetic interactions between the agents were evident. Four patients, two patients each with colorectal cancer refractory to fluoropyrimidines and advanced mesothelioma, had partial responses.

Conclusions: The pemetrexed/irinotecan regimen is well tolerated in patients with advanced solid malignancies at clinically relevant single-agent doses. The recommended dose level of pemetrexed/irinotecan for subsequent disease-directed evaluations involving lightly pretreated patients is 500/350 mg/m^2 every 3 weeks with vitamin supplementation.

Targeting folate-dependent enzymes that are involved in the synthesis of DNA and critical cellular elements has been one of the most effective strategies in our therapeutic armamentarium for treating cancer since the introduction of aminopterin and methotrexate >50 years ago (1). Pemetrexed (Alimta, Eli Lilly and Company, Indianapolis, IN), a multitargeted antifolate, is one of the few new agents of this class that has received regulatory approval for cancer treatment since that time.

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Received 6/30/06; revised 10/16/06; accepted 10/26/06.

Grant support: Eli Lilly and Company, Indianapolis, IN.

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Note: E.K. Rowinsky was affiliated with the Institute for Drug Development, Cancer Therapy, and Research Center during this study.

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doi:10.1158/1078-0432.CCR-06-1606

Pemetrexed, a pyrrolopyrimidine-based antifolate, is a potent inhibitor of several folate-dependent enzymes that are involved in the *de novo* pathways of cellular purine and pyrimidine synthesis, including thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase (2–6). The novel multitargeted action of pemetrexed is generally thought to contribute to its clinical effects. Pemetrexed has shown broad clinical antitumor activity in patients with colorectal, pancreatic, and breast cancers (7) and has received regulatory approval worldwide for treating patients with malignant mesothelioma and non–small cell lung cancer (8).

Multiagent regimens, which consist of the thymidylate synthase inhibitor 5-fluorouracil (5-FU) and irinotecan, a topoisomerase I–targeting camptothecin and prodrug of SN-38 that interferes with DNA replication by inducing double-strand DNA breaks, have shown efficacy in the first- and second-line metastatic settings involving patients with colorectal cancer (9–12). The clinical activity shown by the inhibition of thymidylate synthase in tumor cells, a mechanism of action common to both 5-FU and pemetrexed, suggests that pemetrexed contributes to the activity of irinotecan-based regimens in treating patients with colorectal cancer and other malignancies in which both pemetrexed and irinotecan have shown relevant single-agent activity. In addition, pemetrexed and

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irinotecan have nonoverlapping mechanisms of antitumor activity and resistance, and there has been preclinical evidence of favorable interactions, including synergy, following treatment of pancreatic, colorectal, and breast cancers with both agents in combination in preclinical studies (13, 14). Furthermore, chemotherapy regimens that consist of irinotecan and the prototypical, albeit distinctly different, thymidylate synthase inhibitor 5-FU have been widely incorporated into clinical practice, particularly in treating patients with colorectal cancer because of the repeated demonstration of improved survival in prospectively randomized clinical trials (10–12). Collectively, these observations served as the rationale for evaluating the pemetrexed/irinotecan combination in a phase I trial in patients with advanced solid malignancies. The principal objective of this open-label, dose-finding study was to determine the maximum tolerated dose (MTD) level of pemetrexed and irinotecan. Secondary objectives included an assessment of the pharmacokinetics of the agents in combination, principally to detect major drug-drug interactions and to evaluate safety and preliminary antitumor activity.

Patients and Methods

Eligibility criteria. Patients were eligible for the study if they met the following criteria: histologic or cytologic diagnosis of metastatic or locally advanced cancer; disease refractory to standard therapies or if no therapy of potential benefit exists; bidimensionally measurable or evaluable disease; no prior radiation therapy to bone marrow exceeding 25% of hematopoietic reserves; an Eastern Cooperative Oncology Group performance status of 0 to 2; and adequate hematopoietic (absolute granulocyte count $\geq 1.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L, and hemoglobin ≥ 9 g/dL), hepatic [bilirubin within normal limits and aspartate transaminase and alanine transaminase ≤ 3.0 times upper limit of normal (aspartate transaminase and alanine transaminase ≤ 5 times upper limit of normal is acceptable if liver has tumor involvement)], and renal (calculated creatinine clearance ≥ 45 mL/min) functions. Prior chemotherapy was allowed if it had been completed at least 3 weeks before study entry. Patients who were pregnant or breast-feeding were not eligible for the study. Ineligibility also included prior treatment with pemetrexed or irinotecan, unwillingness or inability to take vitamin B_{12} or multivitamin supplementation, a body surface area (BSA) exceeding 3.0 m^2, or an active infection or other coexisting medical condition whose severity might limit compliance with the study.

Investigators were required to conduct this study according to good clinical practice guidelines and the Declaration of Helsinki. The institutional review boards of participating centers approved the study, and patients gave written informed consent before enrollment.

Study design. This phase I, dose-finding, open-label study of the combination of pemetrexed and irinotecan followed a dose-escalation scheme in which at least three patients were treated at a given dose level for a minimum of two cycles. A cycle was defined as an interval of 3 weeks following the administration of pemetrexed/irinotecan on day 1. If a dose-limiting toxicity (DLT) was observed in a single patient during the first two cycles, three additional patients were to be treated at that dose level. If two patients had DLT, there was no further dose escalation, and three to six additional patients were to be treated at the previous dose level. If, at this dose level, a DLT was not observed in an additional patient during the first two cycles, it represented the MTD level of the combination.

Toxicities were graded according to the National Cancer Institute-Common Toxicity Criteria, version 1 (15). DLT was defined as grade 4 neutropenia (absolute granulocyte count $<0.5 \times 10^9$/L) exceeding 5 days or associated with fever or infection; grade 4 thrombocytopenia (nadir platelets $< 25 \times 10^9$/L); any grade 3 or 4 nonhematologic toxicity except alopecia, nausea, and vomiting, or diarrhea in patients who received optimal treatment with antiemetic or antidiarrheal premedication and supportive measures; and the delay of the next course of treatment by >2 weeks due to unresolved toxicity in patients who had experienced grade 3 thrombocytopenia, grade 4 neutropenia, or any grade 3 nonhematologic toxicity (except alopecia or optimally treated nausea, vomiting, or diarrhea).

Patients who were initially enrolled in the study were considered heavily pretreated and showed unacceptably low tolerance to treatment with pemetrexed and irinotecan (heavily pretreated cohort). In retrospect, these patients were felt not to be entirely representative of those who would likely be evaluated in subsequent disease-directed studies of this regimen; therefore, the protocol was amended to include a second stage using a revised dose level schedule and multivitamin supplementation in lightly pretreated patients defined a priori. Patients meeting the lightly pretreated criteria could not have received more than one prior chemotherapy regimen in the adjuvant setting. One prior chemotherapy regimen for advanced disease was also allowed if administered at least 6 weeks before enrollment with full recovery. No more than six cycles of a regimen containing an alkylating agent (except low-dose cisplatin) or four cycles of a carboplatin-containing regimen were permitted, and no prior mitomycin C was allowed. Prior radiation therapy to <25% of bone marrow was allowed when full recovery preceded study entry. Prior radiotherapy to the whole pelvis was not allowed.

In the same time period that the protocol was being amended to account for the extent of prior treatment, emerging study data regarding

Table 1. Patient characteristics

Characteristic	HPT cohort (*n* = 23)	LPT cohort (*n* = 28)
Gender, *n* (%)		
Female	6 (26)	6 (21)
Male	17 (74)	22 (79)
Age, median (range)	54 (30-78)	64 (35-74)
ECOG performance status, *n* (%)		
0	5 (22)	13 (46)
1	13 (57)	15 (54)
2	5 (22)	0
Prior therapy, *n* (%)		
Prior surgery	10 (43)	19 (68)
Prior radiotherapy	11 (48)	7 (25)
Prior chemotherapy	20 (87)	19 (68)
Fluoropyrimidine-containing regimens	11 (48)	7 (25)
Other regimens	14 (61)	14 (50)
One prior regimen	13 (57)	19 (68)
Two prior regimens	8 (35)	3 (11)
Three prior regimens	6 (26)	0
Prior hormonal, immune, other	4 (17)	3 (11)
Tumor type, *n* (%)		
Mesothelioma	0	12 (43)
Colorectal	5 (22)	5 (18)
Adenocarcinoma, unknown primary	4 (17)	2 (7)
Non–small cell lung cancer	3 (13)	4 (14)
Pancreas	3 (13)	2 (7)
Liver	2 (9)	1 (4)
Neuroendocrine carcinoma	1 (4)	1 (4)
Breast	1 (4)	0
Osteogenic sarcoma	1 (4)	0
Esophagus	1 (4)	0
Squamous cell carcinoma	1 (4)	0
Small intestine	1 (4)	0
Choloangiocarcinoma	0	1 (4)

Abbreviations: HPT, heavily pretreated; LPT, lightly pretreated; ECOG, Eastern Cooperative Oncology Group.

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the principal toxicities of pemetrexed indicated that homocysteine and methylmalonic acid levels, markers for folate and vitamin B_{12} deficiency, were highly correlated with several key toxicities (16). This finding led to the requirement that all subsequent patients participating in pemetrexed trials receive vitamin supplementation with oral folic acid (350-600 μg) given daily 1 to 2 weeks before the first cycle and continued until study therapy discontinuation and vitamin B_{12} (1,000 μg) given i.m. 1 to 2 weeks before the first cycle and repeated every 9 weeks until the study therapy discontinuation. Thus, all lightly pretreated patients enrolled in the second stage of the study also received the aforementioned vitamin supplementation regimen.

Treatment plan. The starting dose level of pemetrexed/irinotecan was 300/175 mg/m^2 in the heavily pretreated cohort, which represents 50% to 60% of doses indicated for each agent as single agents, and 450/250 mg/m^2 in the lightly pretreated cohort. The BSA was calculated at the beginning of each cycle. Pemetrexed was administered as a 10-min i.v. infusion followed immediately by irinotecan administered as a 90-min i.v. infusion on day 1 of a 3-week cycle.

Premedication for pemetrexed consisted of dexamethasone 4 mg orally, twice daily for 3 days starting the day before pemetrexed treatment. Patients also received the following antiemetic medications 30 min before irinotecan: dexamethasone 10 mg i.v., ondansetron 32 mg i.v. or granisetron 10 μg/kg i.v., and lorazepam 1 to 2 mg i.v. Patients were required to have an absolute granulocyte count of at least 1.5×10^9/L and a platelet count of at least 100×10^9/L before treatment on the first day of each cycle. If hematologic or nonhematologic toxicity did not resolve to requisite levels within 2 weeks of the planned day of retreatment, the doses of both agents were to be reduced in the next cycle by one dose level.

Dose adjustments during the study were based on hematologic variable values and maximum Common Toxicity Criteria grade nonhematologic values in the preceding cycle. Treatment may have also been delayed due to insufficient folic acid or vitamin B_{12} supplementation. The occurrence of the following events resulted in reduction by one dose level in the next cycle: grade 4 neutropenia exceeding 5 days, any grade 4 thrombocytopenia, grade 3 thrombocytopenia or grade 4 neutropenia resulting in delayed administration of a subsequent cycle by >2 weeks, grade 3 or 4 nausea or vomiting in patients who received an optimal antiemetic premedication and support, grade 3 or 4 diarrhea in patients who received optimal antidiarrheal premedication and support (for these patients, the dose of irinotecan only was reduced by one dose level in the next cycle), and any grade 3 or 4 toxicity resulting in delayed administration of a subsequent cycle by >2 weeks due to unresolved toxicity.

Clinical assessments, pretreatment, and follow-up. Medical histories, physical examinations, and performance status evaluations were done before treatment and weekly thereafter. Weekly laboratory evaluations included complete blood cell counts with differentials, liver function tests, blood chemistries, and serum creatinine. Vitamin metabolites were assayed on day 1 of cycle 2 and at the summary visit. Before each treatment cycle, creatinine clearance was assessed using the original weight-based Cockcroft and Gault formula (17).

Toxicities were rated using the National Cancer Institute-Common Toxicity Criteria scale (version 1) at the beginning of each cycle. Radiologic studies for disease assessment were conducted pretreatment and before every other cycle. Tumor response was assessed according to standard Southwest Oncology Group criteria (18). A complete response required disappearance of all measurable and evaluable disease on two study visits separated by a minimum of 4 weeks. A partial response required at least a 50% decrease in the sum of products of the perpendicular diameters of all measurable lesions without evidence of progression of any lesion documented in visits separated by at least 4 weeks. A 50% increase in the size or an increase of 10 cm^2 in the sum of products of all measurable lesions over the smallest sum observed or the appearance of any new lesion was considered progressive disease.

Table 2. Phase I extent of exposure to study therapy, vitamin supplementation, and DLT

Dose Level	Pemetrexed/ irinotecan (mg/m^2)	No. patients	Median cycles per patient (range)	Total cycles	Dose intensity (%)	Vitamin supplementation	Patients requiring dose reductions/ total dose reductions	DLT in cycles 1 and 2
Heavily pretreated cohort								
1	300/175	3	5.0 (2-6)	13	98.4	No	1/3	0
				13	111.5*		0	
2	300/250	6	3.0 (1-8)	21	99.1	No	0	Grade 3 nausea and grade 3 vomiting with optimal antiemetic regimen (n = 1)
				21	96.6		1/3	
3	400/250	9	2.0 (1-6)	21	96.7	No	0	Grade 4 neutropenia with treatment delay >2 wk (n = 1)
				21	96.8		0	
4	500/250	5	3.0 (1-9)	19	95.4	No	1/3	Grade 4 febrile neutropenia (n = 2)
				19	98.2		0	
Lightly pretreated cohort (revised schedule with vitamin supplementation)								
1	450/250	4	10.5 (2-18)	41	99.1	Yes	0	0
				41	99.0		0	
2	500/250	9	7.0 (2-19)	79	99.8	Yes	0	0
				79	98.8		1/6	
3	500/300	6	3 (1-19)	34	101†	Yes	0	Grade 4 febrile neutropenia (n = 1)
				34	98.8		1/6	
4	500/350	9	4 (1-10)	39	97.5	Yes	0	0
				39	93.6		4/6	

*One patient received irinotecan 300 mg/m^2 in cycle 1 instead of 175 mg/m^2.
†One patient received doses of pemetrexed slightly greater than 500 mg/m^2.

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Table 3. Principal National Cancer Institute-Common Toxicity Criteria toxicities for pemetrexed/irinotecan

Toxicities	Heavily pretreated cohort (n = 23)				Lightly pretreated cohort (n = 28)			
	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
Hematologic								
Neutropenia	2 (8.7)	2 (8.7)	4 (17.4)	4 (17.4)	8 (28.6)	2 (7.1)	6 (21.4)	2 (7.1)
Anemia	5 (21.7)	8 (34.8)	5 (21.7)	0	14 (50.0)	7 (25.0)	2 (7.1)	0
Thrombocytopenia	5 (21.7)	0	1 (4.3)	1 (4.3)	7 (25.0)	0	0	0
Nonhematologic								
Vomiting	3 (13.0)	6 (26.1)	2 (8.7)	1 (4.3)	7 (25.0)	3 (10.7)	5 (17.9)	2 (7.1)
Diarrhea	6 (26.1)	8 (34.8)	1 (4.3)	0	9 (32.1)	8 (28.6)	2 (7.1)	3 (10.7)
Nausea	11 (47.8)	6 (26.1)	3 (13.0)	0	10 (35.7)	6 (21.4)	6 (21.4)	0
Transaminitis	7 (30.4)	6 (26.1)	1 (4.3)	0	13 (46.4)	3 (10.7)	1 (3.6)	0
Hypoglycemia	0	0	0	0	0	0	1 (3.6)	0
Hyperglycemia	1 (4.3)	1 (4.3)	0	0	9 (32.1)	2 (7.1)	1 (3.6)	0
Hypocalcemia	1 (4.3)	2 (8.7)	0	0	3 (10.7)	1 (3.6)	0	1 (3.6)
Alkaline phosphatase	4 (17.4)	0	0	0	8 (28.6)	1 (3.6)	1 (3.6)	0

Lesions not qualifying for complete response, partial response, or progressive disease were characterized as stable disease.

Pharmacokinetic assessments. Serial blood samples were collected to measure concentrations of pemetrexed, irinotecan, and the active metabolite of irinotecan (SN-38) in the first cycle of treatment. Blood sampling was done: 0 min (pretreatment) and 9 min (1 min before the end of the 10-min pemetrexed infusion); 30 min; and 1, 1.5 (before the end of the irinotecan infusion), 2, 3, 5, 7, 24, and 48 h after the end of the pemetrexed infusion. Heparinized human plasma samples were analyzed for pemetrexed using liquid chromatography electrospray ionization tandem mass spectrometry methods over the concentration ranges of 1,000 to 200,000 ng/mL and 10.0 to 2,000 ng/mL (Taylor Technology, Inc., Princeton, NJ; ref. 19). Both irinotecan and SN-38 concentrations were measured in human plasma samples using a validated high-performance liquid chromatography method over the concentration range of 1.28 to 3,840 ng/mL for irinotecan and 0.48 to 640 ng/mL for SN-38 (AvTech Laboratories, Inc., Kalamazoo, MI; ref. 20).

To determine urinary excretion of pemetrexed, urine samples were collected pretreatment as well as 0 to 7 h and 7 to 24 h posttreatment. Pemetrexed concentrations were measured using a validated liquid chromatography electrospray ionization tandem mass spectrometry method over the concentration range 1,000 to 200,000 ng/mL (Taylor Technology; ref. 21).

Pemetrexed pharmacokinetic variables were estimated using noncompartmental methods (WinNonlin Professional, version 3.1, Pharsight Corp., Menlo Park, CA). Principal pharmacokinetic variables assessed included maximum plasma concentration (C_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration versus time curve from time 0 to infinity ($AUC_{0-\infty}$), volume of distribution at steady-state (V_{ss}), and plasma clearance (CL_p; ref. 22). The fraction of drug excreted unchanged in urine (F_e) was calculated by dividing the cumulative amount of pemetrexed excreted unchanged in urine within 24 h (Ae_{0-24}) by the administered dose (22). Renal clearance (CL_r) was estimated as the product of F_e and CL_p (22).

Assessment of pharmacokinetic interactions. Pemetrexed pharmacokinetic variable estimates from the current study were compared with those for pemetrexed given as a single agent (6). Potential interactions of irinotecan on the pharmacokinetics of pemetrexed were evaluated using analysis of covariance by evaluating the effect of incorporating

Table 4. National Cancer Institute-Common Toxicity Criteria toxicities implicated in the DLT assessment of pemetrexed/irinotecan over all courses by dose level (N = 51)

Dose level	Pemetrexed/ irinotecan (mg/m^2)	No. patients	Neutropenia				Vomiting		Diarrhea	
			Grade 3	Grade 4	Grade 4 >5 days	Grade 3/4 febrile neutropenia	Grade 3	Grade 4	Grade 3	Grade 4
Heavily pretreated cohort (no vitamin supplementation)										
1*	300/175	3	2	0	0	0	0	0	0	0
2	300/250	6	0	1	0	0	1†	0	0	0
3	400/250	9	2	1	1‡	1	0	1	1	0
4	500/250	5	0	2	1	2§	1	0	0	1
Lightly pretreated cohort (vitamin supplementation)										
1‖	450/250	4	0	0	0	0	0	0	0	0
2	500/250	9	3	0	0	0	2	0	1	1
3	500/300	6	1	2	0	2‖	1	2	0	1
4	500/350	9	2	0	0	0	2	0	1	1

*Patients may have been counted in more than one category.
†One patient had DLTs of grade 3 nausea and grade 3 vomiting with optimal antiemetic regimen in cycle 2.
‡One patient had grade 4 neutropenia lasting 6 d (DLT) and grade 3 diarrhea during cycle 1.
§One patient had a DLT of grade 4 febrile neutropenia in cycle 1. A second patient had grade 4 febrile neutropenia (DLT) and grade 4 diarrhea in cycle 2.
‖One patient developed grade 4 febrile neutropenia (DLT) with grade 2 fever in cycle 1.

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Table 5. Summary of pharmacokinetic variables for pemetrexed, irinotecan, and SN-38

	Pemetrexed arithmetic mean (% CV)				Irinotecan arithmetic mean (% CV)	
Dose (mg/m^2)	300	400	450	500	175	250
No. patients	10	9	6	26	2	34
C_{max} (μg/mL)	62.7 (72.7)	80.4 (28.3)	128 (48.6)	104 (30.2)	1,610 (1,560-1,663)*	2,960 (25.3)
$AUC_{0-\infty}$ (μg·h/mL)	94.4 (33.5)	121 (21.2)	174 (41.7)	173 (32.5)	11,400 (9,300-13,500)*	18,500 (40.4)
CL_p ‡	105 (29.3)	103 (24.3)	112 (64.9)	102 (27.4)	30.8 (24.7-36.9)*	28.5 (37.2)
CL_r	39.6 (43.8)	47.7 (47.5)	63.7 (46.6)	47.5 (55.8)	NA	NA
F_e	0.350 (41.2)	0.473 (33.2)	0.564 (40.4)	0.462 (40.5)	NA	NA
V_{ss} (L)	16.4 (28.9)	18.7 (30.2)	17.8 (42.4)	18.1 (23.8)	261 (238-284)*	221 (25.7)
$T_{1/2}$ (h)§	3.69 (2.89-6.43)	3.3 (2.56-5.16)	3.27 (2.61-4.74)	3.07 (1.17-6.50)	8.55 (7.2-10.2)	8.05 (1.79-11.6)

Abbreviations: CV, coefficient of variation; NA, not applicable (only two patients with quantifiable terminal phase data available).
*Arithmetic mean (range).
†N = 1.
‡Units: CL_p (mL/min) for pemetrexed; CL_p (L/h) for irinotecan.
§Geometric mean (range).

study and study*CrCL into the model for CL_p and evaluating the effect of incorporating study and study*BSA into the model for V_{ss}. Ninety percent confidence intervals of the ratio of the geometric means for CL_p and V_{ss} values for pemetrexed given in combination with irinotecan relative to pemetrexed given alone were calculated. Pharmacokinetic variables were logarithmically transformed before analysis.

Results

A total of 51 patients, including 23 patients in the heavily pretreated cohort and 28 patients in the lightly pretreated cohort, were enrolled in this study at two centers in the United States. Pertinent demographic and historical characteristics are summarized in Table 1. The majority of patients had colorectal cancer, non–small cell lung cancer, or mesothelioma.

Dose escalation and MTD levels. In the first stage of the study, involving heavily pretreated patients, four total subjects experienced DLTs, as shown in Table 2. Because one patient treated with pemetrexed/irinotecan at the 300/250 mg/m^2 dose level experienced DLT, consisting of grade 3 nausea and vomiting in cycle 2 despite receiving optimal antiemetic premedication and support, six total patients were treated at this dose level, and no further dose-limiting events were observed. At the next higher pemetrexed/irinotecan dose level (400/250 mg/m^2), one of the six heavily pretreated patients experienced grade 4 neutropenia lasting 6 days in cycle 1, and dose escalation progressed to the 500/250 mg/m^2 dose level, at which time, two of five patients experienced grade 4 febrile neutropenia in cycle 1. Because of consistent, intolerable toxicity in the first cycle, additional experience was ascertained at the previous pemetrexed/irinotecan dose level of 400/250 mg/m^2. At this dose level, the incidence of DLT was considered acceptable, with one of nine new patients experiencing DLT; therefore, it was considered the MTD and recommended for subsequent studies in heavily pretreated patients.

In the lightly pretreated cohort, the pemetrexed/irinotecan regimen given with vitamin supplementation was well tolerated through the highest planned dose level, 500/350 mg/m^2 (Table 2). One of the six patients experienced a DLT, an episode of grade 4 febrile neutropenia in cycle 1, at the dose level of 500/300 mg/m^2. This incidence was considered acceptable, and nine total lightly pretreated patients were treated at the next higher dose level (500/350 mg/m^2) without DLT. Although the MTD level was not strictly determined based on the criteria established a priori, the pemetrexed/irinotecan dose level of 500/350 mg/m^2 consisted of clinically relevant single-agent doses of both pemetrexed and irinotecan, further dose escalation was not done, and this dose level was recommended for further disease-directed studies (23).

Dose administration. In the heavily pretreated cohort, the median number of cycles given was two (range, 1–9). Two pemetrexed doses were reduced due to neutropenia, and one irinotecan dose was reduced due to diarrhea (Table 2). Treatment delay, principally due to unresolved toxicity, was required during nine cycles. There were no dose omissions of pemetrexed or irinotecan.

In the lightly pretreated patients who also received vitamin supplementation, the median number of cycles given was six (range, 1–19 cycles). Dose reductions of pemetrexed were not required, whereas the irinotecan dose was reduced in six cycles due to diarrhea (two cycles) and neutropenia, anemia, fatigue, and vomiting (one cycle each; Table 2). Treatment delay was required in 16 (8.3%) of 193 cycles, principally due to insufficient renal function tests on the scheduled day of treatment (four cycles). There were no dose omissions of pemetrexed or irinotecan.

Toxicity. Most hematologic and nonhematologic events due to pemetrexed/irinotecan therapy were mild to moderate in severity (Table 3). The most common grade 3 or 4 hematologic toxicity in both treatment cohorts was neutropenia, which occurred in 8 (34.8%) patients in the heavily pretreated cohort and 8 (28.6%) patients in the lightly pretreated cohort. Eight patients in each cohort developed grade 3 or 4 febrile neutropenia. Grade 3 or 4 thrombocytopenia was observed in 2 (8.7%) patients in the heavily pretreated cohort. The most frequent grade 3 or 4 nonhematologic toxicities overall were vomiting, nausea, and diarrhea, which are summarized in Tables 3 and 4.

Among the toxicities factored into the assessment of DLT, including protracted neutropenia, febrile neutropenia, nausea/vomiting, and diarrhea (Table 4), there was no discernible relationship between dose level and any toxicity except for febrile neutropenia and neutropenia among heavily pretreated patients. These events occurred more frequently at the MTD level (pemetrexed/irinotecan, 500/250 mg/m^2) and the recommended dose level (pemetrexed/irinotecan, 400/250 mg/m^2).

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Table 5. Summary of pharmacokinetic variables for pemetrexed, irinotecan, and SN-38 (Cont'd)

Irinotecan arithmetic mean (% CV)		SN-38 arithmetic mean (% CV)			
300	350	175	250	300	350
7	8	2	34	7	8
2,830 (35.0)	4,830 (42.7)	22.5 (17.9-27.1)*	35.3 (46.1)	20.1(43.8)	43.3 (54.8)
14,200 (32.2)	23,800 (17.4)	150†	389 (65.7)	230 (117)	520 (70.6)
43.2 (34.7)	31.0 (22.3)	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
323 (37.4)	241 (20.0)	NA	NA	NA	NA
8.35 (7.24-9.44)	8.57 (7.61-9.50)	4.60†	6.87 (2.39-24.0)	5.95 (2.68-34.4)	12.5 (3.02-27.6)

No treatment-related deaths occurred. Adverse events considered related to study drug resulted in discontinuation from the study for two patients: grade 3 neutropenia for an heavily pretreated patient (pemetrexed/irinotecan, 500/250 mg/m^2) and grade 3 vomiting for an lightly pretreated patient (pemetrexed/irinotecan, 500/300 mg/m^2).

Antitumor activity. Four of the 51 patients had partial responses. Of the 23 patients enrolled in the heavily pretreated cohort, a 48-year-old male with metastatic colorectal cancer, which progressed on prior treatment with 5-FU and leucovorin, had a partial response lasting 4 months following treatment with pemetrexed/irinotecan at the 300/175 mg/m^2 dose level. Of the 28 patients comprising the lightly pretreated cohort, three patients experienced partial responses. Two previously untreated patients with unresectable mesothelioma experienced partial response lasting 8 and 6 months following treatment with pemetrexed/irinotecan at the 450/250 and 500/250 mg/m^2 dose levels, respectively. A patient with metastatic colorectal cancer refractory to a 5-FU and leucovorin regimen had a partial response lasting 9 months following treatment with pemetrexed/irinotecan at the 450/250 mg/m^2 dose level.

Pharmacokinetics. Plasma sampling to evaluate pemetrexed and irinotecan pharmacokinetics was done in all 51 patients; pertinent pharmacokinetic variables for pemetrexed, irinotecan, and SN-38 as a function of dose level are presented in Table 5. As evident in Fig. 1, by the large overlap between pemetrexed concentration-time data from the present study and a previous study of single-agent treatment, the pharmacokinetic behavior of pemetrexed in combination with irinotecan was similar to that of pemetrexed as a single agent (6). Furthermore, an analysis of covariance indicated that irinotecan had no major effect on the pharmacokinetics of pemetrexed in the present study

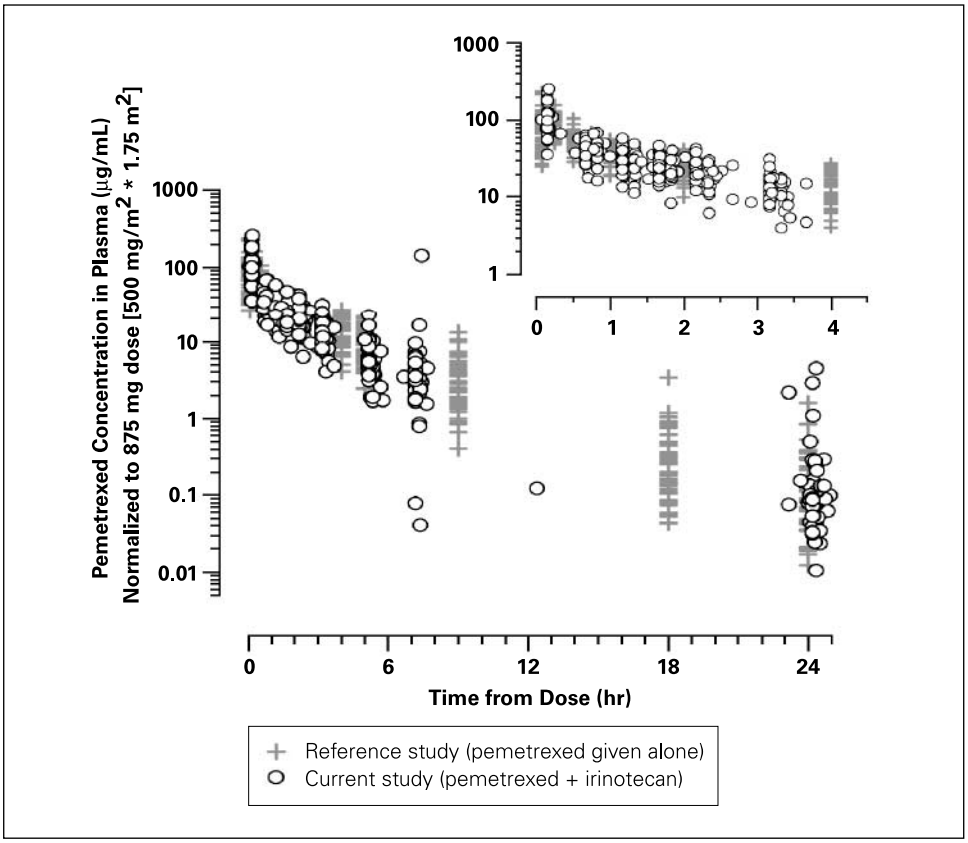

Fig. 1. Comparison of pemetrexed concentration-time data following treatment with pemetrexed and irinotecan in the current study to those following pemetrexed given alone (see Materials and Methods).

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($P > 0.05$) both for the incorporation of study and study*CrCL into the model for CL_p and for the incorporation of study and study*BSA into the model for V_{ss} (Table 6). AUC_{0-8} values for pemetrexed at the recommended dose (500 mg/m^2) averaged 173 μg h/mL, and $t_{1/2}$ values ranged from 1.17 to 6.5 h. At the recommended dose of 350 mg/m^2, irinotecan AUC_{0-8} values averaged 23.8 mg h/mL, and the $t_{1/2}$ values ranged from 7.61 to 9.50 h. For SN-38, $AUC_{0-\infty}$ values at the recommended irinotecan dose of 350 mg/m^2 averaged 520 μg h/mL, and $t_{1/2}$ values ranged from 3.02 to 27.6 h.

Discussion

The inhibition of thymidylate synthase with fluoropyrimidines has been the mainstay of treatment of colorectal cancer and other cancers for nearly four decades (24). When combined with the biochemical modulator leucovorin, 5-FU has shown improved outcomes in colorectal cancer with median survival times of 12 months in first-line therapy of patients with advanced disease (25). The addition of the DNA topoisomerase I inhibitor irinotecan to the fluoropyrimidines has resulted in improved overall survival in patients with metastatic colorectal cancer (11, 12); therefore, it was logical to evaluate the feasibility of combining irinotecan with the novel multitargeted antifolate pemetrexed, whose principal mechanism of action is thymidylate synthase inhibition.

Although the current phase I study identified an initial MTD level (pemetrexed/irinotecan, 400/250 mg/m^2) for the heavily pretreated cohort, the combination of pemetrexed and irinotecan was also evaluated in a lightly pretreated cohort because the regimen was considered more relevant for this group of patients in treating patients with early disease. The lightly pretreated cohort also received supplementation with folic acid and vitamin B_{12} based on reports indicating that vitamin supplementation greatly reduced toxicities associated with pemetrexed treatment (26–29). Although the MTD level, as strictly defined a priori, was not reached in the lightly pretreated cohort, the feasibility and safety of the pemetrexed/irinotecan regimen at clinically relevant single-agent doses of both agents given every 3 weeks along with multivitamin supplementation, as well as irinotecan at its single-agent MTD, led to the decision to terminate pemetrexed dose escalation, which would likely have resulted in a diminished therapeutic index for the regimen. The feasibility and preliminary antitumor activity of the combination given every 3 weeks with vitamins has also been evaluated in another study in patients with advanced colorectal cancer (23). The investigators reported that the MTD level for pemetrexed/irinotecan was 500/350 mg/m^2 and recommended the next lower dose level (500/300 mg/m^2) for disease-directed studies (23). Despite the fact that these doses are the recommended and approved doses for both pemetrexed and irinotecan as single agents, the toxicities observed in the present study were generally tolerable, manageable, predictable, consistent, and nonerratic as a function of dose level. The principal DLTs of the pemetrexed/irinotecan regimen were myelosuppression, nausea, vomiting, and diarrhea. It was also encouraging that objective responses of notable duration occurred in two patients each with advanced mesothelioma and colorectal cancer with demonstrable progression on prior treatment with 5-FU/leucovorin.

Pemetrexed CL_p and V_{ss} estimates obtained from the present study, in which the agent was administered in combination with irinotecan, are consistent with previously reported values (6, 19). Also consistent with previous pharmacokinetic evaluations (19, 21), the systemic CL_p of pemetrexed decreased with decreasing renal function, as estimated by creatinine clearance, and V_{ss} values that correlated with BSA (ref. 19; data not shown). Furthermore, the pharmacokinetic behaviors of irinotecan and SN-38 in the present study are similar to the results reported previously with irinotecan given as a single agent (30, 31). Although the number of patients in the study was too low to detect small drug-drug interactions, there was no evidence of significant pharmacokinetic interactions between pemetrexed and irinotecan (6, 19, 21, 30–34).

Malignant neoplasms with inordinately low thymidylate synthase levels have been reported to be particularly sensitive to chemotherapeutic agents that inhibit the enzyme, including 5-FU and pemetrexed (7, 35). Furthermore, Pavillard et al. (36) have shown an inverse relationship between thymidylate synthase activity and irinotecan-induced cleavable complex formation, which suggests a potential mechanism whereby synergy between pemetrexed and irinotecan might be expected to occur. These authors also observed that the greatest antitumor activity in six human colon carcinoma cell lines occurred when SN-38 was followed by 5-FU. This study was initiated just after the emergence of preclinical data by Guichard et al., which suggested the potential for sequence-dependent cytotoxicity between inhibitors of topoisomerase I and thymidylate synthase (37). In fact, data available in the final publication from that preclinical study suggested that treatment of cancer cells with 5-FU followed by SN-38 was more cytotoxic than the alternate sequence (38). Schedule-dependent cytotoxic activity has previously been shown with the gemcitabine and pemetrexed combination in pancreatic cancer cell lines (39). Thus, it is possible that the activity of the pemetrexed/irinotecan regimen may be even increased by administering irinotecan before pemetrexed. In any case, it would be fair to conclude that the potential for relevant sequence-dependent cytotoxic interactions between inhibitors of topoisomerase I and thymidylate synthase have not been

Table 6. Estimated geometric mean values for pemetrexed in combination with irinotecan versus pemetrexed alone for patients with CrCL = 90 mL/min and BSA = 1.75 m^2

Variable	Treatment regimen	Geometric mean	Ratio	90% Confidence interval
CL_p (mL/min)	Pemetrexed + irinotecan	90.9	1.06	0.963-1.17
	Pemetrexed alone	85.6		
V_{ss} (L)	Pemetrexed + irinotecan	16.1	1.21	1.09-1.35
	Pemetrexed alone	13.2		

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rigorously evaluated in the clinic to date. However, sequence-dependent cytotoxicity between pemetrexed and various types of cytotoxic therapies have been shown in preclinical studies (13). Nevertheless, the dosing recommendations based on the results presented in this report are applicable only to the specific administration scheme used in this study, which is pemetrexed as a brief infusion followed by irinotecan.

The results of this study indicate that the administration of pemetrexed and irinotecan is feasible at clinically relevant single-agent doses of both agents. Further refinement of dosing and/or scheduling and the inclusion of pharmacogenomic information to identify targeted patient populations through biomarker characterization may lead to improvement in efficacy for this regimen.

Acknowledgments

We thank the following Eli Lilly employees for their assistance with the manuscript: David Ohannesian, Ph.D., Scientific Communications Associate; Mary Alice Miller, Ph.D., Scientific Communications Consultant; and Karyn Warsow, M.S., M.P.H., Pharmacokinetics Operations.

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Clin Cancer Res 2007;13:532-539.

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