Cancer Chemother Pharmacol (2005) 55: 522–530
DOI 10.1007/s00280-004-0950-7

ORIGINAL ARTICLE

**Grace K. Dy · Ajit Suri · Joel M. Reid · Jeff A. Sloan
Henry C. Pitot · Steven R. Alberts
Richard M. Goldberg · Pamela J. Atherton
Lorelei J. Hanson · Patrick A. Burch · Joseph Rubin
Charles Erlichman · Alex A. Adjei**

A phase IB study of the pharmacokinetics of gemcitabine and pemetrexed, when administered in rapid sequence to patients with advanced solid tumors

Received: 2 April 2004 / Accepted: 2 July 2004 / Published online: 8 March 2005

Abstract *Background*: We have previously demonstrated that pemetrexed is clinically active when administered 90 min after gemcitabine in a phase I study. The present study was undertaken to evaluate the efficacy, toxicity, and pharmacokinetics of gemcitabine and pemetrexed when pemetrexed is administered immediately after gemcitabine. *Patients and Methods*: A total of 14 patients received 84 cycles of treatment. Gemcitabine 1250 mg/m^2 was administered on days 1 and 8 of each 21-day cycle, and pemetrexed 500 mg/m^2 on day 8 immediately following gemcitabine administration. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria and recorded as maximum grade per patient for all treatment cycles. Pharmacokinetic analyses of plasma gemcitabine and pemetrexed concentrations were performed. *Results*: Neutropenia was the most common severe toxicity. Non-hematologic toxicities, which included nausea, vomiting, fatigue, diarrhea, rash, and elevated transaminases were of mild-to-moderate severity. No increased toxicity was observed with this schedule in comparison to the previous phase I schedule. There was no pharmacokinetic interaction between the two drugs. One partial response was documented in a patient with non-small-cell lung cancer. Eight patients had disease stabilization for five or more cycles. *Conclusion*: Gemcitabine immediately followed by pemetrexed is well tolerated and clinically active, and deserves further evaluation in phase II trials.

Keywords Combination chemotherapy · Gemcitabine · Pemetrexed · Pharmacokinetics · Schedule

G. K. Dy
Department of Medicine, Mayo Clinic and Foundation,
200 First Street SW, Rochester, MN 55905, USA

G. K. Dy · J. M. Reid · J. A. Sloan · H. C. Pitot · S. R. Alberts
R. M. Goldberg · P. J. Atherton · L. J. Hanson · P. A. Burch
J. Rubin · C. Erlichman · A. A. Adjei (✉)
Department of Oncology, Mayo Clinic and Foundation,
200 First Street SW, Rochester, MN 55905 USA
E-mail: adjei.alex@mayo.edu
Tel.: +1-507-5380548
Fax: +1-507-2841803

A. Suri
Eli Lilly and Company, Indianapolis, IN 46285, USA

Present address: R. M. Goldberg
University of North Carolina, Chapel Hill, NC 27514, USA

Introduction

Gemcitabine (2′,2′-difluorodeoxycytidine, Gemzar; Eli Lilly and Company, Indianapolis, Ind.) is a broadly active S phase-specific pyrimidine analog of deoxycytidine approved for the treatment of non-small-cell lung cancer (NSCLC) and pancreatic cancer. It is anabolized sequentially to the nucleoside monophosphate, diphosphate and triphosphate intracellularly. The triphosphate, difluorodeoxycytidine triphosphate, is incorporated into DNA, resulting in chain termination. Moreover, the diphosphate derivative inhibits ribonucleotide reductase, thus depleting intracellular pools of dCTP for DNA synthesis [1].

Pemetrexed disodium (Alimta, LY231514, MTA; Eli Lilly and Company) is a broadly active multitargeted folate analog that suppresses tumor growth by inhibiting various folate-dependent enzymes, primarily thymidylate synthase (TS), dihydrofolate reductase (DHFR) and the purine biosynthetic enzyme, glycinamide ribonucleotide formyl transferase (GARFT) [2]. Pemetrexed has demonstrated single-agent activity during phase II trials against a variety of tumor types, including NSCLC [3, 4], and colorectal [5, 6], breast [7], pancreas [8], gastric

[9], head and neck [10], bladder [11], and cervical cancers [12]. In phase III testing, pemetrexed combined with cisplatin was superior to cisplatin alone in pleural mesothelioma [13].

Although there is discordance between the various in vivo and in vitro preclinical models regarding the optimal sequence of administration, additive cytotoxicity has been consistently demonstrated during simultaneous treatment with pemetrexed and gemcitabine [14–16]. A phase I study of gemcitabine and pemetrexed combination therapy demonstrated a tolerable toxicity profile and significant clinical activity. The schedule recommended as safe, potentially effective, and feasible for further studies was gemcitabine at 1250 mg/m^2 on days 1 and 8, and pemetrexed at 500 mg/m^2 administered as a 10-min intravenous infusion, 90 min after gemcitabine, on day 8 of a 21-day cycle [14]. This regimen has shown activity in a phase II study of pancreatic cancer, and is currently being evaluated in phase II trials in NSCLC and breast cancer and in a phase III trial in pancreatic cancer.

As clinical development of this promising combination proceeds, it is important to determine whether any pharmacokinetic interaction exists between pemetrexed and gemcitabine. In addition, patient convenience will be greatly enhanced if the 90-min interval between the administration of gemcitabine and pemetrexed could be eliminated without adversely affecting toxicity and efficacy. We therefore undertook the present study to characterize any possible pharmacokinetic interaction between these two agents, and to compare the toxicity and efficacy of this combination administered in close succession with similar data from our previous phase I study in which administration of the drugs was separated by a 90-min interval.

Patients and methods

Patient selection

Patients with histologic or cytologic evidence of metastatic or locally advanced cancer for which there was no established life-prolonging or curative therapy available, or who were unresponsive to conventional therapy, and had measurable or evaluable disease were eligible for this study. Eligibility criteria included: age ≥18 years; Eastern Cooperative Oncology Group performance status ≤ 2; adequate bone marrow (platelets ≥100×10^9 cells/l, absolute neutrophil count (ANC) > 1.5×10^9 cells/l, hemoglobin ≥9 g/dl), hepatic (total bilirubin not more than 1.5 times the upper limit of normal, aspartate transaminase or alanine transaminase not more than 3 times normal or less than 5 times normal if elevation caused by liver metastases) and renal (calculated creatinine clearance ≥45 ml/min) functions; prior radiation to ≤ 25% of bone marrow or prior chemotherapy that was completed at least 3 weeks (6 weeks if the treatment was with nitrosourea or mitomycin-C) before study enrollment, the patient having recovered from the acute toxicities of the said therapies; and a life expectancy of at least 12 weeks. Excluded from this study were pregnant or lactating females; patients who had a diagnosis of leukemia, lymphoma or multiple myeloma; patients with symptomatic brain metastases; and patients with clinically significant effusions, albumin < 2.5 g/dl; homocysteine levels ≥12 μM and body surface area more than 3 m^2. Aspirin or nonsteroidal antiinflammatory agents were not allowed within 2 days (5 days for long-acting agents such as piroxicam) of treatment with pemetrexed as these agents may inhibit the renal clearance of pemetrexed. Written informed consent was obtained according to federal and institutional guidelines.

Treatment regimen

Gemcitabine was supplied as a sterile lyophilized powder in vials of either 200 or 1000 mg, formulated as the hydrochloride salt with mannitol and sodium acetate. The vial contents were reconstituted with sterile normal saline to make a solution containing 10 mg/ml of gemcitabine. Pemetrexed was supplied as a sterile lyophilized powder in vials of either 100 or 500 mg, with mannitol as a nonactive component. The vial contents were reconstituted with sterile normal saline yielding a solution containing 10 mg/ml of pemetrexed.

On day 1 of the first therapy cycle, pemetrexed 500 mg/m^2 was administered intravenously over 10 min as a single agent for the purpose of characterizing its pharmacokinetic profile. Gemcitabine was not administered during the first therapy cycle. During subsequent therapy cycles, gemcitabine 1250 mg/m^2 was administered as a 30-min intravenous infusion on days 1 and 8, followed by pemetrexed 500 mg/m^2 as 10-min infusion immediately after gemcitabine administration on day 8 only. Antiemetic prophylaxis consisted of ondansetron 16 mg and dexamethasone 20 mg administered orally before treatment, followed by ondansetron 8 mg orally every 8 h and dexamethasone 4 mg orally every 12 h for 24 h after treatment. Dose escalation was not performed in this study. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (NCICTC).

Pretreatment and follow-up studies

Complete patient histories, physical examinations, complete blood counts, serum electrolytes, chemistries, and coagulation profile were obtained at baseline and prior to each course of treatment. Laboratory studies were performed weekly while patients were on study. Radiologic studies (roentgenograms, computed axial tomographic scans, and magnetic resonance imaging) were performed at baseline and at the end of every second cycle to assess tumor response. A partial response required at least a 50% reduction in the sum of

the products of bidimensional measurements, separated by at least 4 weeks. A complete response was defined as the disappearance of all evidence of tumor sustained for a minimum of 4 weeks. Progressive disease was defined as the appearance of new lesion(s) or an increase in the sum of the bidimensional products of all known lesions by at least 50%. Stable disease was documented when there was persistence of disease without meeting the criteria for progression, partial response or complete response.

Pharmacokinetic analyses

On day 1 of cycle 1, blood samples for plasma pemetrexed concentration determinations were collected into EDTA-containing tubes immediately prior to pemetrexed infusion and at 10, 15 and 30 min and 1, 3, 6, 9, 24 and 48 h after starting the infusion. Blood samples for determination of plasma gemcitabine and dFdU concentrations were collected on day 1 of cycle 2 into heparinized tubes containing tetrahydrouridine (THU) prior to gemcitabine infusion and at 15, 30, 35, 45, 60, 90 and 120 min, and 4, 6, 8, 24 and 48 h after starting the infusion. On day 8 of cycle 2, blood samples for gemcitabine, dFdU, and pemetrexed concentrations were collected into heparinized tubes containing THU prior to gemcitabine infusion and at 15, 30, 35, 45, 60, 90 120 min, and 4, 6, 8, 24 and 48 h after starting the gemcitabine infusion. The 35-min sample was the preinfusion sample for pemetrexed and the pemetrexed infusion began immediately afterwards. This sampling schedule, therefore, provided pemetrexed concentration determinations at 10, 25 and 55 min, and 1.4, 3.4, 5.4, 7.4, 23.4, and 47.4 h after starting the pemetrexed infusion. Immediately after collection, each sample was chilled in an ice-water slurry and centrifuged. The plasma layer then was transferred to polypropylene tubes, capped, frozen immediately and stored at −70°C. All samples were sent to Eli Lilly and Company for analysis.

Assay methodology

Gemcitabine

Gemcitabine was measured in plasma using a high-performance liquid chromatography (HPLC) method consisting of a Waters LC module equipped with a Supelcosil LC-NH_2 HPLC column and a UV detector set at 272 nm. The mobile phase consisted of cyclohexane, 1,2-dichloroethane, methanol, water, glacial acetic acid, and triethylamine (6.3:1.5:2.2:0.01:0.005:0.01 by volume) and was delivered at a rate of 1.5 ml/min. The HPLC method was validated for precision and accuracy to a concentration range of 150–50,000 ng/ml. Samples above the upper limit of quantitation were diluted and reanalyzed to yield results within the validated range. Aliquots of plasma samples were thawed and an internal standard (5-fluoro-2′-deoxyuridine) was added prior to extraction. An amount of 1 ml isopropanol was added to 200 µl plasma and mixed vigorously by vortexing. Following mixing, 2.5 ml ethyl acetate was added, the sample mixture was once again vortexed, and the sample was centrifuged at approximately 3000 rpm for 10 min to pellet the precipitate. The supernatant was transferred to a clean tube and concentrated to dryness under a nitrogen flow. The samples were then reconstituted with 250 µl mobile phase and injected directly onto the HPLC. The linear range for this assay was 0.15–2.5 µg/ml. The limit of quantitation for this assay was determined to be 0.15 µg/ml.

Pemetrexed

Plasma pemetrexed concentrations were analyzed by a liquid chromatography mass spectroscopy/tandem mass spectroscopy (LC/MS/MS) method [17]. Briefly, plasma proteins were precipitated with a perchloric acid solution, and the analytes in the supernatant, as well as the internal standard, [$^2H_4$]-LY231514, were then separated under reverse-phase conditions on a YMC Basic C8 column (Keystone Scientific, Belfonte, Pa.). A gradient mobile phase was used with solvent A containing 0.2% formic acid in a 2% (v/v) acetonitrile and water solution and solvent B containing 0.2% formic acid in a 98% (v/v) acetonitrile and water solution. The HPLC gradient conditions were: 0–0.5 min, 100% A; 4.0–4.2 min, 10% A, 90% B; 5.2 min, 100% A. The compounds were detected and quantified by LC/MS/MS using electrospray ionization. The LC/MS/MS methods for determination of pemetrexed in plasma were validated for the concentrations ranging from 10 to 2000 ng/ml and 1000–200,000 ng/ml using 0.5 ml heparinized human plasma. Validation experiments were conducted to evaluate the linearity response for the assay and to assess interday and intraday precision and accuracy. The lower limit of quantitation was 10 ng/ml and both intraday an interday precision were $\leq 18.1\%$. The mean accuracy of back-calculated values was within 20% of the expected concentration.

Data analysis

Pemetrexed

Pharmacokinetic analysis of plasma pemetrexed concentration-time data was conducted using noncompartmental methods (WinNonlin Professional Network version 3.1, Pharsight Corporation). The maximum observed plasma concentration (C_{max}) and the time of C_{max} (T_{max}) were identified from visual inspection of the plasma concentration-time profile. The elimination rate constant (λ_z) was estimated from the slope of the terminal portion of the log-transformed plasma concentration-time curve. The elimination half-life ($t_{1/2}$) was calculated as $(\ln 2)/(\lambda_z)$.

The area under the plasma concentration-time curve ($AUC_{0\text{-}t}$) was estimated using the linear/log trapezoidal method and extrapolated to infinity using the predicted concentration ($\hat{C}$) at the last measurable sampling time (t) and the apparent terminal elimination rate constant (λ_z):

$$AUC_{0-\infty} = AUC_{0-t} + \frac{\hat{C}}{\lambda_z} \quad (1)$$

Total plasma clearance (CL_p), was calculated using dose and $AUC_{0\text{-}\infty}$:

$$CL_p = \frac{\text{Dose}}{AUC_{0-\infty}} \quad (2)$$

The apparent steady-state volume of distribution (V_{ss}) was calculated using standard noncompartmental first-moment theory methods [18].

The pemetrexed pharmacokinetic parameters determined with and without concomitant gemcitabine administration were compared to determine the influence of gemcitabine on pemetrexed pharmacokinetics using a mixed effects model with treatment as a fixed effect and patient as a random effect.

Gemcitabine

The plasma gemcitabine concentration versus time profiles of seven patients contained five or fewer measurable plasma concentrations, which precluded accurate estimation of the gemcitabine AUC by noncompartmental methods. Nonlinear mixed-effects modeling (NONMEM) was therefore used to fit individual plasma gemcitabine concentration versus time profiles utilizing a Bayesian approach with a two-compartment model parameterized in terms of systemic clearance (CL), intercompartmental clearance (Q), central volume of distribution (V_1) and peripheral volume of distribution (V_2). Interindividual error was modeled using an exponential function, while a combined additive-proportional function was used to describe residual error. Predicted plasma gemcitabine concentrations were in good agreement with observed values indicating the validity of this modeling method (data not shown).

The gemcitabine AUC was estimated as dose/CL. The steady-state volume of distribution was estimated from the central and peripheral volumes of distribution

$$V_{ss} = V_1 + \frac{k_{12}}{k_{21}} \bullet V_1 \quad (3)$$

Compartmental microconstants (k_{10}, k_{12}, k_{21}) and macroconstants (α, β), and C_{max} were estimated using standard two-compartment model relationships [19]. The elimination half-life was estimated as $(\ln 2)/\beta$.

The gemcitabine pharmacokinetic parameters determined with and without concomitant pemetrexed administration were compared to determine the influence of pemetrexed on gemcitabine pharmacokinetics using a mixed effects model with treatment as a fixed effect and patient as a random effect.

Results

Patient demographics

A total of 14 patients (Table 1) received 84 assessable courses of therapy. The median number of courses administered per patient was 5 (range 1–14). The median age of the study participants was 60 years (range 35–73 years). There were nine males and five females enrolled. All patients had an Eastern Cooperative Oncology Group performance status ≤ 1. With regard to prior treatment, 11 patients had prior surgery, 12 had received prior chemotherapy, 1 had prior hormonal therapy, 4 had prior immunologic therapy, and 4 had prior radiation therapy. The most common tumor types were colorectal cancer ($n = 6$) and NSCLC ($n = 3$). Other tumor types included esophageal cancer, anal cancer, mesothelioma, atypical carcinoid and leiomyosarcoma. Mean creatinine clearance for the study patients at the time of pemetrexed administration alone was 136 ml/min (coefficient of variation 34.1%) and 128 ml/min ($\pm 28.0\%$) when administered in combination with gemcitabine (Fig. 1) .

Non-hematologic toxicity

Non-hematologic toxicities were mostly mild-to-moderate in severity, and consisted of nausea, emesis, diarrhea, rash and mild elevation of serum transaminases. Figure 2 displays the treatment-related toxicities for the cohort of ten patients who received a total of 59 treatment courses from our previous phase I study at these same doses, but with a 90-min interval between drug

Table 1 Patient characteristics ($n = 14$)

No. of courses (fully evaluable)		84
Courses/patient	Median	5
	Range	1–14
Age (years)	Median	60
	Range	35–73
Gender	M/F	9/5
ECOG performance status	0	6
	1	8
	2	0
Prior therapy	Surgery	11
	Chemotherapy	12
	Radiation	4
	Immunotherapy	4
	Hormone	1
Tumor type	Colorectal	6
	Leiomyosarcoma	1
	Lung (one carcinoid)	4
	Mesothelium	1
	Esophagus	1
	Anus	1

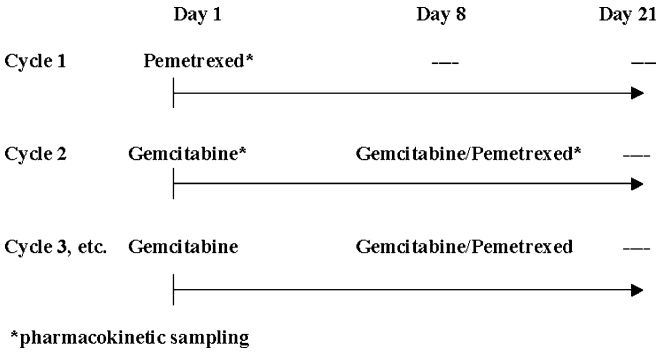

Fig. 1 Schedule of administration and pharmacokinetic sampling in this study

administration [14]. For comparison, the non-hematologic side effects of this present study are shown in Fig. 3.

Hematologic toxicity

In general, anemia and thrombocytopenia were mild-to-moderate in severity. Severe neutropenia was the most common severe hematologic toxicity (grades 3 and 4 in 28% and 15% of patients in cycle 1, respectively). This resulted in the hospitalization of one patient during the second cycle of treatment. Comparisons of the treatment-related hematologic toxicities between the two schedules are shown in Figs. 4 and 5.

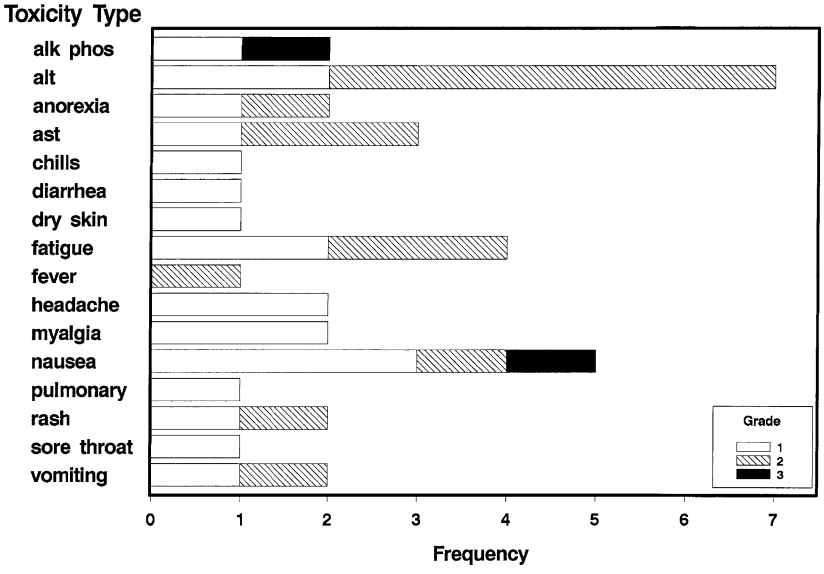

Fig. 2 Treatment-related non-hematologic toxicities of patients on cycle 1 of the phase I schedule

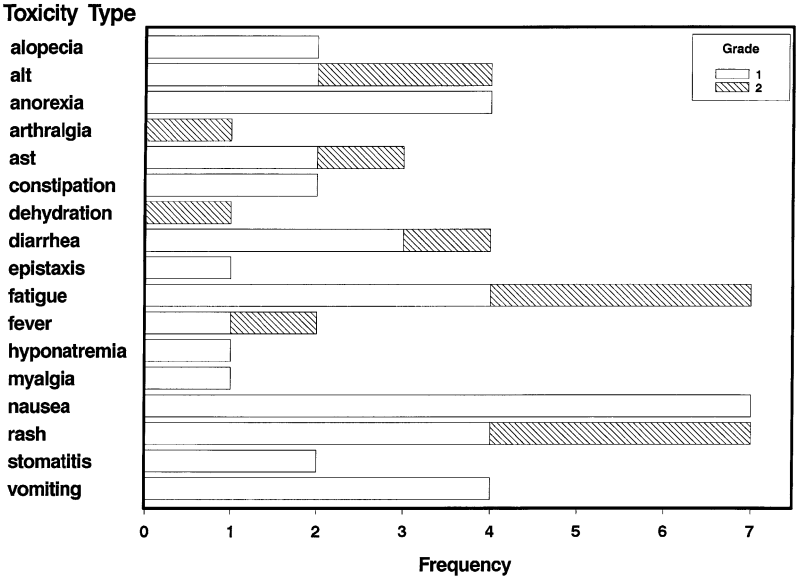

Fig. 3 Treatment-related non-hematologic toxicities of patients on cycle 2 of the modified schedule with gemcitabine and pemetrexed administered in close sequence

Antitumor activity

All 14 patients in this study were assessable for tumor response, and 8 had prolonged disease stabilization on therapy (six or more cycles). Four of these patients received eight or more cycles. One patient with atypical carcinoid received 11 cycles of therapy, and a partial response in NSCLC was maintained for 8 months.

Pharmacokinetics

Pemetrexed

Mean plasma pemetrexed concentration-time profiles for pemetrexed administered alone and in combination with gemcitabine are provided in Fig. 6.

Pharmacokinetic parameter estimates were available for 14 patients when pemetrexed was administered alone and for 13 patients when administered in combination with gemcitabine. One patient who had received only pemetrexed and not pemetrexed in combination with gemcitabine was excluded from the pemetrexed summary statistics. A second patient receiving pemetrexed 126 mg/m^2 was excluded from the summary statistics as this amount represented only 25% of the dose administered to other study patients. Pemetrexed pharmacokinetic parameter estimates from 12 patients were therefore evaluable for the drug interaction assessment. Mean pharmacokinetic parameter estimates for pemetrexed administered alone and in combination with gemcitabine are provided in Table 2. The AUC, CL, V_{ss}, and $t_{1/2}$ values determined when pemetrexed was administered concomitantly with gemcitabine were similar to those determined when pemetrexed was administered alone. The mean C_{max} for pemetrexed when administered in combination with gemcitabine was slightly lower than for pemetrexed administered alone (105 and 124 μg/ml, respectively). Concomitant gemcitabine administration exerted no influence on pemetrexed disposition.

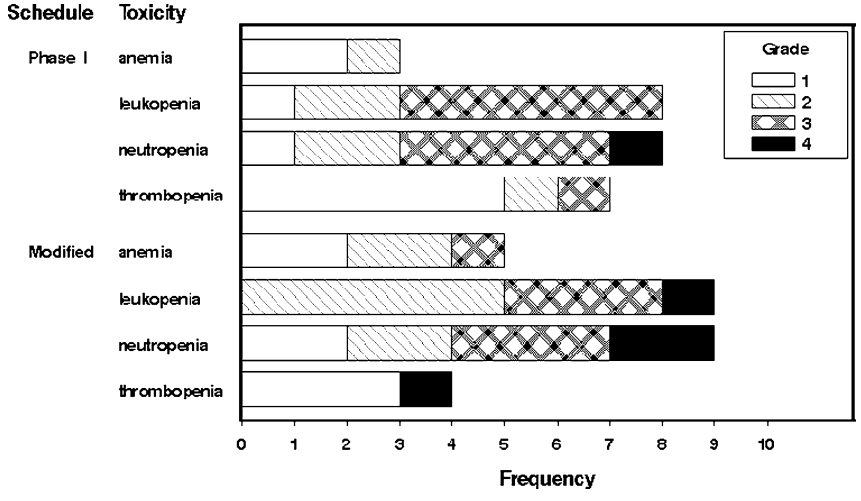

Fig. 4 Comparison of treatment-related hematologic toxicities between the two schedules on the first course of combination therapy

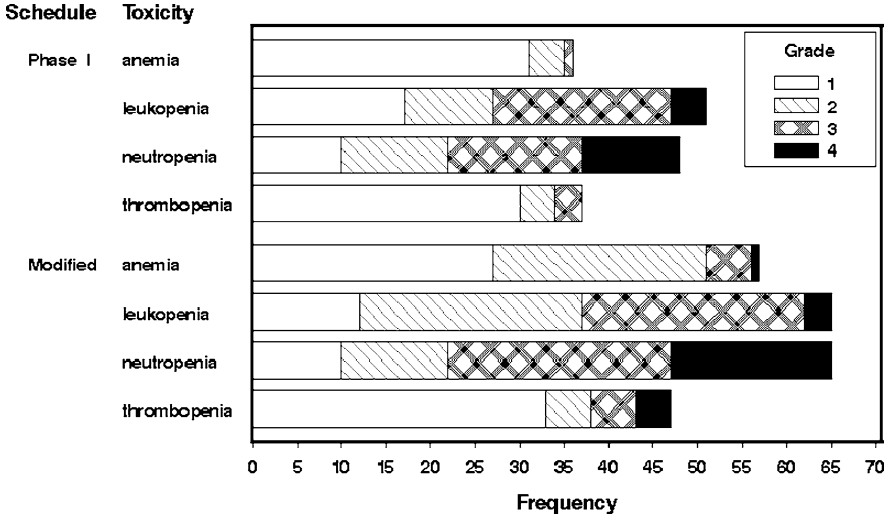

Fig. 5 Comparison of cumulative treatment-related hematologic toxicities between the two schedules of combination therapy

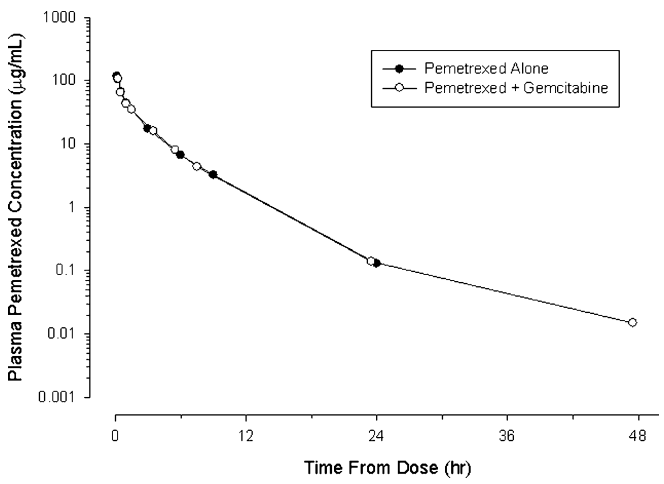

Fig. 6 Mean plasma pemetrexed concentration-time profiles for each treatment

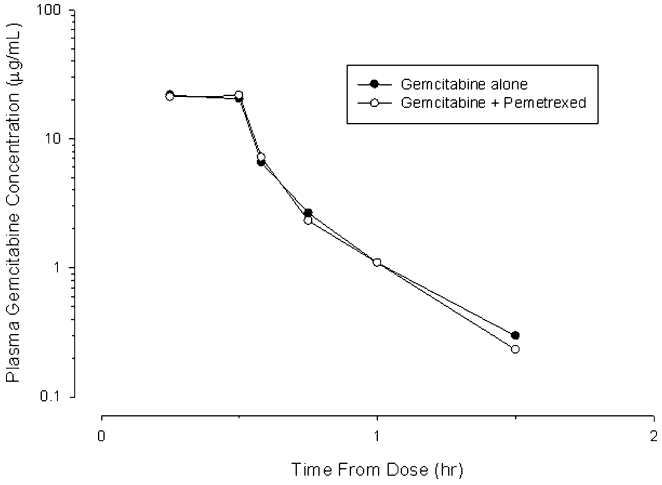

Fig. 7 Mean plasma gemcitabine concentration-time profiles for each treatment

Gemcitabine

Mean plasma gemcitabine concentration-time profiles for gemcitabine administered alone and in combination with pemetrexed are provided in Fig. 7.

One patient receiving gemcitabine at a dose of only 315 mg/m^2 was excluded from the summary statistics as this amount represented only 25% of the dose administered to other study patients. Gemcitabine pharmacokinetic parameter estimates from 13 patients were therefore evaluable for the drug interaction assessment. Mean pharmacokinetic parameter estimates for gemcitabine administered alone and in combination with pemetrexed are provided in Table 3. The C_{max}, AUC, CL, V_{ss}, and $t_{1/2}$ values for gemcitabine administered concomitantly with pemetrexed were virtually identical to those determined when gemcitabine was administered alone. The gemcitabine clearance estimates are consistent with those from an earlier population pharmacokinetic analysis of plasma gemcitabine concentrations obtained from 178 patients with a variety of solid tumors (Gemzar product labeling). Concomitant pemetrexed administration exerted no influence on gemcitabine disposition.

Plots of plasma dFdU concentrations versus time following gemcitabine administered alone and in combination with pemetrexed were identical, suggesting that dFdU pharmacokinetics were not altered by concomitant pemetrexed administration (data not shown).

Discussion

In cancer chemotherapy, a combination of agents is used to maximize antitumor activity without unduly increasing toxicity. Because of its broad spectrum of activity, pemetrexed has been tested in combination with a wide variety of antineoplastic agents [20]. One such combination, based on in vitro synergy data, is pemetrexed and gemcitabine. Following preclinical investigations, it was suggested that cytotoxic synergism may be achieved because these two agents target defined pathways that deplete the intracellular deoxynucleotide triphosphate pools necessary for DNA synthesis. It was hypothesized that cell cycle effects consequent to pemetrexed treatment, such as G_1/S delay and S phase synchronization of tumor cells, may enhance incorporation of gemcitabine into tumor DNA [15, 21, 22]. However, in several hu-

Table 2 Mean (CV%) pharmacokinetic parameter estimates for pemetrexed, administered alone and in combination with gemcitabine

Parameter	Cycle 1 (pemetrexed alone, $n = 12$)	Cycle 2 (pemetrexed + gemcitabine, $n = 12$)
C_{max} (µg/ml)	124 (12%)	105 (11%)
$AUC_{0-\infty}$ (µg*h/ml)	188 (25%)	180 (19%)
CL (ml/min)	99.2 (32%)	101 (26%)
CL (ml/min/m^2)	48.1 (25%)	49.4 (21%)
V_{ss} (l)	16.8 (13%)	17.9 (10%)
V_{ss} (l/m^2)	8.25 (7.6%)	8.85 (6.3%)
$t_{1/2}$ (h)	3.93 (28%)	4.37 (24%)

Table 3 Mean (CV%) pharmacokinetic parameter estimates for gemcitabine, administered alone and in combination with pemetrexed

Parameter	Day 1 (gemcitabine alone, $n = 12$)	Day 8 (gemcitabine with pemetrexed, $n = 12$)
C_{max} (µg/ml)	23.5 (14%)	23.2 (16%)
$AUC_{0-\infty}$ (µg*h/ml)	12.5 (13%)	12.3 (17%)
CL (ml/min)	3530 (18%)	3620 (20%)
CL (ml/min/m^2)	1740 (14%)	1780 (18%)
V_{ss} (l)	25.9 (36%)	25.9 (34%)
V_{ss} (l/m^2)	12.8 (37%)	12.8 (33%)
$t_{1/2}$ (h)	0.228 (23%)	0.238 (16%)

man colon carcinoma cell lines, such as HCT8, LoVo, WiDr, LRWZ, pretreatment with gemcitabine has been found to be synergistic [14, 21]. On the other hand, cytotoxicity of gemcitabine is potentiated by a 24-h preexposure to pemetrexed in HT29 colon cancer cell lines and tumor xenografts [15], whereas synergy in LoVo and WiDr cell lines after a 48-hour preincubation with pemetrexed has also been observed [21]. Interestingly, in the study by Tesei et al. [21], simultaneous administration of both drugs was found to be at least additive in LoVo cell lines only. These results suggest that patterns of interaction with these two agents may be cell-line specific and schedule-dependent. This may be due to variability in the effects on nucleotide pool depletion and induction of thymidylate synthase expression.

A previous clinical study [14] has shown antineoplastic activity across a broad range of tumor types when pemetrexed is administered 90 min after gemcitabine infusion. The administration of gemcitabine immediately followed by pemetrexed was evaluated in this study, as this schedule would be more practical in the clinical setting. Overall, the revised schedule of administration was well tolerated. Eruption of an erythematous, macular rash was ameliorated by premedication with dexamethasone, reaffirming observations from previous clinical studies. No delays or discontinuation of therapy for any patient occurred as a consequence of this side effect. The nonhematologic toxicities encountered in the modified protocol were similar, if not less severe, than the original schedule. Schedule-independent, cumulative-dose myelosuppression of varying degrees was frequently encountered with this combination. Frequency of grade 4 neutropenia was similar (RR for neutropenia in this present study was 1.7; 95% CI 0.79–3.8) between the two cohorts. Despite the occurrence of grades 3 and 4 neutropenia in many patients, hospitalizations for neutropenic fever did not occur.

The results of this study demonstrate that the pharmacokinetics of both pemetrexed and gemcitabine following combination chemotherapy are consistent with those following single-agent therapy, indicating that neither pemetrexed nor gemcitabine exposure is altered when pemetrexed is administered immediately following gemcitabine.

Pemetrexed clearance and volume of distribution estimates from the current study (approximately 100 ml/min and 17 l, respectively) are somewhat lower than those reported by Adjei et al. [14] for a study where pemetrexed was administered 90 min after gemcitabine (CL 130 ml/min, V 20 l). Patients included in the study by Adjei et al. had slightly lower creatinine clearance at the time of pemetrexed administration alone (98.0 ml/min $\pm$29.3%) but were otherwise similar to those in the current study, suggesting that differences in pharmacokinetics for the two dosage regimens are not attributable to patient characteristics. The results do not, however, suggest a difference in pemetrexed pharmacokinetics based on the time interval between administration of the two agents since the current results are very consistent with those determined when pemetrexed was administered as a single agent to 426 cancer patients with various solid tumors (CL 91.8 ml/min, V 16.1 l; reference Pemetrexed package insert). Therefore, the difference in pharmacokinetic results between the two pemetrexed-gemcitabine combination regimens is likely a reflection of the small number of patients ($n=4$) studied by Adjei et al. and the interindividual variability in pemetrexed pharmacokinetics. Figure 8 shows the distribution of pemetrexed clearance values for patients in each of the three study populations.

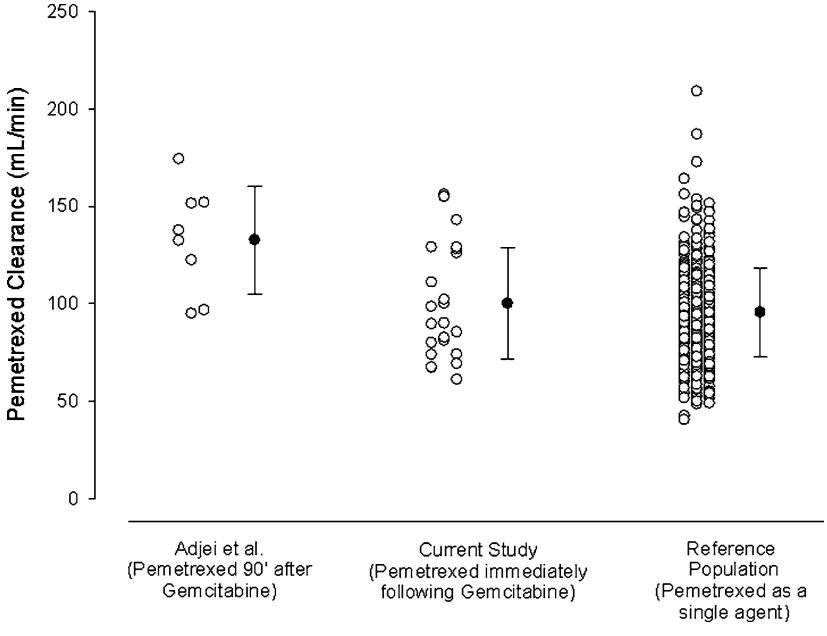

Fig. 8 Comparison of the distribution of pemetrexed clearance values in three separate study populations

Administering pemetrexed immediately after gemcitabine does not compromise the antitumor activity observed with the original phase I schedule. This is consistent with the predicted supra-additive activity upon simultaneous administration of these agents demonstrated in preclinical studies. An objective response was observed in a patient with NSCLC who had failed a platinum-based regimen. This study demonstrates that simultaneous administration of these two agents is feasible and tolerable.

We have compared toxicity and efficacy data from this study to data observed from ten patients treated at this same dose in our earlier phase I study. However, this comparison should be interpreted with caution because of the small numbers in both groups (10 and 14, respectively). In addition, cycle 1 treatment in our study did not include gemcitabine, and this could have affected cycle 1 toxicity, as well as efficacy. Moreover, this study was undertaken prior to routine folate and vitamin B12 supplementation which was shown in later studies to ameliorate pemetrexed-related toxicities [23]. Thus, folate and vitamin B12 supplementation may further improve the tolerability of this regimen.

The schedule incorporating administration of the recommended phase II dose of gemcitabine 1250 mg/m^2 on days 1 and 8 of each 21-day cycle, and pemetrexed 500 mg/m^2 on day 8 in immediate sequence, is well tolerated and clinically active, and therefore should be tested in the phase II setting to better define its efficacy and toxicity. Neither agent has been demonstrated to affect the pharmacokinetic profile of the other, therefore allowing simultaneous administration.

Acknowledgements Supported by grants from the National Institutes of Health (CA77112, CA69912, and RR00585) and Eli Lilly and Company. The authors wish to thank Janet Lensing for excellent data management, Michelle Daiss for protocol management, the nurses at GCRC and E12 Mayo Building for pharmacokinetic sampling and clinical care, and Raquel Ostby for expert secretarial assistance.

References

1. Plunkett W, Huang P, Xu YZ, et al (1995) Gemcitabine: metabolism, mechanisms of action, and self-potentiation. Semin Oncol 22 [Suppl 11]:3–10
2. Shih C, Chen VJ, Gossett LS, et al (1997) LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. Cancer Res 57:1116–1123
3. Rusthoven J, Eisenhauer E, Butts C, et al (1999) Multitargeted antifolate, LY231514, as first-line chemotherapy for patients with advanced non-small-cell lung cancer: a phase II study. J Clin Oncol 17:1194–1199
4. Clarke S, Millward M, Findlay M, et al (1998) Activity of the multi-targeted antifolate MTA (LY231514) in advanced non-small cell lung cancer (NSCLC). Ann Oncol 9 [Suppl 4]:86
5. John W, Picus J, Blanke C, et al (2000) Multitargeted antifolate (pemetrexed disodium, LY231514) activity in patients with advanced colorectal cancer: results from a phase II study. Cancer 88:1807–1813
6. Cripps C, Burnell M, Jolivet J, et al (1999) Phase II study of first-line LY231514 (multitargeted antifolate) in patients with locally advanced or metastatic colorectal cancer: an NCIC Clinical Trials Group study. Ann Oncol 10:1175–1179
7. Miles DW, Smith IE, Coleman RE, et al (2001) A phase II study of pemetrexed disodium (LY231514) in patients with locally recurrent or metastatic breast cancer. Eur J Cancer 37:1366–1371
8. Miller KD, Loehrer PJ, Picus J, et al (2000) Phase II study of the multi-targeted antifolate LY231514 (ALIMTA), MTA, pemetrexed disodium) in patients with advanced pancreatic cancer. Ann Oncol 11:101–103
9. Celio L, Bajetta E, Toffolatti L, et al (2000) Phase II trial of pemetrexed disodium administered every 21 days in patients (pts) with gastric cancer: efficacy and toxicity without and with folic acid. Ann Oncol 11 [Suppl 4]:65
10. Pivot X, Raymond E, Laguerre B, et al (2001) Pemetrexed disodium in recurrent locally advanced or metastatic squamous cell carcinoma of the head and neck. Br J Cancer 85:649–655
11. Paz-Ares L, Tabernero J, Moyano A, et al (1998) Significant activity of the multi-targeted antifolate MTA (LY231514) in advanced transitional cell carcinoma (TCC) of the bladder: results of a phase II trial. Ann Oncol 9 [Suppl 4]:61
12. Goedhals L, van Wijk AL (1998) MTA (LY231514) in advanced carcinoma of the cervix. Ann Oncol 9 [Suppl 2]:761
13. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al (2003) Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 21(14):2636–2644
14. Adjei AA, Erlichman C, Sloan JA, et al (2000) Phase I and pharmacologic study of sequences of gemcitabine and the multitargeted antifolate agent in patients with advanced solid tumors. J Clin Oncol 18:1748–1757
15. Tonkinson JL, Worzalla JF, Teng CH, et al (1999) Cell cycle modulation by a multitargeted antifolate, LY231514, increases the cytotoxicity and antitumor activity of gemcitabine in HT29 colon carcinoma. Cancer Res 59:3671–3676
16. Teicher BA, Chen V, Shih C, et al (2000) Treatment regimens including the multitargeted antifolate LY231514 in human tumor xenografts. Clin Cancer Res 6:1016–1023
17. Chaudhary AK, Schannen V, Knadler MP, et al (1999) Analysis of LY231514 by LC/MS/MS. In: The 47th ASMS Conference on Mass Spectrometry and Allied Topics, Dallas, TX
18. Benet LZ, Galeazzi RL (1979) Noncompartmental determination of the steady-state volume of distribution. J Pharm Sci 68(8):1071–1074
19. Rowland M, Tozer T (1995) Clinical pharmacokinetics. Concepts and applications, 3rd edn. Lippincott Williams & Wilkins, Media, Pennsylvania, p 318
20. Adjei AA (2003) Pemetrexed (Alimta): a novel multitargeted antifolate agent. Expert Rev Anticancer Ther 3(2):145–156
21. Tesei A, Ricotti L, De Paola F, et al (2002) In vitro schedule-dependent interactions between the multitargeted antifolate LY231514 and gemcitabine in human colon adenocarcinoma cell lines. Clin Cancer Res 8:233–239
22. Tonkinson JL, Marder P, Andis SL, et al (1997) Cell cycle effects of antifolate antimetabolites: implications for cytotoxicity and cytostasis. Cancer Chemother Pharmacol 39:521–531
23. Niyikiza C, Baker SD, Seitz DE, et al (2002) Homocysteine and methylmalonic acid: markers to predict and avoid toxicity from pemetrexed therapy. Mol Cancer Ther 1:545–552