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Phase II study of pemetrexed disodium (Alimta®) administered with oral folic acid in patients with advanced gastric cancer

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Background: The aim of this study was to assess the activity of pemetrexed in patients with advanced gastric cancer.

Patients and methods: Thirty-eight eligible patients (median age 60 years) received pemetrexed 500 mg/m^2 every 3 weeks. Since toxicity was considerable in the first six patients, the protocol was amended to supplement subsequent patients with oral folic acid (5 mg/day on days –2 to +2 of every cycle).

Results: Among 36 stage IV patients evaluable for efficacy (six non-supplemented\30 supplemented), there were two complete and six partial responses. The response rate was 21% (95% confidence interval 8% to 32%) according to intention-to-treat analysis. All responding patients were in the supplemented group. The median duration of response was 4.6 months and the median survival was 7.8 months. Five of six non-supplemented patients (83%) developed grade 3/4 neutropenia; two (33%) unsupplemented patients discontinued; two (33%) patients died due to toxicity. In the supplemented group, 12 of 32 patients (37%) had grade 3/4 neutropenia. None of the supplemented patients discontinued treatment due to hematological toxicity. Severe non-hematological toxicities were infrequent.

Conclusions: The activity of pemetrexed is promising in light of the tumor burden in these patients (all patients were stage IV and 39% had three or more organs involved). Toxicities were remarkably decreased with folic acid supplementation. Combination studies are warranted.

Key words: folic acid, gastric cancer, pemetrexed, phase II trial

Introduction

Gastric cancer remains a significant problem for global health as it is the second most common cause of tumor-related death worldwide [1]. The antimetabolite 5-fluorouracil (5-FU) is the most widely used chemotherapeutic agent in advanced gastric cancer, with response rates of ~20% [2]. A variety of chemotherapy regimens have been evaluated that result in higher response rates, although they show little improvement in median survival [3–5]. Recently, results from a large randomized trial of epirubicin, cisplatin and protracted venous infusion 5-FU have shown an improvement in patient survival and response rate [3]. However, much potential for improvement in survival and in tolerability exists. Thus, a search for new drugs is needed in order to improve the therapeutic outcome of patients suffering from this aggressive malignancy.

The investigational agent pemetrexed disodium (Alimta®; Eli Lilly & Co., Indianapolis, IN) is a novel antifolate analog which predominantly inhibits the thymidylate synthase (TS) enzyme, but is also active against dihydrofolate reductase and glycinamide ribonucleotide formyltransferase—folate-dependent enzymes involved in *de novo* purine biosynthesis [6]. In the clinical setting, this agent administered at a dose of 500 or 600 mg/m^2 once every 21 days has shown promising activity against a variety of solid tumors [7].

The broad range of antitumor activity of pemetrexed prompted the present study. However, toxicity at the starting dose of 500 mg/m^2 was considerable; in the first six patients enrolled, there was at least one episode of severe toxicity, two patients discontinued and two died, all due to toxicity related to the study drug. This unexpected high rate of toxicity, as well as treatment-associated fatalities, resulted in the accrual being halted prematurely.

Experimentation in mice revealed that nutritional folic acid (FA) supplementation preserved the antitumor activity of pemetrexed while dramatically reducing toxicity [8]. In addition, preliminary results from an ongoing phase I trial combining pemetrexed and high-dose intermittent oral FA (5 mg/day on days –2 to +2 of every cycle) indicated that the addition of folate ameliorates toxicities, permitting dose escalation of the agent up to at least 925 mg/m^2 in heavily pretreated patients [9]. Based on these findings, the study protocol was amended to supplement all patients treated with pemetrexed on this trial with oral FA to

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improve patient safety. We report on the results of this phase II study, which provided an opportunity, for the first time, to prospectively evaluate the effect of folate supplementation on the efficacy and safety profile of pemetrexed in a homogeneous subset of patients.

Patients and methods

Patient selection

For inclusion in the trial, patients had to be ≥18 years of age, have histologically proven adenocarcinoma of the stomach or gastro-esophageal junction with stage IIIB or IV disease, according to the American Joint Committee on Cancer Staging criteria [10], and at least one measurable disease site, and to have received no prior systemic chemotherapy or radiation therapy for gastric cancer. Lesions could not be amenable to curative surgery or radiotherapy. An Eastern Cooperative Oncology Group (ECOG) performance status ≤2 and a life expectancy of at least 12 weeks were required. Women of childbearing potential must have taken adequate precautions to prevent pregnancy. Patients were required to have adequate organ function as defined by the following: neutrophils, $\geq 1.5 \times 10^9$/l; hemoglobin, ≥9 g/dl; platelets, $\geq 100 \times 10^9$/l; bilirubin, ≤1.5× the upper limit of normal (ULN); aspartate transaminase (AST) or alanine transaminase (ALT) ≤3.0× ULN (AST or ALT ≤5× ULN was acceptable if documented liver metastases were present); and calculated creatinine clearance, ≥45 ml/min, using the modified Cockcroft and Gault calculated creatinine clearance formula. The study was conducted only after the appropriate approvals had been obtained from the human subjects research committees of the participating institutions. All patients signed a written informed consent document before enrollment. Patients were excluded if they had a history of second primary malignancy. Patients with clinically significant effusions (pleural or peritoneal) or albumin <2.5 g/dl were ineligible. Pregnant women, patients with active infection or symptomatic brain metastasis were also excluded from the study. Patients who could not discontinue therapy with aspirin and other non-steroidal anti-inflammatory agents for 2 days before, the day of and 2 days after administration of pemetrexed were excluded from the trial.

Pre-treatment investigations, performed within 3 weeks of commencing treatment, included full history and examination, complete blood count, blood chemistries, urinalysis, electrocardiogram, calculated creatinine clearance and assessment of vital signs. During therapy, hematology was measured at the start of every cycle and on a weekly basis thereafter. Blood chemistries were performed before the start of treatment and 1 week after every cycle. Urinalysis, vital signs and calculated creatinine clearance were also assessed at the start of each cycle.

Treatment

Pemetrexed was administered at a dose of 500 mg/m^2 as a 10-min intravenous infusion, once every 21 days. Dexamethasone 4 mg, a prophylactic measure for skin rash, was given orally in the USA and intramuscularly in Italy twice a day, starting the day before and continuing until the day after study drug administration. Supplemental FA was given orally at a dose of 5 mg once a day, on days –2 to +2 for every cycle. Therapy was continued unless the patient met withdrawal criteria or had progressive disease. All adverse events were scored according to National Cancer Institute Common Toxicity Criteria. Grade 4 neutropenia lasting >5 days required a 15% dose reduction. Grade 3 and grade 4 thrombocytopenia required 15% and 50% dose reductions, respectively. A 25% dose reduction was required in case of concomitant grade 4 neutropenia and grade 3 thrombocytopenia. Grade 3 and 4 mucositis required a 25% and 50% dose reduction, respectively. Once a dose reduction had occurred, it was not permitted to re-escalate for subsequent courses. Patients who required more than two dose reductions for toxicity were removed from the study. Patients were required to meet all the following criteria in order to begin the next cycle of treatment: neutrophils $\geq 1.5 \times 10^9$/l; platelets $\geq 100 \times 10^9$/l; calculated creatinine clearance ≥45 ml/min; resolution or improvement of clinically significant non-hematological adverse events to grade 1 or 0. If a patient could not be retreated within 42 days from the last course of pemetrexed, they were excluded from further treatment.

Efficacy assessment and statistical analysis

The study plan was to enroll eligible patients from one American and five Italian centers. Participating Italian institutions were coordinated by means of the Italian Trials in Medical Oncology (ITMO) Group. The primary objective of the study was to evaluate the response rate; secondary objectives being the estimation of the toxicity profile and the time-to-event efficacy measures. A two-stage design was utilized to permit early study termination if significant antitumor activity was not observed. Thirteen qualified patients had to be enrolled into the first stage and if one or more patients responded to pemetrexed, 22 additional patients would be accrued. If fewer than seven of 35 patients responded, the study drug would be rejected as inactive in this indication. This procedure tested the null hypothesis (H_0) that the true response rate was ≤10% versus the alternative hypothesis (H_A) that the true response rate was at least 25%. The significance level (probability of rejecting the H_0 when it is true) was 0.06, and the power (probability of rejecting the H_0 when the H_A is true) was 80%. The efficacy analysis was performed on data from all patients who qualified for the protocol and who received at least two doses of pemetrexed. A patient who discontinued from study due to unacceptable drug toxicity prior to receiving two doses was also included in the efficacy analysis.

Tumor measurements were assessed by radiographic scan within 4 weeks prior to study entry and were restaged after every second cycle. Patients with tumor responses required a confirmatory disease assessment at least 4 weeks later. All responses were confirmed by independent radiology review and determined using standard Southwest Oncology Group criteria. Response duration was defined as the time from treatment initiation to the appearance of objective evidence of disease progression. Overall survival was measured from the date of study enrollment to the date of death and was estimated by the Kaplan–Meier method. The safety analysis was performed on data from all 38 patients who received at least one dose of pemetrexed.

Results

From July 1997 to May 2001, 38 patients entered the study. Six were enrolled in the early group of unsupplemented patients, and 32 were enrolled from February 1999 when the accrual started again, after the study protocol was amended to administer oral FA with pemetrexed. Thirty (79%) of a total of 38 patients were treated at the National Cancer Institute of Milan, with the remaining eight patients treated at one of the outside institutions. Thirty-six patients had adenocarcinoma of the stomach and two cases were diagnosed with adenocarcinoma of the gastro-esophageal junction. Table 1 lists the patient demographics.

Efficacy

Thirty-six patients (95%) qualified for efficacy analysis. Two patients in the supplemented group were not evaluable for response due to insufficient therapy, as per protocol. One patient with liver metastases and pulmonary lymphangitis had an early deterioration in their physical condition not considered to be related to study drug and died soon after administration of the first cycle. Another patient who complained of significant upper gastrointestinal bleeding after the first dose of pemetrexed had a

Table 1. Patient characteristics (n = 38)

Characteristic	Patients, n (%)
Age, years	
Median	60
Range	43–76
Sex	
Male	29 (76)
Female	9 (24)
ECOG performance status	
0	26 (68)
1	12 (32)
Disease stage[a]	
IV	38 (100)
Sites of metastases	
Liver	24 (63)
Lymph node	24 (63)
Lung	7 (18)
Other	16 (42)
Number of disease sites	
1	12 (32)
2	11 (29)
≥3	15 (39)
Prior surgery	
None	12 (32)
Curative	14 (36)
Exploratory/palliative	12 (32)

[a]According to the American Joint Committee on Cancer Staging Criteria.
ECOG, Eastern Cooperative Oncology Group.

rapid progression of gastric primary tumor as documented by an abdominal computed tomography scan.

Eight of 36 evaluable patients achieved confirmed objective regressions. Among the supplemented patients, a complete response was observed in two patients, while six patients achieved a partial response. The response rate was 21% (95% confidence interval 8% to 32%) according to intention-to-treat analysis. A complete response occurred in patients who had tumor recurrence after curative surgery, while no patient retaining the primary tumor experienced objective regression at this site. Six of eight responders were males. Sites of response included liver, lymph nodes, lung and peritoneum. Stabilization of disease was observed in 12 patients (33%). The median duration of response was 4.6 months (range 3–10), the median time to disease progression 2.8 months and the median survival 7.8 months (range 0.7 to >24). The probability of being alive at 1 year was 27%. Six of 17 patients who received second-line chemotherapy after progression on pemetrexed treatment experienced partial remission.

Toxicity

All 38 patients who received at least one dose of pemetrexed were evaluated for toxicity. A total of 144 cycles of pemetrexed were administered. The median number of cycles per patient was three (range 1–8). The mean delivered dose intensity was 158.8 mg/m^2/week (planned 166.7 mg/m^2/week), which corresponds to 95% of the planned dose intensity. There were 35 dose delays (24% of doses administered). Common reasons for dose delays were scheduling conflicts (51%), decrease in creatinine clearance (20%) and increase in transaminases (9%). Five patients had one dose reduction for the following reasons: increased transaminases in two patients, and one patient each, leukopenia, thrombocytopenia and stomatitis.

All six patients in the non-supplemented group had at least one episode of severe toxicity. Two of these patients discontinued treatment due to grade 4 leukopenia and grade 2 vomiting, respectively. In addition, two patients had a lethal toxicity. Acute myocardial infarction occurred soon after the first cycle of therapy in a 76-year-old patient with lymph node and liver metastases who was known to have a history of myocardial infarction. The patient was found to have grade 3 or 4 toxicities of anemia, neutropenia, stomatitis and diarrhea during his hospital stay. Despite therapeutic measures, the patient died due to heart failure. Another patient, a 66-year-old man with liver metastases, developed grade 3 stomatitis and vomiting, and grade 2 diarrhea after the second cycle of pemetrexed. The patient was hospitalized for severe dehydration and died due to acute renal failure.

In the supplemented group, there was one death that occurred in a 62-year-old patient with liver metastases. After the first cycle of therapy, the patient experienced grade 3 stomatitis and grade 2 skin rash that led to a dose reduction as per protocol. One week after the second course, the patient developed febrile neutropenia, grade 4 thrombocytopenia, watery diarrhea and grade 3 increased bilirubin. Despite aggressive measures, the patient died due to hepatic failure possibly related to study drug. In fact, it was unclear whether hepatic failure represented a toxic event or was also secondary to disease progression, as the baseline work-up revealed extensive metastatic disease in the liver along with normal bilirubin and transaminase levels. The exact cause of death remained unknown because the patient was unevaluable for response based on failure to obtain a second tumor assessment and post mortem examination.

Hematological toxicities are summarized in Table 2. Percentages of severe hematological events were higher in the non-supplemented group than in the supplemented group. Five of six non-supplemented patients (83%) developed grade 3/4 neutropenia; severe neutropenia occurred in 10 (67%) of 15 cycles completed. In the supplemented group, 12 of 32 patients (37%) experienced grade 3/4 neutropenia. There was no evidence of cumulative toxicity. No patient in the supplemented group discontinued treatment due to hematological toxicity.

Severe non-hematological toxicities occurred infrequently, but were observed mainly in the non-supplemented group (Table 3). Patients not receiving supplemental FA experienced the following toxic effects: grade 3/4 mucositis (two patients), grade 4 diarrhea

Table 2. Hematological toxicity with or without folic acid supplementation (worst grade per patient)

Event	No. of patients (n = 38)	Grade, n (%)[a]	
		3	4
Non-supplemented group			
Neutropenia	6	1 (16)	4 (67)
Leukopenia	6	3 (50)	2 (33)
Thrombocytopenia	6	1 (16)	0 (0)
Anemia	6	1 (16)	0 (0)
Supplemented group			
Neutropenia	32	5 (15)	7 (22)
Leukopenia	32	9 (28)	1 (3)
Thrombocytopenia	32	1 (3)	1 (3)
Anemia	32	3 (9)	0 (0)

[a]Toxicity graded according to National Cancer Institute Common Toxicity Criteria.

(two patients), grade 3 nausea (two patients), grade 3 vomiting (one patient), grade 4 infection (one patient) and grade 3 fatigue (one patient). Severe non-hematological events observed in the 32 supplemented patients were grade 3 mucositis (one patient) and grade 3 fatigue (one patient). Transient deterioration in liver function tests were observed in all patients, with grade 2–3 increases in serum levels of transaminases (24%) and alkaline phosphatase (5%). However, no clinical symptoms associated with these changes were noted.

Discussion

Results of this clinical trial represent the first published evidence of the activity of pemetrexed in gastric cancer. Furthermore, this is the first report on efficacy and safety of pemetrexed with FA supplementation in a clearly defined subset of patients. Responses were usually observed after two courses of pemetrexed and occurred in a variety of metastatic sites, including liver and retroperitoneal lymph nodes, with a median survival of 7.8 months. The response rate achieved was comparable to single-agent activity observed with the most active conventional drugs, but also with newer agents, including taxanes and irinotecan [2].

Some characteristics of the study population should be considered when analyzing the efficacy data. All patients enrolled presented with distant metastases, with 39% of cases showing involvement of at least three organs, a high-risk prognostic factor [11]. It is known that patients with metastatic gastric cancer may have poorer outcomes than those with locally advanced disease in terms of both response rate and short-term survival [3]. In the majority of patients (63%) in this study, the primary tumor had not been resected. Interestingly, in a European phase II trial of docetaxel in advanced untreated gastric cancer, responses occurred more frequently in patients who had primary tumor resection [12]. It is likely that patients whose gastric tumor was not removed have more advanced disease with a larger tumor burden. These data suggest that the 21% response rate achieved with pemetrexed monotherapy in this patient population is promising. In light of this, it is worth pointing out that no activity against this malignancy was observed in a phase II study of raltitrexed, a specific TS inhibitor [13]. A study in which ~50% of patients had received previous 5-FU-based therapy, which might have contributed to the lack of tumor responses due to the up-regulation of TS expression [14]. However, it is possible that the ability of pemetrexed to inhibit more than one folate-dependent enzyme may help to increase the spectrum of tumors potentially sensitive to the drug.

Adverse events observed during this trial were predictable based upon the mechanism of action of pemetrexed. In the majority of clinical trials, the dose-limiting toxicity of pemetrexed was neutropenia; other non-dose-limiting toxicities included transient

Table 3. Non-hematological toxicity (worst grade per patient) (n = 38)

Toxicity	Grade, n (%)[a]			
	1	2	3	4
Nausea	10 (26)	0 (0)	2 (5)	0 (0)
Vomiting	2 (5)	1 (3)	1 (3)	0 (0)
Infection	2 (5)	0 (0)	0 (0)	1 (3)
Diarrhea	3 (8)	4 (10)	0 (0)	2 (5)
Skin rash	10 (26)	4 (10)	0 (0)	0 (0)
Mucositis	4 (10)	1 (3)	2 (5)	1 (3)
Fatigue	7 (18)	0 (0)	2 (5)	0 (0)
Transaminases	14 (37)	6 (16)	3 (8)	0 (0)
Alkaline phosphatase	15 (39)	1 (3)	1 (3)	0 (0)
Bilirubin	0 (0)	2 (5)	3[b] (8)	0 (0)
Creatinine	1 (3)	1[c] (3)	0 (0)	0 (0)

[a]Toxicity graded according to National Cancer Institute Common Toxicity Criteria.
[b]One non-supplemented and one supplemented patient died.
[c]Non-supplemented patient died.

transaminase elevations, skin rash, mucosal toxicity, fatigue and diarrhea [7]. However, such toxicities were not a serious problem in those phase II trials in which patients had a generally good performance and nutritional status [7].

No detailed information on patients' nutritional status was available, but it is plausible that the unexpectedly high rate of hematological toxicity observed in the non-supplemented patients enrolled in this study reflects their generally poor functional folate status due to the gastric carcinoma. In support of this conclusion, the comparison of clinical data from patients receiving versus those not receiving FA supplementation suggests that, although the groups are small and non-randomized, the concomitant use of FA is able to markedly improve the safety profile of pemetrexed in this patient population.

Since hematological toxicity of pemetrexed has been correlated with drug exposure, another possible explanation for the increase in hematological toxicity may be the occurrence of higher plasma concentrations of drug in non-supplemented patients. It is of interest that in a recently reported trial with pemetrexed 500 mg/m^2, administered without supplemental FA in 35 patients with advanced head and neck cancer, hematological toxicity was also higher than that previously observed in other trials with this antifolate [15]. When the pharmacokinetics of pemetrexed in head and neck cancer patients was compared with that in patients with other cancer types, a large degree of overlap between the two populations without any significant difference was observed. Since head and neck cancer patients with advanced disease have generally poor nutritional status, it was suggested that this condition could have played a major role in the incidence and severity of toxicities. Also, studies with other antifolates have reported that poor nutritional status contributes to the likelihood that a patient will experience severe toxicity when exposed to these drugs [16–18]. The importance of FA supplementation in reducing the toxic effects and increasing the therapeutic index of pemetrexed has been shown preclinically, and a multivariate analysis involving a large number of patients showed an association between high pretreatment levels of serum homocysteine, which reflects a low folate status of the patient, and more severe hematological or non-hematological toxicities [8, 19]. It is noteworthy that the main circulating folate, methyltetrahydrofolate, is a substrate for the enzyme methionine synthase, which converts homocysteine to methionine in a B_{12}-dependent reaction, and deficiency in folate or vitamin B_{12} results in a rise in plasma homocysteine [20, 21]. Accordingly, following a programmatic change to supplement patients with daily low-dose FA and vitamin B_{12}, it has recently been reported that pemetrexed-induced toxic effects can be proactively managed [22].

In summary, we conclude that high-dose intermittent oral FA allowed administration of pemetrexed at the dose and schedule explored with a highly satisfactory safety profile and with no compromise in efficacy. Further investigations combining pemetrexed with other known active agents against gastric carcinoma are warranted to put the role of this agent in proper perspective [23]. Accordingly, a phase II trial of pemetrexed in combination with the platinum analog oxaliplatin in locally advanced or metastatic disease has been planned.

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