A phase I/II and pharmacogenomic study of pemetrexed and cisplatin in patients with unresectable, advanced gastric carcinoma

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This phase I/II study was conducted to determine the maximum recommended dose of pemetrexed when given in combination with a fixed dose of cisplatin, and the efficacy, toxicity and association of 5,10-methylenetetrahydrofolate reductase (MTHFR) variants with this pemetrexed–cisplatin combination, in patients with unresectable, advanced gastric carcinoma. Patients 18–70 years of age, with stage IV disease or post-surgery recurrence, no earlier palliative chemotherapy, 0 or 1 Eastern Cooperative Oncology Group performance status, were included. The cisplatin dose was 75 mg/m^2. In phase I, the initial dose of pemetrexed was 600 mg/m^2, escalated in 100 mg/m^2 increments. In phase II, efficacy, including overall response rate, overall survival, as well as toxicity and MTHFR pharmacogenetics were investigated. Phase I enrolled 16 patients; 700 mg/m^2 was defined as pemetrexed recommended dose. Thirteen serious adverse events were reported; the most common grade 3/4 toxicities were haematologic (10 of 13, 76.9%). Phase II enrolled 73 patients, 69 qualified for safety and 68 for efficacy analysis; 65 for pharmacogenomic analysis. Overall response rate was 23.5% (14.1%, 35.4%), disease control rate 55.9%, median overall survival 11.8 months (95% confidence interval, 7.2–18.5 months), progression-free survival 4.9 months (95% confidence interval, 2.8–7.1 months), and median response duration 5.4 months. Patients with MTHFR A1298C variants had median overall survival of 6.6 months, significantly shorter than patients with the wild type (median 18.5 months, $P=0.001$). The pemetrexed–cisplatin combination in patients with advanced gastric cancer generates modest efficacy and a manageable toxicity profile. The reduced overall survival in patients with MTHFR A1298C polymorphism variants deserves further investigation. *Anti-Cancer Drugs* 21:777–784 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Gastric cancer is the second most frequent cause of cancer mortality in the world [1] with high incidence in eastern Europe, and parts of Central and South America, whereas in most parts of Asia gastric cancer remains the leading cause of cancer-related deaths, with the highest incidence in Korea, Japan and China [2]. The only potentially curative treatment is surgical resection. However, even after receiving this intervention many patients relapse with local recurrence or distant metastases [3]. In addition, approximately 20–30% of patients have inoperable disease at diagnosis. Therefore, the majority of patients need a palliative systemic treatment at some point in their disease [4]. Randomized trials have shown that, compared with best supportive care, chemotherapeutic regimens may improve survival and quality of life in patients with advanced gastric cancer [5]. Although there are no standard combination regimens for advanced gastric cancer [6], 5-fluorouracil (5-FU) and cisplatin (CDDP) are the most extensively used single agents [7]. More recently introduced agents, evaluated for the treatment of gastric cancer, include paclitaxel [8], docetaxel [9], irinotecan [10], oxaliplatin [11] and capecitabine [12]. Efficacy outcomes for these agents have not, however, shown superiority to older drugs.

Pemetrexed (Alimta; Eli Lilly, Indianapolis, USA) is a multi-targeted antifolate currently approved for malignant pleural mesothelioma and non-small cell lung cancer (NSCLC) [13]. Pemetrexed inhibits thymidylate synthase and dihydrofolate reductase, an enzyme involved in

Clinical trial registry: Results from this study have been presented on the clinical trial registry located at ClinicalTrials.gov (ID: NCT00320515).

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one-carbon transfer reactions important for *de novo* purine and pyrimidine metabolism. In addition, pemetrexed also inhibits glycinamide ribonucleotide formyl transferase, which is one of two enzymes important for purine synthesis.

Pemetrexed has firmly established its promising efficacy in the treatment of mesothelioma [14] and NSCLC [13,15]. The broad range of clinical activity of pemetrexed against solid tumours has prompted studies in patients with advanced gastric cancer [16]. Cisplatin has a wide spectrum of activity against epithelial cancers and is one of the most widely used cytotoxic agents for the treatment of human cancers [17]. The effect of the combination of cisplatin–pemetrexed has been studied in different forms of cancer. Two phase II and one phase III study have evaluated pemetrexed in combination with cisplatin as first-line chemotherapy for advanced NSCLC [15] and a phase III clinical trial of pemetrexed–cisplatin in mesothelioma resulted in a highly satisfactory safety profile [14]. Pemetrexed in combination with cisplatin has also shown synergistic activity in gastric cancer cells [18]. A recent phase II study in gastric cancer patients indicated that the combination of cisplatin (75 mg/m^2) and pemetrexed (500 mg/m^2) had a modest activity and acceptable toxicity profile, setting the ground for pemetrexed dosing in this study [19].

We conducted a phase I/II study to investigate the combination of pemetrexed–cisplatin in patients with advanced gastric cancer. For the phase I portion, the primary objective was to determine the dose of pemetrexed, in combination with cisplatin, to be recommended for the phase II portion; the secondary objective was to evaluate the DLT of pemetrexed with cisplatin. For the phase II, the primary objective was to assess the response to pemetrexed–cisplatin. The secondary objectives included duration of response, progression-free survival, overall survival (OS) and safety and toxicity as well as the association between the clinical outcome of pemetrexed–cisplatin therapy and two polymorphisms (C677 T and A1298C) in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene that have been reported earlier as having a predictive role on the toxicity and efficacy of antifolate and fluoropyrimidine agents [20].

Patients and methods

Eligibility criteria

Patients with histologically proven gastric adenocarcinoma, stage IV disease not amenable to curative surgery, or disease recurrence after surgery, were eligible. The required disease status for phase II was measurable disease as defined by the Response Evaluation Criteria in Solid Tumours (RECIST). For phase I, patients with non-measurable lesions could be enrolled. Other inclusion criteria were age 18–70 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1; estimated life expectancy ≥ 12 weeks; adequate bone marrow reserve, and hepatic and renal function.

Patients were excluded from the study if they had earlier palliative chemotherapy for advanced disease; known or suspected brain metastasis; active infections; concurrent administration of any other tumour therapy; a second primary malignancy; clinically detectable ascites or pleural effusions; inability to take folic acid (FA), vitamin B_{12}, or dexamethasone; inability to interrupt aspirin or non-steroidal anti-inflammatory drugs for 2 days before, the day of, and 2 days after pemetrexed treatment.

The study was conducted according to Good Clinical Practise and the Declaration of Helsinki; the protocol was approved by the Institutional Review Board at each site of clinical study. All patients provided written informed consent before any study procedures were performed.

Study design

This single-arm, open-label, multicentre study (H3E-AA-S038) was designed to identify the dose and efficacy of pemetrexed, when used in combination with cisplatin, for the treatment of patients with unresectable advanced gastric carcinoma. Patients were recruited by oncologists between October 2001 and August 2003 from Taiwan (phase I), and between March 2004 and July 2008 from three additional countries (Argentina, South Korea and Mexico) for phase II.

Treatment plan

Pemetrexed and cisplatin were administered intravenously on day 1 every 21 days. The maximum number of treatment cycles that patients received was not predetermined and continued until disease progression or intolerance occurred. The cisplatin dose was 75 mg/m^2 for both phases I and II. For phase I, the initial pemetrexed dose was defined as 600 mg/m^2, based on earlier results [19,21], and was administered at four levels: DL1 (600 mg/m^2), DL2 (700 mg/m^2), DL3 (800 mg/m^2) and DL4 (900 mg/m^2).

The patients also received daily oral FA (350–600 μg) given beginning 1–2 weeks before first dose of pemetrexed in cycle 1 and continuing until 3 weeks after discontinuation from study therapy; vitamin B_{12} was administered as a 1000 μg intramuscular injection. A vitamin B_{12} injection was administered approximately 1–2 weeks before treatment with pemetrexed–cisplatin and was repeated approximately every 9 weeks until 3 weeks after the last dose of pemetrexed. The patients also had to take dexamethasone (4 mg orally twice a day, the day before, the day of and the day after pemetrexed) to prevent rash. Treatment was continued until the disease progressed or ended if unacceptable toxicity occurred.

Dose-escalation schema

The initial number of patients enrolled per DL was three. If 0 of three patients had dose-limiting toxicity (DLT), the pemetrexed dose was escalated to the next level. If one of three patients had DLT, three more patients were enrolled at this DL. If ≥ 2 of three or ≥ 2 of six patients had DLT, their dose was not escalated further and this DL was defined as the maximum tolerated dose (MTD).

Dose-limiting toxicity

The recommended dose of pemetrexed for phase II was defined as the highest DL at which less than two of six patients ($< 33\%$) experienced DLT. The following toxicities were defined as DLTs: death due to toxicity, neutropenia $< 0.5 \times 10^9$/l lasting for 5 days or longer; neutropenia $< 1.0 \times 10^9$/l with fever $\geq 38.5^\circ$C; thrombocytopenia $< 10.0 \times 10^9$/l; thrombocytopenia $< 50.0 \times 10^9$/l with bleeding; serum aspartate aminotransferase and alanine aminotransferase $> 20 \times$ ULN; and other non-haematological grade 3/4 toxicities with the exception of nausea and vomiting.

Baseline and treatment assessments

To be eligible for phase II, the patients were required to have a documented measurable disease. In phase I, the patients who had non-measurable lesions were eligible. In phases I and II, disease assessment was recorded at baseline and before the start of every other treatment cycle. Baseline radiological imaging studies for tumour assessment were undertaken within 4 weeks before study enrollment. The method used at baseline was consistently used for tumour assessment throughout the study and was repeated approximately every 6 weeks (± 1 week), before the start of every other treatment cycle. In patients who showed a complete response (CR) or partial response (PR), confirmation of response was performed 4–5 weeks after the first documented response. Thereafter, a responding patient was evaluated every 6 weeks (± 1 week), before the start of every other cycle.

Genotyping methodology

Two common non-synonymous MTHFR variants have been associated with functional changes in this gene. The C677T variant (Ala222Val, rs1801133) has been associated with MTHFR decreased activity, increased level of homocysteine and an altered distribution of folate [22]. The A1298C variant (Glu429Ala, rs1801131) has also been related to reduced MTHFR activity, but to a lesser degree compared with C677T [23]. These two DNA polymorphisms were genotyped using matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (Sequenom, San Diego, California, USA) assays. All patients participating in the pharmacogenomic study consented to the collection of blood and DNA extraction for the analyses of these two polymorphisms.

Statistical analyses

A response rate of 40–50% was anticipated for pemetrexed–cisplatin as the first-line chemotherapy for advanced gastric cancer and a response rate of 30% was considered the minimum activity level of interest for this patient population. With the planned 60 evaluable patients, this study had 82% power not to reject the treatment if the true response rate was 45%, and 95% power not to reject the treatment if the true response rate was 50%.

All patients enrolled in this study, who met the inclusion criteria, had measurable disease and received at least one dose of the study therapy, qualified for the efficacy analyses, including the primary end point of the response rate and all time-to-event analyses. The response rate and its two-sided 95% confidence interval (CI) were calculated based on an exact binomial probability at an α level of 0.05.

Progression-free survival (PFS) was defined as time from enrollment to the date of objective disease progression or death during the study (whichever occurred first). Duration of tumour response was measured from the time of first documentation of CR or PR until the time to disease progression. Overall survival time was defined as the time from the date of enrollment to the date of death due to any cause. All time-to-event variables were estimated by the Kaplan–Meier method.

All patients who received at least one dose of the study therapy were evaluated for safety. All adverse events were assessed according to National Cancer Institute Common Toxicity Criteria (CTC V.3). In terms of dose delays and reductions, the reduction proportion of pemetrexed and cisplatin was determined by the severity grading of toxicities (CTC-AE). Any patient who required a dose reduction continued to receive the reduced dose for the remainder of the study. Two dose reductions for each study drug were allowed in this study. Any patient who had two earlier dose reductions and experienced a toxicity that required another dose reduction was discontinued from study therapy. Treatment could have been delayed for up to 42 days from day 1 of the preceding cycle to allow a patient sufficient time to recover from study drug-related toxicity (summarized in Table 1).

The primary outcome measure in phase II was the objective best overall response rate. The tumour response rate including an exact 95% CI was reported.

Table 1 Dose reduction and delay

	Pemetrexed		Cisplatin	
	n	%	*n*	%
Dose reduction	13	3.49	16	4.29
Dose delay	41	10.99	41	10.99
Dose delay due to adverse event	39	10.46	39	10.46
Reduction or delay	49	13.14	53	14.21

The association of MTHFR genotype with the RECIST response was evaluated using the Fisher's exact test and further by a logistic regression adjusted for age, body mass index, sex, race (Asian/non-Asian) and ECOG PS. The association of MTHFR genotype with survival was investigated using the Kaplan–Meier method and the log-rank test. Cox proportional hazards regression was used for multivariate survival analysis adjusted for the same confounders as above. MTHFR gene polymorphisms were analyzed in a binary manner of all wild types and variants.

Two MTHFR gene polymorphisms (C677T and A1298C) were investigated:

(1) C677T: defined as three categories (C/C, C/T and T/T), further coded as a binary variable with 0 for wild type (C/C) and one for variant genotype (C/T or T/T).
(2) A1298C: defined as three categories (A/A, A/C and C/C), further coded as a binary variable with 0 for wild type (A/A) and one for variant genotype (A/C or C/C).

Results

Patient characteristics

The phase I study enrolled 16 patients. All 16 patients qualified for the safety analysis and 13 patients qualified for the efficacy analysis. The phase II portion of the study enrolled 73 patients, four patients were excluded from all analyses because of data quality issues. Sixty-nine patients qualified for the safety analysis, 68 patients qualified for the efficacy analysis and 65 patients qualified for the pharmacogenomics analysis. Patient characteristics are summarized in Table 2. There were 59 men and 26 women who qualified for analysis with a median age of 51.5 years for phase I and 57.0 years for phase II. Most patients ($n = 54$; 63.5%) had an ECOG PS of 0. Of the 43 patients who had earlier surgery, 21 patients (48.8%) were treated with palliative intent.

Maximum tolerated dose

According to the phase I dose-escalation schema, if ≥ 2 of three or ≥ 2 of six patients had DLT, the dose was not escalated further and this DL was defined as the MTD. The recommended dose of pemetrexed was defined as the highest DL at which less than two of six patients ($< 33\%$) experienced DLT.

Three patients were enrolled at DL one (600 mg/m^2), of whom none experienced DLT (DL1, $n = 3$). Three patients were enrolled at DL2 (700 mg/m^2), of whom one experienced DLT (neutropenia $< 0.5 \times 10^9$/l lasting for 5 days or longer). A further three patients were enrolled, of whom none experienced DLT (DL2, $n = 6$). Three patients were enrolled at DL3 (800 mg/m^2); however, one patient withdrew from the study before completing cycle 1. Therefore, another patient was also enrolled. Of these four enrolled patients, one patient had

Table 2 Patient demographics characteristics at baseline

	Phase I				Phase II
	Pemetrexed treatment level				Pemetrexed + cisplatin
Demographic variable	600 mg/m^2 ($N=3$)	700 mg/m^2 ($N=6$)	800 mg/m^2 ($N=7$)	Total ($N=16$)	Total ($N=69$)
Age (years)					
Median (min–max)	50.0 (44–52)	62.5 (30–64)	50.0 (43–68)	51.5 (30–68)	57.0 (25–72)
Sex (*n*, %)					
Male	2 (66.7)	4 (66.7)	4 (57.1)	10 (62.5)	49 (71.0)
Female	1 (33.3)	2 (33.3)	3 (42.9)	6 (37.5)	20 (29.0)
Ethnic origin (*n*, %)					
Eastern Asian	3 (100.0)	6 (100.0)	7 (100.0)	16 (100.0)	47 (68.1)
Hispanic					21 (30.4)
Caucasian					1 (1.4)
ECOG PS (*n*, %)					
0	2 (66.7)	3 (50.0)	7 (100.0)	12 (75.0)	42 (60.9)
1	1 (33.3)	3 (50.0)	0 (0.0)	4 (25.0)	27 (39.1)
Prior surgery (*n*, %)					
Curative	2 (66.7)	1 (16.7)	1 (14.3)	4 (25.0)	18 (26.1)
Palliative	1 (33.3)	2 (33.3)	3 (42.9)	6 (37.5)	15 (21.7)
Prior radiotherapy (*n*, %)					
Curative	1 (33.3)	0 (0.0)	0 (0.0)	1 (6.3)	2 (2.9)
Palliative	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
Prior chemotherapy (*n*, %)	1 (33.3)	1 (16.7)	0 (0.0)	2 (12.5)	6 (8.7)
Prior chemotherapy around surgery (*n*, %)	1 (33.3)	1 (16.7)	0 (0.0)	2 (12.5)	5 (7.2)
Prior chemotherapy around radiotherapy (*n*, %)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)

ECOG PS, Eastern Cooperative Oncology Group performance status.

DLT (other non-haematological toxicities of CTC grade 3 or 4; hypokalaemia). Three more patients were enrolled at DL3, of whom two patients experienced DLTs (neutropenia $< 1.0 \times 10^9$/l with fever = 38.5°C and other non-haematological toxicities of CTC grade 3 or 4, tumour haemorrhage) (DL3, $n = 7$).

The criterion for MTD was met at DL3 (800 mg/m^2) because ≥ 2 of six patients had DLT at this dose. The recommended dose for the phase II part of this study was therefore defined as DL2 (700 mg/m^2).

Efficacy

The overall response rate for phase II was 23.5% ($n = 16$; 95% CI, 14.1–35.4%). Of the 68 patients evaluable for efficacy, one (1.5%; 95% CI, 0.0–7.9%) had objective CR, 15 (22.1%; 95% CI, 12.9–33.8%) had PR, 22 (32.4%) had stable disease, 24 (35.3%) had progressive disease, and six (8.8%) were classified as unknown. The disease control rate was 55.9% ($n = 38$; 95% CI, 43.3–67.9%) (Table 3). The median duration of response was 5.4 months (95% CI, 4.6–6.9 months).

The median OS was 11.8 months (95% CI, 7.2–18.5 months) and the median PFS was 4.9 months (95% CI, 2.8–7.1 months). Although efficacy was not a phase I objective, the overall response rate was 46.2%. Of 13 patients with measurable disease, six had a RECIST response, comprising two CR and four PR.

Safety

During the phase I portion of the study a total of 10 patients died: seven patients from study disease and three from adverse events. Thirteen serious adverse events were reported in seven patients. The most common grade 3/4 toxicities were haematologic (10 of 13–76.9%).

Table 3 Phase II overall response rate

Response	Statistic	Pemetrexed + cisplatin (N=68)
Responder (CR or PR)	n (%)	16 (23.5)
	95% CI	(14.1, 35.4)
Non-responder (SD, PD, U)	n (%)	52 (76.5)
	95% CI	(64.6, 85.9)
Disease control (CR, PR, SD)	n (%)	38 (55.9)
	95% CI	(43.3, 67.9)
Disease control Failure (PD or U)	n (%)	30 (44.1)
	95% CI	(32.1, 56.7)

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; U, unknown.

During the phase II portion of the study, 31 patients died of study-related disease; one patient died of an indeterminate cause and one patient died of a non-study-related cause. A total of 69 patients (100%) reported at least one treatment-emergent adverse event (TEAE), of these 66 (95.7%) were possibly study drug-related. Of the 69 patients, 51 (73.9%) reported at least one grade 3/4 TEAE, with 43 (62.3%) being possibly related to the study drug. Grade 3/4 haematological TEAEs were reported in 41 patients (59.4%). The most frequently reported haematological TEAEs were neutropenia ($n = 28$, 40.6%), anaemia ($n = 13$, 18.8%), leucopenia ($n = 7$, 10.1%) and lymphopenia ($n = 7$, 10.1%).

Dose administration (phase II)

A total of 373 cycles were administered to 69 patients. Thirteen dose reductions (3.5%) were reported for pemetrexed and 16 dose reductions (4.3%) for cisplatin. The mean achieved doses per week were 214.10 mg/m^2 for pemetrexed (91.8% of planned doses) and 22.77 mg/m^2 (91.1% of planned doses) for cisplatin.

A total of 18 transfusions were performed during phase II, 17 with packed red blood cells and one with whole blood.

MTHFR genotype and clinical outcome (phase II)

Among the 65 patients qualified for pharmacogenomic studies, 46 were of East Asian ethnicity and 19 were of non-Asian ethnicity (18 Hispanic and one Caucasian). Four patients were excluded from the pharmacogenomic analysis because of lack of genotyping data. The allelic frequency of the two MTHFR polymorphisms (C677T and A1298C) was calculated in 65 patients (Table 4). Among the non-Asian population, the frequency of heterozygous A1298C was 42.1% and heterozygous C677T was 57.9%, whereas those frequencies in the Asian population were 21.7 and 30.4%, respectively. No individuals homozygous for the variant alleles (677TT/1298CC) were found.

Patients with A1298C variants had a median OS of 6.6 months (95% CI, 4.9–11.0 months) (Table 5 and Fig. 1), which was determined through the log-rank test to be significantly ($P = 0.001$) shorter than that of patients with the wild type (median 18.5 months, 95% CI, 10.0–18.5 months). Patients with wild type (both 677CC and 1298AA) had a slightly higher response rate compared

Table 4 MTHFR genotypes (C677T and A1298C) on efficacy-qualified patients

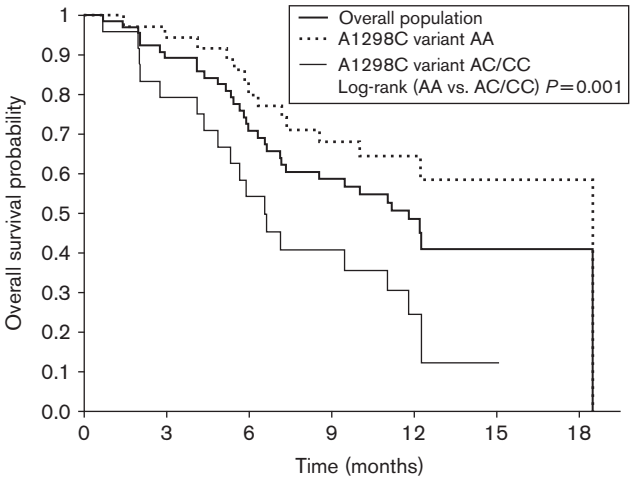
	C677T in Asians				C677T in non-Asians			
A1298C	C/C	C/T	T/T	Total	C/C	C/T	T/T	Total
A/A	12	13	7	32 (69.6%)	2	6	1	9 (47.4%)
A/C	9	1	0	10 (21.7%)	3	5	0	8 (42.1%)
C/C	4	0	0	4 (8.7%)	2	0	0	2 (10.5%)
Total	25 (54.4%)	14 (30.4%)	7 (15.2%)	46 (100%)	7 (36.8%)	11 (57.9%)	1 (5.3%)	19 (100%)

Table 5 Overall survival and progression-free survival according to SNPs

Prognostic factor	No.	Responder or events	Response rate (95% CI) or median (95% CI)	*P* value for fisher test or Log-rank test	Adjusted odds ratio or HR (95% CI)	*P* value for logistic regression or Cox regression
Response rate						
C677T						
CC						
CT/TT	32	8	25.0 (11.5, 43.4)	0.775	1.04 (0.25, 4.40)	0.953
	33	7	21.2 (8.9, 38.9)			
A1298C						
AA						
AC/CC	41	10	24.4 (12.4, 40.3)	1.00	1.63 (0.34, 7.85)	0.540
	24	5	20.8 (7.1, 42.2)			
OS						
C677T				0.766		–
CC	32	15	12.3 (6.6, NA)		1	
CT/TT	33	17	11.0 (6.3,18.5)		0.78 (0.36, 1.68)	0.521
A1298C				0.001		–
AA	41	14	18.5 (10.0, 18.5)		1	
AC/CC	24	18	6.6 (4.9, 11.0)		5.78 (2.43, 13.72)	<0.001
PFS						
C677T						
CC	32	22	5.9 (2.8, 7.1)	0.786	1	–
CT/TT	33	19	4.2 (2.6, 7.9)		1.39 (0.69, 2.84)	0.359
A1298C						
AA	41	22	5.9 (3.7, 8.3)	0.086	1	–
AC/CC	24	19	2.7 (2.0, 7.1)		2.43 (1.16, 5.09)	0.019

Logistic and Cox regression were performed with adjustment for age, BMI, sex, race (Asian or non-Asian), ECOG (0 or 1).
CI, confidence interval; HR, hazards ratio; OS, overall survival; PFS, progression-free survival.

Fig. 1



Kaplan–Meier curves for overall survival – overall population and MTHFR A1298C variants.

with patients with the variant type (25.0% for CC patients vs. 21.2% for CT/TT patients and 24.4% for AA patients vs. 20.8% for AC/CC patients). However, this difference was not statistically significant as tested by the Fisher's exact test and multivariate logistic regression. These same trends, favourable for the wild type, apply to the PFS; in particular, patients with the 1298AA wild type had a longer median PFS than patients carrying the 1298 variant allele (5.9 vs. 2.6 months). This difference was significant when analysed by the multivariate Cox regression adjusted by the confounders ($P = 0.019$) but did not, however, reach significance when analysed by the log-rank test ($P = 0.086$).

No significant associations were detected between toxicity end points and these two genetic variants (data not shown).

Discussion

This phase I/II study showed that pemetrexed and cisplatin can be safely administered every 21 days as first-line therapy in patients with advanced gastric carcinoma. The results of the phase I portion of this study defined the recommended dose of pemetrexed as 700 mg/m^2 for the phase II portion of the study. In an earlier study, Thodtmann *et al.* [21] recommended 500 mg/m^2 of pemetrexed and 75 mg/m^2 of cisplatin as the dose for phase II. The DLT for that study was myelosuppression. It should be noted that this earlier study was conducted before the introduction of vitamin supplementation. It is now established that the tolerability and therefore effectiveness of pemetrexed is related to the patient's folate status, and that severe toxicity can be significantly reduced by vitamin B_{12} and FA supplementation. In this study, the use of high-dose FA and vitamin B_{12} allowed increase of pemetrexed recommended dose to 700 mg/m^2 while having a manageable level of toxicity.

Of the 68 patients evaluable for efficacy, there was one CR and there were 15 PRs, for an objective response rate of 23.5% (95% CI, 14.1–35.4%). The median OS was 11.8 months (95% CI, 7.2–18.5 months) and the median PFS was 4.9 months (95% CI, 2.8–7.1 months). The overall

response rate of 23.5% to the pemetrexed–cisplatin regimen evaluated in this study did not reach the expected minimal response rate of 30%. Moreover, the anti-tumour activity was modest compared with that observed in older 5-FU-based regimens used to treat gastric cancer [6,24,25].

Recently, three phase III studies were published regarding the use of novel agents for the treatment of gastric cancer. The V325 study showed that the response rate of docetaxel, CDDP and 5-FU was 37% [26]. Another study indicated that the response rates for patients treated with epirubicin and cisplatin plus either fluorouracil (ECF) or capecitabine (ECX) or with triplet therapy comprising epirubicin and oxaliplatin plus either fluorouracil (EOF) or capecitabine (EOX) were higher than 37.7% [27]. The Arbeitsgemeinschaft Internistische Onkologie group also confirmed that the OS for oxaliplatin and 5-FU/LV (FLO) was similar to that of CDDP and 5-FU/LV (PLF). However, in a recent phase III study, the response rates were 34.8% for FLO and 24.5% for FLP [28], indicating that the pemetrexed–CDDP combination is less effective than combinations containing 5-fluorpyridimebase for the treatment of gastric cancer.

Although in the current study, the effectiveness of the pemetrexed–cisplatin combination in gastric cancer was modest compared with the studies reported earlier, this combination was well tolerated in most patients. The most common grade 3/4 toxicity was neutropenia (40.6% of patients).

As grade 4 neutropenia (5.8%) and/or grade 3 thrombocytopenia (1.5%) were infrequent, dose reductions were required in only 3.5% of the total administered pemetrexed doses and 4.3% of cisplatin doses. The percentages of doses delivered were above 91% for both drugs.

Two MTHFR variants were genotyped in 65 patients aiming to explore their association with clinical outcomes. As described earlier, the frequency of both polymorphisms varies by geographical origin [29]. In this study, the individuals heterozygous to A1298C and C677T were more frequently present in the non-Asian populations. The frequency of heterozygous individuals among Asians was lower in this study (A1298C: 21.7% and C677T: 30.4%) than in earlier studies (A1298C: 38.6% and C677T: 49.8%) [30]. However, the distribution of homozygous and heterozygous is consistent with earlier data [31].

Recent studies have investigated the potential role of MTHFR polymorphisms on toxicity and response to cancer and anti-inflammatory therapy [20]. In this study, we found that A1298C patients had median OS of 6.6 months, which was significantly shorter than the OS in patients with the wild type. Data from progression-free survival also support the unfavourable prognostic role of A1298C variants in advanced gastric cancer patients treated with pemetrexed. These data are in agreement with earlier studies [32,33] reporting poorer prognosis in A1298C patients. These data suggest that A1298C variants (A/C and C/C patients) could be negative efficacy predictors. In contrast, there are data indicating an association of A1298C variants with improved survival in oesophageal cancer patients, which have been published recently [34].

Regarding C677T variants our data indicate, similar to other reports, that this allele is not associated with improved outcomes in gastric cancer patients [33]. However, other investigators have reported associations of the C677T variant with increased response rate and improved survival [32,34]. In contrast with earlier studies in which MTHFR variants were associated with safety profiles [35], in our study the A1298C variant is associated with efficacy. Furthermore, we did not observe additive effects in patients carrying both variants. It is important to notice that both MTHFR polymorphisms were analysed from circulating blood and not from somatic tissue. The study of these variants in tumour DNA, and in large sample cohorts, might further explain the association with efficacy and help clarify current discrepancies.

In conclusion, the combination of pemetrexed–cisplatin in treating patients with advanced gastric cancer generates modest activity and a manageable toxicity profile.

The reduced OS in patients with MTHFR A1298C variants deserves further investigation.

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