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A randomised, double-blind, phase II study of two doses of pemetrexed in the treatment of platinum-resistant, epithelial ovarian or primary peritoneal cancer

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ABSTRACT

Purpose: We conducted a randomised phase II study to assess the safety and efficacy of standard versus high-dose pemetrexed in platinum-resistant epithelial ovarian cancer (PR-EOC). The expression of ten genes was also examined as potential biomarkers of pemetrexed/platinum activity.

Patients and methods: Patients received pemetrexed 500 mg/m^2 (Pem500) or 900 mg/m^2 (Pem900) on day 1 of each 21-d cycle. Responses were defined per RECIST for measurable disease or by Gynaecologic Cancer Intergroup (GCIG) CA-125 criteria for non-measurable disease.

Results: Of 102 patients randomised, 98 were evaluable for toxicity (47 Pem500, 51 Pem900) and 91 were evaluable for efficacy (43 Pem500, 48 Pem900) of whom 68 had measurable disease and 23 had CA-125-defined disease. The overall RR was 9.3% (95% CI: 2.6–22.1%) on Pem500 and 10.4% (95% CI: 3.5–22.7%) on Pem900. The median progression-free survival (PFS) was 2.8 months on both arms, and the median survival was 11.9 and 10.3 months, respectively. Lower mRNA expression of excision repair cross-complementation group 1 (ERCC1) and reduced folate carrier 1 (RFC1) were associated with longer PFS and time to treatment failure, respectively. Grade 3/4 toxicities, including fatigue, nausea and vomiting, were numerically greater on Pem900. Pemetrexed-related SAEs occurred in 17% and 28% of Pem500 and Pem900 patients, respectively.

Conclusions: Pemetrexed has activity in PR-EOC equivalent to other agents in platinum-resistant disease; however, Pem500 has the preferable toxicity profile. ERCC1 and RFC1 may merit examination as predictive biomarkers in PR-EOC.

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1. Introduction

Epithelial ovarian cancer (EOC) presents as stage III or IV disease in over 70% of patients. Despite radical surgery and high response rates to first-line treatment with paclitaxel and carboplatin, at least 65% of patients with advanced EOC have refractory disease and die of disease progression.[1] Thus a need for better therapies remains, particularly in platinum-resistant EOC (PR-EOC), defined as relapse within 6 months or less following treatment with a platinum-containing chemotherapy.[2]

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Pemetrexed, a multitargeted antifolate inhibitor of thymidylate synthase (TS), dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyltransferase (GARFT), is approved for the treatment of malignant pleural mesothelioma in combination with cisplatin[3] and for second-line monotherapy treatment of non-small cell lung cancer (NSCLC).[4] Recently, the European Medicines Agency (EMEA) granted approval for the use of pemetrexed in first-line non-squamous histology NSCLC. Pemetrexed has demonstrated preclinical activity against ovarian carcinoma cell lines in combination with cisplatin.[5] The overexpression of folate receptor alpha, an important folate transporter, has been observed as ovarian epithelial cells undergo malignant transformation.[6] Observations such as these indicated that pemetrexed may have clinical activity in ovarian carcinoma. Recent phase I studies have suggested that pemetrexed doses higher than the approved dose of 500 mg/m^2 are well tolerated with vitamin supplementation.[7,8]

Miotti and colleagues reported that internalisation of physiologic folate depends not only on the level of folate receptor alpha (FR-α) expression but also on the expression of another folate transport system that demonstrated characteristics consistent with a reduced folate carrier (RFC), which is a low-affinity/high-capacity system that facilitates passive diffusion of folate across the membrane.[9] Corona and colleagues have reported that RFC may have a more important role than FR-α in the transport of 5-methyltetrahydrofolate in ovarian cancer cell lines.[10] Additionally, Wang and colleagues reported evidence for a novel high-affinity transport system that appears to be highly specific for pemetrexed and may be a third method of transport of pemetrexed and folate analogues into human cells.[11] Cancers that overexpress folate analogue transporters may exhibit preferential uptake of pemetrexed and contribute to its antitumour activity. FR-α overexpression has been observed as ovarian epithelial cancer cells undergo malignant transformation.[12]

As it is critical in cytotoxic agent development to determine if there is a dose–response relationship underlying efficacy, and given the variable expression of folate analogue transporters that may modify pemetrexed uptake and activity in certain tumour types, we conducted a randomised, phase II, 22-centre study to determine if high-dose pemetrexed (900 mg/m^2) was superior to the standard dose (500 mg/m^2) in patients with PR-EOC or platinum-resistant primary peritoneal cancer (PR-PPC). The standard dose of pemetrexed (500 mg/m^2) was chosen as the lower dose because it has established efficacy in a variety of solid malignancies.[3,4] Additionally, the expression of ten genes was examined by real-time polymerase chain reaction (RT-PCR) to identify biomarkers that might have prognostic or predictive value in a subset of patients.

2. Patients and methods

2.1. Eligibility

Patients with histologically proven PR-EOC or PR-PPC were enrolled. Inclusion criteria included one or two prior platinum-based regimens (carboplatin, cisplatin or another platinum) for primary disease; measurable disease, defined by the Response Evaluation Criteria in Solid Tumours (RECIST);[13] or non-measurable disease, defined by RECIST, with CA-125 $\geqslant 2\times$ upper limit of normal at least 2 weeks before enrolment; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; estimated life expectancy $\geqslant$24 weeks; adequate bone marrow reserve and hepatic and renal function; age $\geqslant$18 years and platinum-resistant or refractory disease.

Exclusion criteria included the following: >2 prior chemotherapies; pregnancy or breastfeeding; serious concomitant disorders; inability to interrupt aspirin and/or non-steroidal anti-inflammatory drugs for 2 d before, the day of and 2 d after pemetrexed (5 d prior for long-acting agents); inability to take folic acid, vitamin B_{12} supplementation or corticosteroids; or clinically significant third-space fluid that could not be managed with drainage.

The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the appropriate ethical review board. Each patient gave written informed consent before the study entry.

2.2. Study design

The primary objective of this randomised, double-blind, parallel study design compared response rates of two doses of pemetrexed. Secondary objectives were to assess time-to-event efficacy variables (time to response, duration of response, time to progressive disease [TtPD], time to treatment failure [TtTF], progression-free survival [PFS] and overall survival [OS]) and the safety and toxicity profile of the two doses. Biomarkers potentially predictive of pemetrexed activity and toxicity were examined. Random assignment of patients was balanced for number of prior platinum-based regimens (1 or 2), ECOG PS (0 or 1, versus 2) and investigational site.

2.3. Treatment plan

Patients were randomised to receive standard-dose pemetrexed (Pem500 arm; 500 mg/m^2) or high-dose pemetrexed (Pem900 arm; 900 mg/m^2) intravenously on day 1 every 21 d. Patients were also required to receive 350–1000 μg folic acid, taken orally daily at least 1–2 weeks before cycle 1 until 3 weeks after the last pemetrexed dose, and 1000 μg vitamin B_{12} intramuscularly every 9 weeks, starting at least 1–2 weeks before cycle 1, then every 9 weeks until 3 weeks after the last pemetrexed dose. To prevent skin rash, 4 mg of dexamethasone (or equivalent) was given orally, twice daily for 3 d, starting 1 d prior to pemetrexed administration.

Patients who required a pemetrexed dose reduction continued to receive the reduced dose. Pemetrexed doses were reduced or delayed (for up to 3 weeks) for toxicities deemed clinically significant based on platelet and neutrophil nadir counts and maximum non-haematologic toxicity from the preceding cycle. Toxicities were assessed prior to each cycle using the Common Terminology Criteria for Adverse Events (CTCAEs) v3.0.[14]

2.4. Baseline and treatment assessments

Physical examinations, medical histories and ECOG PS evaluations were performed before treatment and prior to every cycle. Laboratory evaluations included complete blood cell counts with differentials, liver function tests, blood chemistries and serum creatinine.

Tumour response was assessed every two cycles according to RECIST. Elevated CA-125 levels were followed as target lesions if no other target lesions were present as defined in the Gynaecologic Cancer Intergroup (GCIG) criteria.[15,16] CA-125 was measured at the start of every other cycle where it served as a 'non-target' lesion in the presence of measurable disease and at the start of every cycle when it served as a target lesion in the absence of measurable disease. CT scans were performed every two cycles. Objective responses were confirmed by a second assessment approximately 6 weeks after the first documentation of response.

2.5. RT-PCR

Patients eligible and willing to participate gave consent to the collection, analysis and storage of the pretreatment tumour tissue sample that was previously taken for diagnosis. This sample was formalin-fixed and embedded in paraffin blocks (FFPE tumour tissue). Markers analysed included those involved in pemetrexed cellular transport: reduced folate carrier (RFC)[10] and folate receptor alpha (FR-α);[12] activation: folylpolyglutamate synthase (FPGS)[17] and γ-glutamyl hydrolase (GGH)[18] and cytotoxic activity: TS,[19] GARFT,[17] DHFR[17] as well as genes involved in the mechanism of action of platinum- and taxane-based therapies: excision repair cross-complementation group 1 (ERCC1),[20] microtubule associated protein tau (MAPT)[21] and glutathione-S-transferase-pi (GSTπ).[22] Patients were ordered from lowest to highest marker expression, as measured by the difference between RT-PCR cycle times for the 10 markers and the endogenous reference gene β-actin (see Supplemental Methods for details of marker analysis).

2.6. Statistical analyses

Confidence intervals (CIs) for parameters to be estimated were constructed with a significance level of $\alpha = 0.05$ (two-sided alpha). The sample size of 100 patients was chosen based on a 'selection design'[23] using the primary end-point of tumour response rate. This design assumed that the primary goal of the trial was to select the regimen with the observed greater tumour response rate for a larger, phase III trial; therefore, type I error or alpha error was not controlled. Assuming a 10-percentage-point difference between the true response rates of the regimens (that the response rate of the inferior dose was 0.1 compared with 0.2 for the better dose), there was approximately a 90% chance of correctly selecting the regimen with the higher response rate. The Fisher exact test was used to test for differences in the best study response between the two doses. Kaplan–Meier techniques[24] were used to assess time-to-event variables. The log-rank test[23] was used to test the null hypothesis that there was no difference in distribution of event times between the two doses. All hypotheses were tested at a two-sided alpha level of 0.05. All 95% CIs were two-sided.

For the translational research analysis, available patient tumour tissue (20 patients-Pem500 and 22 patients-Pem900) was dichotomised into low- and high-expression groups at the point that maximised the association between an individual marker and response. Power was assessed assuming an equal dichotomisation. Additional assessments were made at 10% intervals, with 20–80% of patients having a marker expression level associated with greater tumour response, to evaluate the sensitivity of power to unequal group size. All tests of effects were conducted at a two-sided alpha level of 0.20 given the exploratory nature of the analysis. Significance of the expression group effects was evaluated by testing the effect statistic against the asymptotic distribution of a maximum chi-square value as described by Miller and Siegmund.[25] No other adjustments for multiple comparisons across markers or outcome measures were made.

3. Results

3.1. Patient characteristics

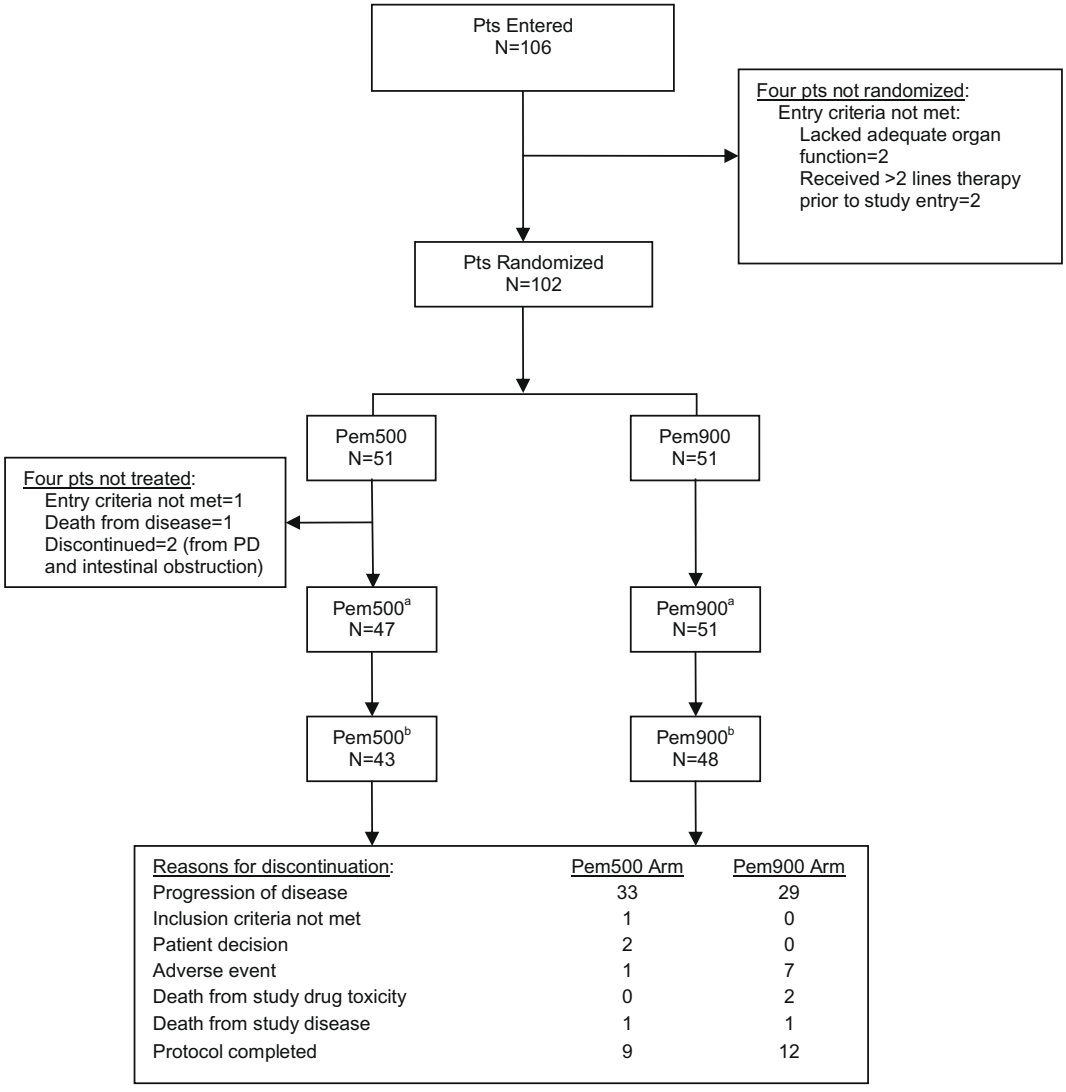
Between June 2005 and March 2007, an entry of 106 patients was made from 22 centres, and 51 patients were randomised to each treatment arm. Four patients on Pem500 discontinued without receiving study drug (see CONSORT diagram). Forty-seven patients on Pem500 and 51 patients on Pem900 were assessable for safety. Forty-three patients on Pem500 and 48 patients on Pem900 were protocol-qualified and evaluable for efficacy. Patient characteristics were generally well balanced between treatment arms (Table 1). Two exceptions were that 6 patients (11.8%) had progressed on prior platinum therapy in the Pem900 arm versus 0 in the Pem500 arm, and that numerically more Stage IV patients were included in the Pem500 arm versus in the Pem900 arm (20% versus 6%) (Table 1). More evaluable patients on Pem900 had a platinum-free interval <3 months: 21 patients (43.8%; N = 48) compared with 13 patients (30.2%; N = 43) on Pem500, although this difference was not statistically significant ($P > 0.05$).

3.2. Treatment compliance

Before the first dose of study therapy and during therapy, compliance with folic acid supplementation was monitored by conducting medical interviews. No significant correlation was detected between folic acid compliance violations and toxicity.

3.3. Tumour response and time-to-event measures

Four (9.3%) (95% CI: 2.6–22.1%) patients on Pem500 and five (10.4%) (95% CI: 3.5–22.7%) patients on Pem900 had a best study response of partial response (Table 2). The combined overall response rate was 9.9% (95% CI: 4.6–18.0%). No statistically significant differences between the two treatment arms were observed for any secondary efficacy time-to-event end-points (Table 2).



Reasons for discontinuation:	Pem500 Arm	Pem900 Arm
Progression of disease	33	29
Inclusion criteria not met	1	0
Patient decision	2	0
Adverse event	1	7
Death from study drug toxicity	0	2
Death from study disease	1	1
Protocol completed	9	12

[a] Patients treated and therefore included in the safety analysis.
[b] Patients included in the final efficacy analyses. Reasons pts were not evaluable were entry criteria not met: pt was not platinum-resistant (2 on Pem500 and 3 on Pem900), pt did not have ovarian cancer (1 on Pem500), and pt did not meet CA-125 criteria (1 on Pem500).

CONSORT

3.4. *Translational research*

In the exploratory analysis of gene-expression by RT-PCR, levels for ERCC1 and RFC were significantly associated with differences in more than one clinical efficacy measure (Table 3). High FPGS and low GSTπ mRNA expression were significantly associated with response, and low GARFT expression was significantly associated with TtTF (Table 3).

ERCC1 had the strongest and most consistent association with clinical outcomes, with lower mRNA levels significantly associated with longer PFS, TtPD and TtTF (Table 4). Lower levels of RFC were also significantly associated with improved best overall response and longer TtTF (Table 4).

A logistic regression comparing severe toxicities with marker expression was performed. None of the biomarkers were associated with severe toxicities (defined as Grade 3 or 4 neutropaenia, thrombocytopaenia, infection, diarrhoea and mucositis).

3.5. *Toxicity and safety*

Numerically higher percentages of patients on Pem900 ($n = 51$) experienced drug-related serious adverse events (SAEs) (27.5% versus 17.0% on Pem500 [$n = 47$]) and discontinued because of drug-related adverse events (9.8% versus 2.1% on Pem500). Two (3.9%) patients on Pem900 died of sepsis and neutropenic sepsis, respectively, events which were possibly drug-related. Both deaths occurred in patients who were compliant with vitamin supplementation.

Grade 3/4 haematologic toxicities were generally greater on the Pem900 arm (Table 5), although the differences were modest and not statistically significantly different. The most

Table 1 – Patient demographics and baseline characteristics .

Variable	Pem500 arm (*N* = 43)	Pem900 arm (*N* = 48)
Mean age (range), years	57.7 (38.3–76.5)	63.2 (29.6–78.2)
Origin, *n* (%)		
Caucasian	42 (97.7)	46 (95.8)
East/southeast Asian	1 (2.3)	2 (4.2)
ECOG PS, *n* (%)		
0	13 (30.2)	18 (37.5)
1	26 (60.5)	27 (56.3)
2	4 (9.3)	3 (6.3)
Basis for diagnosis, *n* (%)		
Histopathological	43 (100.0)	48 (100.0)
Pathological		
Epithelial ovarian cancer	33 (76.7)	41 (85.4)
Primary peritoneal	10 (23.3)	7 (14.6)
Carboplatin and taxol as part of primary or secondary regimens, *n* (%)	40 (78.4)	46 (90.2)
Progression on prior platinum-based therapy, *n* (%)	0 (0)	6 (11.8)
FIGO disease stage at diagnosis, *n* (%)		
I	0	1 (2.1)
IA–IIA	2 (4.7)	2 (4.2)
IIB–IIIA	1 (2.3)	5 (10.4)
IIIB	3 (7.0)	5 (10.4)
IIIC	28 (65.1)	30 (62.5)
IV	8 (18.6)	3 (6.3)
Unspecified	1 (2.3)	2 (4.2)
Measurable disease, *n* (%)		
Yes	32 (74.4)	36 (75.0)
CA-125 only	11 (25.6)	12 (25.0)

N, number of randomised patients; SD, standard deviation; *n*, number of patients; ECOG, Eastern Cooperative Oncology Group; PS, performance status; FIGO, Federation of Gynaecology and Obstetrics.
* Patients who were evaluable for efficacy.

Table 2 – Comparison of best study response and time-to-event parameters between treatment arms.

Best study response	Pem500 arm (*N* = 43)[a]	Pem900 arm (*N* = 48)[a]	Fisher's exact *P*-value
ORR, *n* (%)	4 (9.3)	5 (10.4)	1.000
(95% CI)	(2.6–22.1)	(3.5–22.7)	
PR, *n* (%)	4 (9.3)	5 (10.4)	
SD, *n* (%)	14 (32.6)	14 (29.2)	
CR + PR + SD, *n* (%)	18 (41.9)	19 (39.6)	
PD, *n* (%)	21 (48.8)	24 (50.0)	
Unknown, *n* (%)	4 (9.3)	5 (10.4)	
Time-to-event parameter	Pem500 arm (*N* = 43), months (95% CI)	Pem900 arm (*N* = 48), months (95% CI)	Log rank *P*-value
Median time to response	2.1 (1.4–3.4)	1.5 (1.1–2.3)	0.270
Median duration of response	4.0 (3.1–6.0)	4.3 (3.2–6.1)	0.799
Median PFS	2.8 (2.6–4.2)	2.8 (2.1–4.2)	0.786
Median OS	11.9 (7.9–14.8)	10.3 (7.7–14.8)	0.974

ORR, overall response rate; CI, confidence interval; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease, PFS, progression-free survival; OS, overall survival.
a The reasons for patients to not be evaluable for efficacy: on the Pem500 arm, one patient did not meet the CA-125 inclusion criterion, one patient did not have ovarian or primary peritoneal cancer, and two patients did not have platinum-resistant disease. On the Pem900 arm, three patients did not have platinum-resistant disease.

prominent Grade 3/4 non-haematologic toxicities included fatigue, vomiting, and ascites, all of which were Grade 3 or lower.

Nausea dropped from the description because it occured in less than 5% of patients and is not therefore present in Table 5. The median number of cycles delivered was four (range 1–11) and three (range 1–8) on Pem500 and Pem900, respectively. Four patients (8.5%) on Pem500 required a single dose reduction and 15 patients (31.9%) required a total of 24 cycle delays, seven of which were clinically relevant (that is, not due to scheduling conflicts). Eight patients (15.7%) on Pem900 required dose reductions and 21 patients (41.2%)

Table 3 – Association of biomarker RNA expression with best response rate, TtPD, TtTF and PFS.

Marker	Response rate P-value[a]	TtPD P-value[a]	TtTF P-value[a]	PFS P-value[a]
TS	0.151 (low)[b]	0.706 (low)[b]	0.244 (low)[b]	1.000 (low)[b]
FPGS	**0.034 (high)**[b]	0.105 (low)[b]	0.187 (low)[b]	0.226 (low)[b]
GARFT	0.053 (low)[b]	0.694 (low)[b]	**0.012 (low)**[b]	0.978 (low)[b]
GGH	0.177 (low)[b]	0.221 (low)[b]	0.584 (low)[b]	0.738 (low)[b]
TP	0.246 (low)[b]	0.127 (low)[b]	0.060 (low)[b]	0.065 (low)[b]
FRα	0.849 (high)[b]	0.511 (low)[b]	0.080 (low)[b]	0.556 (low)[b]
RFC1	**0.014 (low)**[b]	0.267 (low)[b]	**0.008 (low)**[b]	0.584 (low)[b]
ERCC1	0.096 (low)[b]	**0.041 (low)**[b]	**0.028 (low)**[b]	**0.049 (low)**[b]
GSTπ	**0.021 (low)**[b]	0.157 (low)[b]	0.058 (low)[b]	0.070 (low)[b]
MAPT	1.000 NA[c]	1.000 NA[c]	1.000 NA[c]	1.000 NA[c]

TS, thymidylate synthase; FPGS, folylypolyglutamate synthase; GARFT, glycinamide ribonucleotide reductase formyl transferase; GGH, γ-glutamyl hydrolase; TP, thymidine phosphorylase; FRα, folate receptor alpha; RFC1, reduced folate carrier 1; ERCC1, excision repair cross-complementation group 1; GSTπ, glutathione-S-transferase pi; MAPT, microtubule associated protein tau.

a Asymptotic probability of the observed maximum chi-square statistic under the null hypothesis of no association between best study response, TtPD or TtTF and marker expression level, limiting the search to the central 50% of values. Calculated with the formula of Miller and Siegmund.[22]

b High or low refers to whether association was observed for the high or low expression subgroup of a given gene in conjunction with a positive effect on the given response or time-to-event parameter. Statistically significant associations are bolded.

c Not applicable, samples from only 3 patients were assessable for MAPT expression.

Table 4 – Association of high versus low ERCC1 and RFC expression subgroups with overall response rate, TtTP, TtTF and PFS.[a]

Response category	High expression subgroup[b] (Delta CT below threshold), *n* (%)	Low expression subgroup[b] (Delta CT at or above threshold), *n* (%)	OR[c] (95% CI) P-value
Association of high versus low ERCC1 expression with overall response rate[b]			
PR	1 (10.0)	4 (17.4)	
SD	1 (10.0)	13 (56.5)	
PD	8 (80.0)	6 (26.1)	
			10.4 (1.73–62.8) 0.096
Association of high versus low RFC expression with overall response rate[b]			
PR	1 (4.76)	4 (36.4)	
SD	8 (38.1)	6 (54.6)	
PD	12 (57.1)	1 (9.1)	
			36.7 (4.31–312) **0.014**
Gene	High expressionmedian TtTP, months (95% CI) [*n*]	Low expressionmedian TtTP, months (95% CI) [*n*]	P-valueHR (95% CI)
Association of high versus low ERCC1 and RFC expression subgroups with TtTP			
ERCC1	1.82 (1.35–2.83) [9]	4.40 (2.76–9.17) [27]	**0.041** 5.09 (1.70–15.2)
RFC	2.76 (2.17–3.22) [23]	4.86 (4.40–.) [11]	0.267 3.57 (1.05–12.1)
Association of high versus low ERCC1 and RFC expression subgroups with TtTF			
ERCC1	2.37 (1.14–2.83) [14]	3.88 (2.79–5.49) [22]	**0.028** 4.11 (1.65–10.2)
RFC	2.76 (2.17–2.83) [23]	5.49 (4.11–6.57) [11]	**0.008** 6.28 (2.23–17.7)
Association of high versus low ERCC1 and RFC expression subgroups with PFS			
ERCC1	2.17 (1.45–2.83) [9]	4.40 (2.79–5.52) [27]	**0.049** 3.81 (1.51–9.61)
RFC	2.79 (2.53–4.21) [23]	5.52 (4.40–7.39) [11]	0.584 1.94 (0.815–4.62)

ERCC1, excision repair cross-complementing 1; RFC, reduced folate carrier; TtTP, time to tumour progression; TtTF, time to treatment failure; PFS, progression-free survival; OR, odds ratio; CI, confidence interval; CT, reverse transcriptase-polymerase chain reaction threshold cycle value; delta CT, the difference between the marker of interest CT and the endogenous β-actin CT within each patient; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; HR, hazard ratio.

a Pem500 $n = 20$; Pem900 $n = 22$; total patients with assessable samples for both arms $n = 42$. Numbers may not add to 42 due to lack of successful amplification/signal from certain samples.

b The threshold delta CT value provided the best association between high- and low-expression subgroups of patients and best study response or time-to-event parameter. Patients with a delta CT value at or above the threshold were classified as having low relative gene-expression levels; patients with a delta CT value below the threshold were classified as having high relative gene-expression levels.

c Overall OR of better clinical outcome from logistic regression analysis.

Table 5 – Incidence of CTC Grade 3 and 4 haematologic and non-haematologic toxicities in treated patients by treatment arm.[a]

Toxicity	Pem500 arm (N = 47), n (%)				Pem900 arm (N = 51), n (%)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Haematologic								
Anaemia	1 (2.1)	7 (14.9)	3 (6.4)	2 (4.3)	3 (5.9)	12 (23.5)	6 (11.8)	1 (2.0)
Leukopaenia	0	3 (6.4)	2 (4.3)	1 (2.1)	1 (2.0)	0	3 (5.9)	2 (3.9)
Neutropaenia	1 (2.1)	1 (2.1)	1 (2.1)	5 (10.6)	1 (2.0)	2 (3.9)	4 (7.8)	3 (5.9)
Febrile neutropaenia	0	0	1 (2.1)	2 (4.3)	0	1 (2.0)	2 (3.9)	0
Thrombocytopaenia	1 (2.1)	1 (2.1)	1 (2.1)	1 (2.1)	2 (3.9)	0	3 (5.9)	3 (5.9)
Non-haematologic								
ALT, SGPT	0	1 (2.1)	1 (2.1)	0	1 (2.0)	0	3 (5.9)	0
Ascites	1 (2.1)	1 (2.1)	3 (6.4)	0	1 (2.0)	4 (7.8)	2 (3.9)	0
Fatigue (asthenia, lethargy, malaise)	15 (31.9)	18 (38.3)	3 (6.4)	0	17 (33.3)	13 (25.5)	7 (13.7)	0
Ileus	0	0	3 (6.4)	0	2 (3.9)	1 (2.0)	2 (3.9)	0
Vomiting	8 (17.0)	13 (27.7)	2 (4.3)	0	12 (23.5)	10 (19.6)	3 (5.9)	0

CTC, Common Toxicity Criteria; ALT, alanine transaminase; SGPT, serum glutamic pyruvic transaminase.

a Reported as ⩾5% of patients experiencing a Grade 3/4 toxicity in at least one treatment arm except for febrile neutropaenia for which all patients are accounted.

required a total of 35 cycle delays, 18 of which were clinically relevant. Mean dose intensity for Pem500 was 96.9% and for Pem900, it was 94.0%.

4. Discussion

Growing evidence has shown that doses higher than the currently approved dose, although tolerable, do not improve upon the efficacy observed with standard, 500 mg/m^2 pemetrexed with vitamin B_{12} and folic acid supplementation in several tumour types including ovarian, breast and non-small cell lung cancer.[26,27] Reasons for this lack of dose-responsiveness are not readily apparent but, as has been noted for other cytotoxic drugs,[28] may in part be that (1) the limit of bioavailability (transport, etc.) may have been reached; (2) the dose–response relationship may be flat within the range examined or (3) high-dose therapy may only be effective for a subpopulation of cells within the complex tumour makeup. The greater toxicity (Grade 3/4 platelets and fatigue) seen in patients on Pem900 is likely due to greater drug exposure to normal tissues.

Platinum-resistant epithelial ovarian cancer remains a significant clinical challenge. Taxane- and platinum-based regimens, in conjunction with radical surgery, represent the current standard of care in primary EOC. Available salvage therapies such as liposomal doxorubicin and topotecan have response rates of only 10–20% in PR-EOC.[29,30] In ongoing phase-I/II studies, pemetrexed is combined with carboplatin in patients with platinum-sensitive ovarian cancer.[31,32] The current study tested whether Pem900 was superior to Pem500 in the treatment of recurrent PR-EOC. Response rates of approximately 10% were observed on both treatment arms; however, Pem500 exhibited a better safety profile. An exploratory analysis of 10 genes on a subset of patients suggests that ERCC1 and RFC may warrant further investigation as biomarkers predictive of pemetrexed efficacy in this patient population.

The role of ERCC1, a key component of the nucleotide excision DNA repair pathway, in the response of PR-EOC is not completely unexpected. Evidence has emerged that this DNA repair enzyme may be predictive of platinum-efficacy in several tumour types including lung, ovarian, bladder, colorectal, gastric and oesophageal cancers.[33] In a study of 26 patients with ovarian cancer, those resistant to platinum-based therapy had ERCC1 mRNA levels 2.6-fold higher than those who were sensitive.[34] Smith et al. recently showed that ovarian cancer patients with high ERCC1 expression or the C/C genotype at codon 118 may benefit from platinum and paclitaxel.[35] This study also revealed that those with low ERCC1 expression or the C/T or T/T genotype may benefit from platinum therapy without paclitaxel. The data obtained in the current study also suggest that ERCC1 may be a particularly good marker not only for platinum sensitivity, but also, more broadly, for pemetrexed sensitivity in PR-EOC. Thus, further analysis of this marker in PR-EOC appears warranted.

Our finding that low expression of RFC is related to improved efficacy is somewhat unexpected. A small molecule antifolate, pemetrexed, is subject to transport by several of the folate receptors/transporters, although the bulk of its transport is attributed to RFC.[36] Conventional Wisdom has held that these receptors/transporters would function to transport exogenous antifolates into tumour cells and hence render cells sensitive to these agents. An inverse relationship between RFC function and pemetrexed activity has been observed in the human colon carcinoma cell line HCT-15 and in a transfected clonal variant expressing a RFC-null cDNA.[37] These authors postulated that loss of RFC function potentiates pemetrexed sensitivity due to cellular folate pool contraction in the face of preserved pemetrexed polyglutamylation and increased target enzyme inhibition. The folate transporter accounting for this activity in the absence of RFC has recently been cloned and designated the proton-coupled folate transporter (PCFT).[38] It will be interesting to determine if PCFT expression/activity in low RFC-expressing ovarian tumours is elevated and might thereby explain pemetrexed's greater activity in patients whose tumours have this profile.

It is likely that markers associated with response (FPGS [high], RFC [low] and GSTπ [low]) may be more predictive of pemetrexed effects than those associated with time-to-event

parameters since the latter may be merely prognostic and, therefore, not particularly relevant in selecting patients for pemetrexed treatment. It is important when considering these results, however, to consider the relatively small sample size (42/102 enrolled patients) used in the biomarker analysis of the current study.

Recently, a large pharmacogenetic study of the Scottish Randomised Trial in Ovarian Cancer (SCOTROC1), by Marsh and colleagues, showed that genotype associations of single nucleotide polymorphisms (SNPs) implicated in previously reported studies between outcome and toxicity in carboplatin- and taxane-treated patients could not be replicated.[39] Some of the 27 SNPs examined in the 914 patient cohort in the aforementioned study included those in ERCC1 as well as in GSTπ and MAPT. Although this type of study differs significantly from the candidate gene approach taken herein, such results from the genetic association study of SCOTROC1 underscore the difficulty of identifying surrogate markers for efficacy and toxicity, even from large patient cohorts.

In conclusion, higher doses of pemetrexed do not appear to improve response rates, PFS or OS above the standard approved dose of 500 mg/m^2 every 21 d in the treatment of PR-EOC. Although pemetrexed has activity at parity with other approved agents in this setting, Pem500 has a better safety profile than Pem900 and seems to possess the best therapeutic index. Preliminary evidence of efficacy prediction by several of the markers examined in this study (in particular, ERCC1 and RFC) should likely be addressed in larger prospective clinical trials.

Conflict of interest statement

The following disclosures are made on behalf of the authors listed.

Ignace Vergote, MD, is the Principal Investigator for this study, H3E-MC-JMHF.

Hilary Calvert, MD, is a consultant for Eli Lilly and Company; receives honoraria for speaking events and research funding from Eli Lilly and Company; and has testified on behalf of Eli Lilly and Company.

Marek Kania, MD, is an employee of Eli Lilly and Company and has stock ownership.

Christopher Kaiser, PhD, is an employee of Eli Lilly and Company and has stock ownership.

Annamaria Hayden Zimmermann, MS, is an employee of Eli Lilly and Company and has stock ownership.

Jalid Sehouli, MD, is a consultant for Eli Lilly and Company and receives honoraria and research funding for ovarian cancer speaking events and studies.

Role of the funding source

The study sponsor, Eli Lilly and Company, was involved in the study design and data analysis. The sponsor and the non-Lilly authors contributed to data interpretation. Lilly employees assisted the authors with the preparation of the manuscript. Manuscript submission was made at the discretion of the non-Lilly authors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2008.12.013.

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