11. Nardi M, Cognetti F, Pollera CF, *et al.* Intraperitoneal recombinant alpha 2 interferon alternating with cisplatin as salvage treatment for minimal residual disease ovarian cancer: a phase II study. *J Clin Oncol* 1990, **8**, 1036–1041.
12. Iaffaioli RV, Frasci G, Facchini G, *et al.* Alpha 2b interferon by intraperitoneal administration via temporary catheter in ovarian cancer. Preliminary data. *Eur J Gynecol Oncol* 1991, **12**, 69–75.
13. Berek JS, Welander C, Schink JC, Grossberg H, Montz FJ, Zigelboim J. Phase I–II trial of intraperitoneal cisplatin and α-interferon in patients with persistent epithelial ovarian cancer. *Gynecol Oncol* 1991, **40**, 237–243.
14. Repetto L, Chiara S, Guido T, *et al.* Intraperitoneal chemotherapy with carboplatin and interferon alpha in the treatment of relapsed ovarian cancer: a pilot study. *Anticancer Res* 1991, **11**, 1641–1643.
15. Mangioni C, Egres A, Landoni F, *et al.* A phase II study of cisplatin in a weekly schedule for advanced epithelial ovarian cancer. 14th International Cancer Congress, Budapest 1986, 3859 (abstract).
16. Young RC, Perez CA, Hoskins WJ. Cancer of the ovary. In De Vita VT, Hellman S, Rosenberg SA, eds. *Cancer, Principles and Practice of Oncology*, 4th edition. Philadelphia, J.B. Lippincott Company, 1993, 1226–1263.
17. Dunnick WR, Jones RB, Doppman JL, *et al.* Intraperitoneal contrast infusion for assessment of intraperitoneal fluid dynamics. *AJR* 1979, **133**, 221–223.
18. Kaplan ES, Meier P. Non parametric estimation for incomplete observations. *J Am Stat Assoc* 1958, **53**, 557–580.
19. Howell SB, Zimm S, Markman M, *et al.* Long term survival of advanced refractory ovarian carcinoma patients with small-volume disease treated with intraperitoneal chemotherapy. *J Clin Oncol* 1987, **5**, 1607–1612.
20. Markman M, Reichman B, Hakes T, *et al.* Impact on survival of surgically defined favorable responses to salvage intraperitoneal chemotherapy in small-volume residual ovarian cancer. *J Clin Oncol* 1992, **10**, 1479–1484.
21. Zambetti M, Gianni L, Escobedo A, Pizzetti P, Spatti GB, Bonadonna G. First-line chemotherapy with intraperitoneal cisplatin and intravenous cyclophosphamide in ovarian carcinoma. A preliminary report. *Am J Clin Oncol* 1989, **12**, 118–122.
22. Canetta R, Bragman K, Smaldone L, *et al.* Carboplatin: current status and future prospects. *Cancer Treat Rev* 1988, **15**(suppl.B), 17–32.
23. Quesada JR, Talpaz M, Rios A, *et al.* Clinical toxicity of interferons in cancer patients: a review. *J Clin Oncol* 1986, **4**, 234–243.
24. McVie JG, ten Bokkel Huinink W, Dubbelman R, *et al.* Phase I study and pharmacokinetics of intraperitoneal carboplatin. *Cancer Treat Rev* 1985, **12**(suppl.A), 35–41.
25. De Vries EGE, Biesma B, Willemse PHB, *et al.* A double-blind placebo-controlled study with granulocyte–macrophage colony-stimulating factor during chemotherapy for ovarian carcinoma. *Cancer Res* 1991, **51**, 116–122.
26. Rusthoven J, Levin L, Eisenhauer E, *et al.* Two phase I studies of carboplatin dose escalation in chemotherapy-naive ovarian cancer patients supported with granulocyte-colony stimulating factor. *JNCI* 1991, **83**, 1748–1753.
27. Los G, Mutsaers PHA, van der Vijgh WJF, *et al.* Direct diffusion of *cis*-diamminedichloroplatinum (II) in intraperitoneal rat tumors after intraperitoneal chemotherapy: a comparison with systemic chemotherapy. *Cancer Res* 1989, **49**, 3380–3384.
28. Markman M, George M, Hakes T, *et al.* Phase II trial of intraperitoneal mitoxantrone in the management of refractory ovarian carcinoma. *J Clin Oncol* 1990, **8**, 146–150.

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A Phase I–II Study of *N*-(Phosphonacetyl)-L-Aspartic Acid (PALA) Added to 5-Fluorouracil and Folinic Acid in Advanced Colorectal Cancer

D.I. Jodrell, W. Oster, D.J. Kerr, P.A. Canney, H. Yosef, W.P. Steward, S.B. Kaye and J. Cassidy

***N*-(phosphonacetyl)-L-aspartic acid (PALA) inhibits the enzyme L-aspartic acid transcarbamoylase (ATCase) which is important in *de novo* pyrimidine synthesis. Low dosages of PALA modulate the *in vitro* activity of 5-fluorouracil (5-FU) and PALA (250 mg/m²) inhibits pyrimidine synthesis in patients. PALA (250 mg/m² day 1) was combined with an established 5-FU/folinic acid (FA) regimen [FA (200 mg/m² over 2 h days 2+3) and bolus and 22 h infusional 5-FU (300–500 mg/m² days 2+3)] without the need for dose reduction of 5-FU or FA. 35 patients were entered. Treatment was well tolerated; 4/27 patients experienced ≥ ECOG grade 3 toxicity at full 5-FU dosage (500 mg/m² bolus/infusion). However, the response rate in 33 evaluable patients was only 6.1% [95% confidence intervals (C.I.) 0.2–21.8%]. Median response duration was short (4 months, 95% C.I. 3–6 months) and overall median survival was 10 months (95% C.I. 7–16 months). Although PALA (250 mg/m²) can be combined with full dosage 5-FU/FA, the combination has poor activity in colorectal cancer.**

Key words: PALA, 5-fluorouracil, folinic acid, colorectal cancer
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INTRODUCTION

TO DATE, response rates achieved in patients with metastatic colorectal cancer have been disappointing. The fluorinated pyrimidine, 5-fluorouracil (5-FU) is the most extensively used drug in metastatic colorectal cancer, although a meta-analysis [1] has shown that the response rate achieved using conventional bolus administration schedules is only 11%. Because of this low response rate, interest has focused on the modulation of 5-FU activity.

N-(phosphonacetyl)-L-aspartic acid (PALA) is an inhibitor of the enzyme L-aspartic acid transcarbamoylase (ATCase) which is important in *de novo* pyrimidine synthesis. At high concen-

trations (10^{-4} M), PALA inhibits cell growth *in vitro* [2]. At lower concentrations, PALA is not cytotoxic but does act as a modulator of the antitumour activity of 5-FU [3]. A major locus of action of 5-FU is the enzyme thymidylate synthase (TS). The 5-FU metabolite FdUMP is a potent inhibitor of TS [4], forming an irreversible ternary complex with TS and the cofactor 5,10-CH_2-FH_4. In addition to TS inhibition, the incorporation of 5-FU metabolites into RNA and DNA has been shown to be an alternative mechanism of action [5]. The reduction in synthesis of uridine nucleotides caused by the administration of PALA allows increased anabolism of 5-FU to FdUMP, FUTP and other metabolites, and also enhances the potential for binding of FdUMP to TS as a result of reduction in the competing natural substrate (dUMP). Similarly, reduction of UTP reduces competition for incorporation of FUTP into RNA [6].

In patients initially treated with PALA administered at high dosage (2 g/m²/week) in combination with 5-FU (480 mg/m²/week), it was necessary to reduce 5-FU dosage in 80% of patients because of marked toxicity [7]. This reduction in the dosage of 5-FU may have been the cause for the lack of antitumour activity seen in such patients. It has been shown that lower dosages of PALA (250 mg/m²) are sufficient to inhibit *de novo* pyrimidine synthesis in patients [8] and this dosage of PALA was successfully combined with full dose 5-FU (750 mg/m²/week).

The co-administration of folinic acid (FA, 5-CHO-FH_4) represents an alternative approach to the modulation of 5-FU activity. The TS inhibitory effects of the 5-FU metabolite FdUMP (see above) may be limited by reduced intracellular concentrations of 5,10-CH_2-FH_4. The use of 5-FU in combination with FA, which is readiy converted to 5,10-CH_2-FH_4, increases the formation of the ternary complex. There have been a number of clinical studies which have shown that the combination of 5-FU with FA enhances the activity of 5-FU in patients with metastatic colorectal cancer [1].

Although the addition of FA to bolus 5-FU has been shown to improve response rates [1], this may occur at the expense of increased toxicity. A widely used 5-FU/FA combination administered to outpatients at weekly intervals [2 h infusion of FA (500 mg/m²) with a bolus dose of 5-FU (600 mg/m²) administered 1 h into the FA infusion] was associated with a high incidence (40%) of dose-limiting diarrhoea [9]. However, the combination of infusional FA with both bolus and infusional 5FU, administered over a 48-h period, has been shown to be as active (response rate = 54%) as bolus alone schedules, and is much better tolerated [10]. No WHO grade 3 toxicities were experienced by the 37 patients reported. Therefore, a similar regimen combining bolus and infusional 5-FU with FA was chosen for this study. Because of experience with PALA at high dosage and other modulators of 5-FU activity leading to an increase in toxicity, in the first 8 patients treated, 5-FU dosages were reduced for both the bolus and infusion (300 mg/m² in 4, 400 mg/m² in 4). Dosages were increased following the successful treatment of each of these cohorts.

Therefore, this study investigated the feasibility of combining low-dose PALA (250 mg/m²) with FA (200 mg/m²), and increasing dosages of bolus and infusional 5-FU. The aim of the study was to administer PALA in combination with 'full dosage' 5-FU/FA, and to assess the toxicity of such a regimen and to collect preliminary response data.

Table 1. Drug regimen used (repeated every 2 weeks)

Drug	Dosage (mg/m²)	Time (h)	Infusion duration
Day 1			
PALA	250	0	15–20 min
Day 2			
FA	200	24	2 h
5-FU	300–500	26	Short infusion (10 min)
5-FU	300–500	26	22 h
Day 3			
FA	200	48	2 h
5-FU	300–500	50	Short infusion (10 min)
5-FU	300–500	50	22 h

PATIENTS AND METHODS

This was an open study in patients with histologically proven colorectal cancer. All patients had evidence of metastatic disease. No patient had received previous 5-FU chemotherapy or any other anticancer therapy (including radiotherapy) in the 4 weeks prior to treatment. All patients had measurable disease as assessed unidimensionally or bidimensionally by clinical examination, X-ray, ultrasonography, computed tomography (CT) scanning or magnetic resonance imaging (MRI) scanning. Patients with poor ECOG performance status (PS > 2) were excluded, as were patients with a life expectancy of less than 3 months or patients who were unable to provide written informed consent. Ethical committee approval for the study was granted by Greater Glasgow Health Board–The West Ethical Committee.

Prior to entry into the study all patients underwent a complete physical examination and full blood count, plasma biochemical profile and chest X-ray were obtained. All patients entered had adequate peripheral blood counts (haemoglobin ≥ 10.0 g/dl, WBC ≥ 3.0 × 10^9/l, platelets ≥ 100 × 10^9/l) and adequate renal and hepatic function (plasma creatinine ≤ 150 μmol/l, bilirubin ≤ 20 μmol/l). Previous malignancy, cerebral metastases and severe concurrent illness were also considered to be exclusion criteria.

PALA was synthesised by the National Cancer Institute of the United States and was supplied by U.S. Bioscience in 5-ml vials containing 500 mg of PALA. 5-FU and FA are both available commercially. PALA, FA and 5-FU were further diluted in 0.9% NaCl for administration purposes.

The treatment regimen is shown in Table 1 and Figure 1. It is known that 24 h are required for PALA to fully deplete uridine nucleotides. In practice, PALA was administered in an outpatient clinic on the day prior to a 48-h admission for 5-FU/FA. Prophylactic antiemetics or antidiarrhoeal agents were not routinely administered. Treatment was repeated every 2 weeks provided that non-haematological toxicities (mucositis and

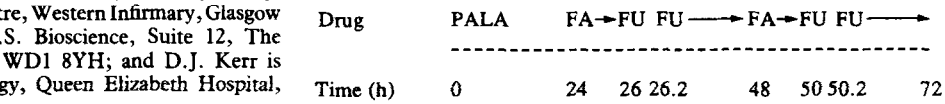

Figure 1. Drug regimen used (repeated every 2 weeks).

Correspondence to J. Cassidy.
D.I. Jodrell, P.A. Canney, H. Yosef, W.P. Steward, S.B. Kaye and J. Cassidy are at the Beatson Oncology Centre, Western Infirmary, Glasgow G11 6NT, U.K.; W. Oster is at U.S. Bioscience, Suite 12, The Courtyards, Croxley Centre, Watford WD1 8YH; and D.J. Kerr is at the Department of Clinical Oncology, Queen Elizabeth Hospital, Birmingham B15 2TH, U.K.
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diarrhoea) had resolved, WBC $\geq 3.0 \times 10^9$/l and platelets $\geq 100 \times 10^9$/l. The initial 5-FU dosage for both bolus and 22-h infusion was 300 mg/m^2. This was increased to 400 mg/m^2 in the absence of dose-limiting toxicity (DLT) in the first 4 patients. A final dose escalation to 500 mg/m^2 was allowed in the absence of DLT in 4 patients treated at 400 mg/m^2. There was no intrapatient dose escalation.

5-FU dosage (bolus and infusion) was reduced by 20% in patients with unacceptable (ECOG grade III) toxicity or ECOG grade II toxicity which had not resolved within 14 days of the planned treatment time. Any patients experiencing life-threatening (ECOG grade 4) toxicity were only retreated if clinically appropriate and then at 50% of the starting 5-FU dosage. After four cycles of treatment (8 weeks) patients' measurable disease was reassessed. Treatment was continued for a further four cycles in patients who had either stable or responsive disease. Treatment was continued beyond eight cycles (16 weeks) in stable or responding patients at the discretion of the individual clinicians participating in the study. In patients with progressive disease at four cycles (or prior to this if clinically apparent), treatment was discontinued and further treatment was at the discretion of the individual clinicians. Objective responses were assessed by standard WHO criteria, i.e. complete response (CR), complete resolution of all known disease determined by two observations at least 4 weeks apart; partial response (PR), 50% reduction in dimensions (the product of bidimensionally measurable disease where appropriate) of indicator lesions without evidence of progression in other disease sites or the appearance of new lesions; progressive disease (PD), the appearance of new lesions or a 25% or greater increase in existing lesions; stable disease (SD), disease status fulfilling none of the above criteria.

In addition to progressive disease or unacceptable toxicity, patients could be withdrawn if they elected to discontinue therapy or the individual physician felt it was not in the patient's best interest to continue therapy.

Treatment-related toxicity was recorded at each clinic visit and graded according to standard ECOG criteria.

RESULTS

Patient characteristics

35 patients with advanced colorectal cancer were entered into the study. Patient characteristics are shown in Table 2. Performance status was good (ECOG grade 0 or 1) in all patients.

Table 2. Patients' characteristics

Sex	
Male	16
Female	19
Age (years)	
Median	60
Range	14–69
Primary site	
Rectum	14
Colon	21
Number of metastatic sites	
Median	2
Range	1–3
Performance status (by ECOG grade)	
0	13
1	22
2	0

Table 3. The incidence of toxicity during the dose escalation phase

Toxicity (worst toxicity with any course)	5-FU 300 mg/m^2 (n = 4)	5-FU 400 mg/m^2 (n = 4)
Mucositis		
ECOG 1	1	1
ECOG $\geq$ 2	0	1
Diarrhoea		
ECOG 1	2	0
ECOG 2	2	1
ECOG $\geq$ 3	0	0
Nausea		
ECOG 1	3	2
ECOG 2	1	1
ECOG $\geq$ 3	0	0
Vomiting		
ECOG 1	0	1
ECOG 2	0	1
ECOG $\geq$ 3	0	0

The most common site of metastatic disease was the liver (15/35 patients) and 17/35 patients had a single site of metastatic disease. One patient had previously received radiotherapy (not to indicator lesion) and 1 patient had previously received chemotherapy (elsamitrucin in a phase II study).

Dosage escalation phase

4 patients were treated with 5-FU dosages of 300 and 400 mg/m^2, in the absence of DLT (no ECOG grade $\geq$ 3, see Table 3), 5-FU dosage for both the bolus and 22-h infusion was escalated to 500 mg/m^2 and used in the subsequent 27 patients. However, in 1 patient treated at 400 mg/m^2, 5-FU dosage was reduced by 25% at the eighth cycle because of hand–foot syndrome severe enough to interfere with function. This patient achieved PR in liver metastases, and time to progression was 10 months, and survival 16 months from the start of treatment. Infusion-related phlebitis was noted in 1 patient treated at the starting dose, but this was not dose limiting. No haematological toxicity was documented at either of these dose levels. 1 patient, treated at 5-FU 300 mg/m^2, was reassessed as stable disease after four cycles but died suddenly at home prior to his fifth cycle of treatment. Postmortem was not performed, but there was no history to suggest an infective cause, and he had reported no diarrhoea with that course of treatment. It is not possible to comment on whether 5-FU-induced cardiotoxicity contributed to his death. One patient (SD at cycle 10) refused further treatment. He had experienced only ECOG grade 1 toxicity associated with the study medication but was unable to tolerate the 48-h admission period psychologically.

The antitumour activity of PALA/5-FU/FA

2 patients were considered to be non-evaluable; 1 patient developed grade 3 diarrhoea and grade 4 leucopenia, and died following her fourth cycle of treatment and before reassessment; 1 patient with subacute obstruction developed complete intestinal obstruction following his second cycle of therapy. We felt justified in not including him as progressive disease because 10 months following his laparotomy he developed symptoms associated with liver metastases and local recurrence in the pelvis, and was successfully treated (documented partial remission) with 5-FU/FA alone. Of 25 evaluable patients treated

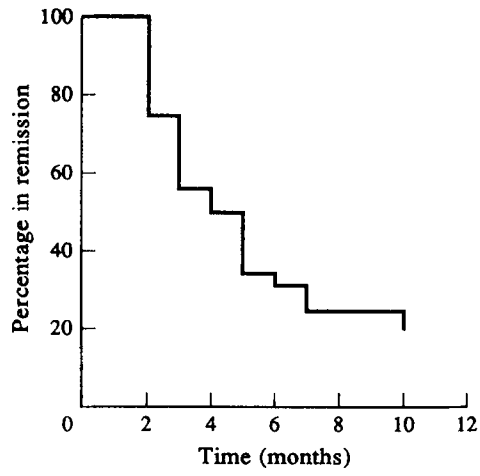

Figure 2. Kaplan–Meier plot of time to disease progression from start of treatment (n = 33). Median = 4 months (95% C.I. 3–6 months).

at the proposed full dosage of 5-FU (500 mg/m^2), only 1 patient (4%) achieved a partial remission using standard WHO criteria. In addition, 1 patient, receiving 400 mg/m^2 5-FU, also achieved a partial response and was treated at a maximally toleratd 5-FU dosage (see above). Therefore the overall response rate for 33 evaluable patients was only 6.1% [95% confidence interval (C.I.) 0.2–21.8%]. In addition, at 5-FU 500 mg/m^2, a further 13 patients (52%) were assessed as having SD following four cycles (8 weeks), but of these, 4 had progressed when reassessed after four further cycles leaving 9/25 (36%) stable at 16 weeks. The median time to progression was 4 months (95% C.I. 3–6 months, Figure 2) and the median overall survival was 10 months with 95% C.I. 7–16 months (Figure 3). In summary, of the 25 patients treated with full dosage 5-FU (500 mg/m^2), 60% had progressive disease on therapy although 15/25 remain alive (follow-up 7–15 months, median 11 months).

The incidence of toxicity at full 5-FU dosage (500 *mg/m^2*)

27 patients were treated at this dose level but only 26 were evaluable. Toxicities encountered in the fixed-dose phase of the study are shown in Table 4. Toxicity is recorded as the worst toxicity experienced in individual patients in any course. The incidence of ECOG $\geq$ 3 toxicity was low (4/27 patients—diarrhoea alone, 3 patients; diarrhoea, nausea and vomiting, 1 patient). However, 1 patient died following treatment having developed grade 3 diarrhoea and grade 4 leucopenia. This toxicity occurred following cycle four of treatment and preceding cycles had been administered with minimal toxicity. In 2 patients developing grade 3 diarrhoea following cycles four and five, progressive disease was documented at reassessment and treatment was discontinued. In one of these patients, diarrhoea continued despite receiving no further chemotherapy and may well have been disease related. Dosage reduction of 20% resulted in 1 patient continuing therapy without further diarrhoea. In 1 patient, dose reduction of 25% was performed following two successive courses in which treatment was delayed for 1 week because of leucopenia. Hand–foot syndrome was noted in 1 further patient at the maximum 5-FU dosage. Alopecia was noted in 3 patients (grade 1, 2 patients; grade 2, 1 patient). Anaemia requiring transfusion occurred in 2 patients whilst on study.

Two toxicities were noted during the study which had not been anticipated prior to the study and were not assessed prospectively. For this reason they are discussed here but the true incidence has not been documented. A number of patients noted marked superficial phlebitis following the intravenous infusion. In some patients, this resulted in a painless brown discolouration along the course of the vein, but in occasional patients, marked erythema and pain was a feature. Patients were also noted to frequently complain of blood-stained mucous discharge from their nose. In no patient was this associated with thrombocytopenia or clotting abnormalities.

DISCUSSION

This paper has described the coadministration of PALA with a 'full dose' combination of 5-FU and FA. PALA was administered at a dosage (250 mg/m^2) which has been shown to inhibit *de novo* pyrimidine synthesis in man [7]. The combination of bolus and infusional 5-FU and short infusion FA used in this study has been reported as being highly active (response rate 54%) in patients with metastatic colorectal cancer [10].

The study regimen (Table 1) was generally well tolerated with only 4/27 patients experiencing $\geq$ ECOG grade 3 toxicity at the maximum 5-FU dosage administered (500 mg/m^2, Table 2). In 3/4 patients, this toxicity comprised diarrhoea alone. These toxicity data are similar to those reported for the combination of 5-FU and FA in the absence of PALA [10], and although this was not a randomised prospective study, there was no apparent increase in toxicity as a result of the addition of PALA at this dosage. In 1 patient severe toxicity, including grade 4 neutropenia, was encountered, and this patient probably died as

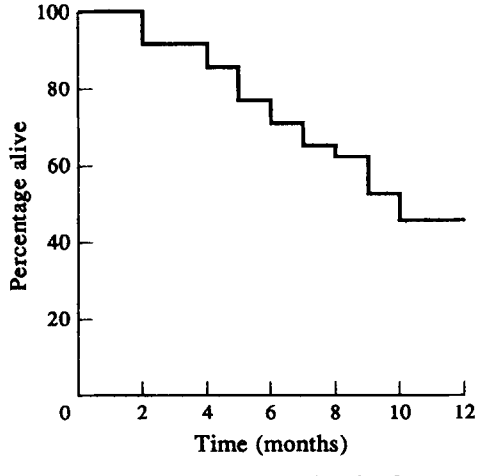

Figure 3. Kaplan–Meier plot of survival duration from start of treatment (n = 35). Median = 10 months (95% C.I. 7–16 months).

Table 4. The incidence of toxicity at full 5-FU dosage (500 *mg/m^2*)

Toxicity (worst with any course)	ECOG 0	ECOG 1	ECOG 2	ECOG 3
Mucositis	16 (62%)	4 (15%)	6 (23%)	0
Diarrhoea	13 (50%)	6 (23%)	3 (12%)	4 (15%)
Nausea	10 (38%)	13 (50%)	2 (8%)	1 (4%)
Vomiting	19 (73%)	5 (19%)	1 (4%)	1 (4%)

a result of drug-induced toxicity. Sporadic severe toxicity has been previously reported in patients receiving 5-FU, and this phenomenon has been associated with evidence of reduced activity of the enzyme dihydropyrimidine dehydrogenase (DPD) [11], but clinical material which might allow the diagnosis of such an enzyme deficiency was not available.

The most disappointing outcome of this study was the poor response rate. Although this study comprised only 33 evaluable patients leading to wide confidence intervals on the reported response rate (6.1%, 95% C.I. 0.2–21.8%), the upper limit of the confidence interval remains below 25%, and does not approach the 54% initially reported for this particular 5-FU/FA regimen [10]. In a separate study, a regimen using a reduced dosage of 5-FU (400 mg/m^2) but similar infusion schedule was reported to give a response rate of 24% (95% C.I. 11–37%) [12]. It is not possible to identify reasons for this poor response rate. Only 1 patient (in the dose escalation phase) had received previous chemotherapy and WHO performance status was good (ECOG grade 0 or 1) in all patients. In addition, 17/35 patients had a single site of metastatic disease. The ages of patients treated in this study (median 60 years, range 41–69) are also similar to those previously reported (median 62 years, range 38–79). It is accepted that response rates may vary between studies as a result of interpretation of response criteria and the difficulty in obtaining reproducible bidimensional measurement using present imaging techniques. However, in this study, response duration was short (4 months, 95% C.I. 3–6 months) and overall survival (10 months, 95% C.I. 7–16 months) was also shorter than that previously reported for this regimen (18 months) [10]. Although it cannot be shown from the data presented, it is unlikely that PALA has a negative impact on the antitumour activity of 5-FU/FA, and therefore it must be concluded that a suboptimal 5-FU/FA regimen had been selected for this study, despite the promising results from previous publications [10, 12].

In addition, the chosen 5-FU/FA regimen is costly to administer in terms of both drug costs and, in our experience in Glasgow, inpatient care. Patients were admitted for 2 nights every 2 weeks, and this is likely to have a negative impact upon quality of life. In 1 patient, this was stated as the cause of significant psychological morbidity and premature cessation of therapy.

The dose intensity of the 5-FU/FA regimen used in the study reported here is 5-FU 1000 mg/m^2/week and FA 200 mg/m^2/week. In comparison, a regimen has been reported where the 5-FU dose intensity (in combination with FA) has been escalated to 2600 mg/m^2/week and FA dose intensity to 500 mg/m^2/week [13]. In combination with PALA (250 mg/m^2/week), exciting preliminary response data have been reported [6/7 (86%) in evaluable untreated patients, 7/16 (44%) in evaluable previously treated patients] using this dose-intense regimen [14], in which the 5-FU and FA were coadministered as a 24-h infusion, 24 h after the administration of PALA.

It is unlikely that dose intensity alone is the most important factor in the administration of drugs with antimetabolic effects. However, the relative lack of toxicity and poor response rate noted in this study demonstrate that further studies are required to optimise the dosage and scheduling of 5-FU and FA. It will be difficult to define the exact role of PALA in the modulation of 5-FU anticancer activity until this has been achieved, and carefully designed prospective randomised studies must be a priority to answer these questions.

1. Advanced Colorectal Cancer Meta-Analysis Project. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. *J Clin Oncol* 1992, **10**, 896–903.
2. Swyryd EA, Seaver SS, Stark GR. *N*-(phosphonacetyl)-L-aspartate, a potent transition state analog inhibitor of aspartate transcarbamylase, blocks proliferation of mammalian cells in culture. *J Biol Chem* 1974, **249**, 6945–6950.
3. Martin DS, Stolfi RL, Sawyer RC, Spiegelman S, Casper ES, Young CW. Therapeutic utility of utilizing low doses of *N*-(phosphonacetyl)-L-aspartic acid in combination with 5-fluorouracil: a murine study with clinical relevance. *Cancer Res* 1983, **43**, 2317–2321.
4. Cohen SS, Flaks G, Barner HD, *et al.* The mode of action of 5-fluorouracil and its derivatives. *Proc Natl Acad Sci USA* 1958, **44**, 1004.
5. Jolivet J, Curt GA, Clendeninn NJ, *et al.* Antimetabolites. In Pinedo HM, ed. *Cancer Chemotherapy*. Excerpta Medica, 1983, 7–8.
6. Spiegelman S, Nayak R, Sawyer R, Stolf R, Martin D. Potentiation of the antitumor activity of 5-FU by thymidine and its correlation with the formation of (5-FU) RNA. *Cancer* 1986, **45**, 1129–1134.
7. Bedikian AY, Stroehlein JR, Karlin DA, Bennetts RW, Bodey GP, Valdivieso M. Chemotherapy for colorectal cancer with a combination of PALA and 5-FU. *Cancer Treat Rep* 1981,**65**, 9–10, 747–753.
8. Casper ES, Vale K, Williams LJ, Martin DS, Young CW. Phase I and clinical pharmacological evaluation of biochemical modulation of 5-fluorouracil with *N*-(phosphonacetyl)-L-aspartic acid. *Cancer Res* 1983, **43**, 2324–2329.
9. Petrelli N, Herrera L, Rustum Y, *et al.* A prospective randomised trial of 5-fluorouracil versus 5-fluoruouracil and high-dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma. *J Clin Oncol* 1987, **5**, 1559–1565.
10. De Gramont A, Krulik M, Cady J, *et al.* High dose folinic acid and 5-fluorouracil bolus and continuous infusion in advanced colorectal cancer. *Eur J Cancer* 1988, **24**, 1499–1503.
11. Lilenbaum RC, Harris BE, Diasio RB, Naes J, Lyss AP. Heterozygosity for dihydropyrimidine dehydrogenase (DPD) deficiency may result in life-threatening (Gr4) toxicity from fluorouracil. *Proc Am Soc Clin Oncol* 1991, **10**, 120.
12. Johnson PWM, Thompson PI, Seymour MT, *et al.* A less toxic regimen of 5-fluorouracil and high-dose folinic acid for advanced gastrintestinal adenocarcinomas. *Br J Cancer* 1991, **64**, 603–605.
13. Ardalan B, Sridhar KS, Reddy R, *et al.* A phase I study of short high dose 5-fluorouracil and high dose leucovorin and low dose phosphonacetyle-L-aspartic acid (PALA) in patients with advanced malignancies. *Int J Radiat Oncol Biol Phys* 1992, **22**, 511–514.
14. Ardalan B, Donofrio K, Sridhar KS, *et al.* Is there hope after 5-FU bolus? A phase II study of short term protracted infusion of high dose 5-fluorouracil with leucovorin and low dose phosphonacetyl-L-aspartic acid (PALA) in colorectal carcinoma. *Proc Am Soc Clin Oncol* 1992, **11**, 186.

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