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Phase I–II Study of the Addition of α-2a Interferon to 5-Fluorouracil/Leucovorin. Pharmacokinetic Interaction of α-2a Interferon and Leucovorin

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5-Fluorouracil (5-FU) activity has been improved by the use of leucovorin (LV) or α-2a interferon (α-IF). We investigated the feasibility and activity of addition of α-IF to a 5-FU/LV regimen. A phase I study with 26 patients (14 previously untreated, 12 previously treated) with disseminated cancer was conducted. 15 patients were treated with 5-FU/LV and 11 with 5-FU/LV/α-IF. The 5-FU/LV regimen consisted of escalating doses of 5-FU bolus intravenously on days 2 and 3, combined with repeated oral LV on days 1, 2 and 3. Treatment was every 2 weeks. In the 5-FU/LV/α-IF schedule, 18 × 10^6 U α-IF subcutaneously daily was added on days 1, 2 and 3. The phase I study was followed by a phase II study of 5-FU/LV/α-IF at the established 5-FU dose in 29 previously untreated patients with disseminated colorectal cancer. The optimal 5-FU dose in both parts of the phase I study was 750 mg/m^2/day. Mucositis, diarrhoea and leucopenia were dose limiting. Although α-IF added its own toxicity (fever, flu-like symptoms, fatigue), it did not decrease the optimal dose of 5-FU. In the phase II study 28 patients were evaluable for response: three complete responses and 12 partial responses were observed (response rate 54%, 95% confidence interval, 34 to 72%). Pharmacokinetics of oral LV was performed in patients treated with and without α-IF: significantly higher serum levels of LV and 5-methyltetrahydrofolate were found after α-IF addition. Influence of α-IF on gastrointestinal absorption or renal clearance could be excluded. In conclusion, this 5-FU/LV/α-IF combination seems active in metastatic colorectal cancer. The pharmacokinetic interaction between α-IF and LV may play a role in the activity of this regimen. Controlled studies are necessary to establish the value of addition of α-IF to 5-FU/LV regimens.
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INTRODUCTION

BETTER UNDERSTANDING of 5-fluorouracil (5-FU) metabolism has led to advances in the treatment of metastatic colon cancer. One way to improve 5-FU activity is the use of leucovorin (LV). This reduced folate stabilises the binding of the active 5-FU metabolite fluorodeoxyuridine monophosphate to its target enzyme thymidylate synthase. Several preclinical [1–4] and clinical [5, 6] studies confirmed the efficacy of LV addition to 5-FU in terms of enhancement of tumour response but also of toxicity. However, agreement on the optimal schedule and dose of LV still has to be established. Some studies using intravenous LV favour low doses (20 mg/m^2) in view of efficacy, toxicity and costs [7, 8]. Commercially available LV comprises equal amounts of the biologically active *l*-LV and the inactive *d*-LV. One potential disadvantage of (high-dose) intravenous LV is the accumulation of *d*-LV in the plasma, resulting in competition with the active *l*-LV. Oral administration results in the selective uptake of the *l*-isomer at a rate four to five times greater than for *d*-LV. After oral administration, *l*-LV is rapidly metabolised in the small bowel mucosa and liver to its active metabolite 5-methyltetrahydrofolate (MTHF) [9–12].

Another way to improve 5-FU activity is the use of α-2a interferon (α-IF). Synergism between 5-FU and α-IF has been demonstrated *in vitro* [13], and single-centre phase II studies show response rates of 26–62% in patients with advanced colorectal carcinoma [14–17]. Only one multicentre trial has been reported with the combination 5-FU/α-IF, reaching a 42% response rate [95% confidence interval (CI), 27 to 58%] [18]. Toxicity in these clinical studies is considerable. The basis for the synergism has not yet been fully elucidated. At least seven potential mechanisms of interaction of interferon and 5-FU have been reported [13]. Among these are alterations of intracellular [19] or serum [20, 21] pharmocokinetics of 5-FU.

In the present studies 5-FU was combined with both modulating agents. We started a phase I study with increasing doses of 5-FU administered for 2 consecutive days combined with a fixed dose of oral LV (5-FU/LV) to determine the optimal dose of 5-FU. Thereafter, the optimal dose of 5-FU combined with the same dose of LV and 18 × 10^6 U α-IF subcutaneously (5-FU/LV/α-IF) was determined. The modulating agents LV and α-IF were given for 3 days, starting 1 day for 5-FU infusion. Treatment was every 2 weeks. Serum levels of total LV and MTHF were measured in 3 patients receiving 5-FU/LV and in 3 patients receiving 5-FU/LV/α-IF to evaluate if α-IF influences pharmacokinetics of LV. The phase I study was followed by a

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phase II study of 5-FU/LV/α-IF at the established optimal dose of 5-FU in patients with disseminated colorectal carcinoma.

PATIENTS AND METHODS

Patients

Eligibility criteria for the phase I study were end-stage disseminated cancer, WHO performance status (PS) ≤ 2, age 16–75 years, leukocytes > 3×10^9/l, platelets > 100×10^9/l, serum creatinine < 200 μmol/l, serum bilirubin < 100 mmol/l and no diseases, other than the malignancy, not controllable with outpatient treatment. Additional eligibility criteria for the phase II study were measurable or evaluable histologically documented colorectal cancer and no prior chemotherapy. Informed consent was obtained from all patients and the studies were approved by the Medical Ethical Committee of the University Hospital Groningen.

Drug treatment

The phase I 5-FU/LV chemotherapy schedule consisted of 60 mg LV (Ledervorin, Lederle, Etten-Leur, The Netherlands) administered orally on days 1 to 3, every 8 h for nine doses, starting at 8.00 a.m. 5-FU (Roche, Mijdrecht, The Netherlands) bolus injection was given on days 2 and 3 at an initial dose of 500 mg/m²/day, and was escalated by 250 mg/m²/day to a maximum of 1000 mg/m²/day. Treatment courses were given every 2 weeks (day 15 = day 1).

The phase I 5-FU/LV/α-IF schedule was essentially identical, insofar that 18×10^6 U α-IF (Roferon-α, purchased from Roche, Mijdrecht, The Netherlands) subcutaneously administered, was added on days 1 to 3. At least 3 patients were treated at each dose level. There was no 5-FU escalation in individual patients. Evaluation of toxicity according to WHO guidelines [22] was performed weekly. Mucositis, nausea/vomiting or diarrhoea grade 2 and haematological toxicity grade 3 were considered dose limiting. A minimum of two treatment courses were given. If no objective or subjective progression was evident treatment was continued until progression or until a maximum of eight treatment courses.

The phase I study was followed by a phase II study of 5-FU/LV/α-IF at the optimal 5-FU dose.

Criteria for response

Evaluation of response was performed after every four courses. Response criteria were complete remission (CR), the disappearance of all perceptible tumour and partial response (PR), at least 50% reduction in product of largest perpendicular diameter of the indicator lesion with no increase in the size of other measurable disease and no appearance of new lesions for a period of at least 4 weeks. Stable disease (SD) was defined as no change in size of measurable lesion or a decrease in tumour size by less than 50% or an increase of 25% with no appearance of new lesions; SD required a minimum of 8 weeks' duration. Progressive disease (PD) was the appearance of any new lesions and/or growth of any existing lesion by more than a quarter from the start of the treatment. Survival time was calculated on the basis of the period from the start of the treatment to death.

Analytic procedure of LV and MTHF

LV and MTHF used for analytical procedures were provided by Lederle. Serum concentrations of total LV and MTHF were measured by reversed phase high performance liquid chromatography (HPLC) based on the description of Van Tellingen *et al.* [23]. Two hundred and fifty microlitres of patient serum with 25 μl 1% ascorbic acid and 100 μl water were placed for 2 min on ice. After the addition of 250 μl perchloric acid (1.5 mol/l), the sample was vortexed for 10 s. Then, 100 μl potassium acetate (8 mol/l, pH 8) was added and the sample was mixed. After addition of 50 μl citric acid phosphate buffer (0.4 mol/l, pH 4.4) the sample was vortexed for 20 s and placed on ice for 5 min. After centrifugation (14 000 *g*, 5 min), the supernatant was filtered through a 0.45 μm 13 mm disposable filter unit (Supelco, Supelchem BV, Leusden, The Netherlands). Fifty microlitres were injected into a 40 μm Octadecyl (C18) column and thereafter into a 5-micron RP-18 Lichrosorb HPLC column using a WISP 710A automatic injector (Waters, Etten-Leur, The Netherlands). This column was kept at 55°C with a 655 A-52 column oven (Hitachi, Merck, Amsterdam, The Netherlands). The chromatographic system consisted of a 6000 A HPLC pump (Waters Associates) and a 759 A u.v. absorbance detector (Separations, ABI Applied Biosystems, Hendrik Ido Ambacht, The Netherlands) at 305 nm, 0.005 AUFS, combined with a Spectra-Physics SP 4290 integrator. LV and MTHF were separated with a mobile phase of 5% acetonitril in 60 mmol/l phosphate buffer at pH 3.7 at a flow rate of 1.0 ml/min. Retention times for LV and MTHF were 15 and 12.5 min, respectively. A standard curve was prepared and run with each set of 10 patient samples. Detection limit, defined as three times the noise level, was 100 μg/l serum for LV as well as for MTHF. Variation coefficient of the analytic procedure was 6.6% (concentration = 300 μg/l serum; $n = 10$) for LV, and 6.1% (concentration = 300 μg/l serum; $n = 10$) for MTHF.

Sampling procedure

In 3 patients on the 5-FU/LV regimen and in 3 patients on the 5-FU/LV/α-IF regimen blood specimens were obtained for pharmacokinetic analysis of repeated oral doses of LV. Sixty milligrams of LV were administered at $t = 0, 8, 16, 24, 32, 40, 48, 56$ and 64 h. Patients were fasting from 2 h before until 2 h after each LV dose. Blood was drawn just before (trough level) and 2 h after (established peak level) each dose. The last blood sample was taken at 72 h. All samples were collected in red-top vacutainer tubes (Venoject; Omnilab, Breda, The Netherlands) containing 0.1 ml of 7.5% ascorbic acid. Blood was allowed to clot fully at 6°C and serum was separated by centrifugation. To 1 ml serum, 10 μl of 7.5% ascorbic acid were added, and samples were stored at −80°C until analysis. All samples were collected and analysed in duplicate.

Statistical analysis

The χ^2 test corrected for continuity was applied for the differences between the toxicities of 5-FU/LV treatment and 5-FU/LV/α-IF treatment. The multiple unpaired Wilcoxon test was used to compare the differences in pharmacokinetic data of 5-FU/LV treatment and 5-FU/LV/α-IF treatment. Values of $P < 0.05$ were considered significant. Survival times were calculated using the Kaplan–Meier method.

RESULTS

Phase I study of 5-FU/LV and 5-FU/LV/α-IF

Fifteen patients were entered into the 5-FU/LV part and 11 patients into the 5-FU/LV/α-IF part of the phase I study. Patients' characteristics are summarised in Table 1. Toxicity of the 5-FU/LV part is summarised in Table 2. All patients experienced some degree of toxicity, leucopenia and mucositis being most frequent; nausea/vomiting were also frequent. At the

Table 1. Patients' characteristics of the phase I and phase II studies

	Phase I		
	5-FU/LV	5-FU/LV/ α-IF	Phase II
No. of patients	15	11	29
Median age, years (range)	56 (34–66)	55 (32–68)	53 (27–69)
Male/female	8/7	9/2	13/16
Performance status (WHO)			
0	1	3	11
1	6	4	16
2	8	4	2
Diagnosis			
Colorectal carcinoma	7	6	29
Gastric carcinoma	6	3	
Ovarian carcinoma	1		
Adenocarcinoma, unknown primary	1		
Others		2	
Prior chemotherapy			
No	9	5	29
Yes, with 5-FU	2	5	
Yes, without 5-FU	4	1	
Prior radiation			
No	14	7	27
Yes	1	4	2

the 1000 mg/m² 5-FU dose level diarrhoea was also frequently observed. Conjunctivitis occurred in 1 patient at the 750 mg/m² level during two courses. Hand-foot syndrome (HFS) was experienced in 5 patients (eight courses). 3 of these 5 patients were at the 750 mg/m² dose level, while one was at the 500 mg/m² level and another at the 1000 mg/m² level. At the 1000 mg/m² level dose reduction was performed in 5 out of 7 patients (in 3 patients because of grade 3 non-haematological toxicity and in 2 patients because of persistent grade 3 leucopenia), while at the 750 mg/m² level dose reduction was performed only in 1 patient (due to persistent grade 3 leucopenia). 3 out of 15 patients treated with 5-FU/LV were not evaluable for response. In the remaining 12 patients, 6 had PD, 5 had SD for 4–13 weeks, and 1 patient with a colorectal carcinoma had a PR lasting 4 weeks (response rate 1/12 = 8%, 95% CI, 0.2 – 38.5%).

Table 2. Toxicity of the 5-FU/LV part of the phase I study

Dose level 5-FU (mg/m²)	500	750	1000
No. of patients	3	5	7
No. of courses	11	19	18
Leucopenia			
Grade 1	0	2	1
Grade 2	1	5	3
Grade 3	1	4	3
Thrombopenia			
Grade 1	0	0	0
Grade 2	0	0	1
Mucositis			
Grade 1	1	5	1
Grade 2	1	6	2
Grade 3	0	0	1
Nausea/vomiting			
Grade 1	1	1	1
Grade 2	0	0	3
Grade 3	0	0	1
Diarrhoea			
Grade 1	1	0	2
Grade 2	0	1	3
Grade 3	0	0	1

Data indicate number of courses.

Then, the 5-FU/LV/α-IF part of the phase I study was started and its toxicity is summarised in Table 3. All but 1 patient experienced toxicity, myelosuppression and gastrointestinal toxicity were most frequent and dose limiting. Conjunctivitis occurred in 1 patient at the 750 mg/m² dose level during six courses. HFS occurred in 3 patients for 13 courses. These patients were all treated at the 750 mg/m² level and all received four or more courses. Alopecia grade 3 was also observed in these 3 patients. In all patients receiving α-IF flu-like symptoms with fever up to 39°C were observed. This could be suppressed partially by acetaminophen. No dose reduction of 5-FU was necessary. In view of the frequently observed grade 3 leucopenia and mucositis, the 750 mg/m² dose level was considered to be the optimal dose of 5-FU when combined with LV and α-IF. In this part of the phase I study, 1 patient was not evaluable for response. In the remaining 10 patients 7 patients had PD, 2 had a PR lasting 10 weeks and 26 weeks, and 1 patient had a CR for 10 weeks (response rate 3/10 = 30%, 95% CI, 6.6 – 65.3%). The patients with a response had all gastrointestinal malignancies (two gastric carcinomas and one colorectal carcinoma).

Toxicities in 5-FU/LV and the 5-FU/LV/α-IF were compared: 5-FU/LV/α-IF treatment tended to result in more side-effects compared to 5-FU/LV treatment, but the differences were too small to reach significance, with exception of α-IF-related symptoms such as fever and fatigue.

Phase II study of 5-FU/LV/α-IF

To date, 29 patients, all with measurable disease, were entered into the phase II study. Patients' characteristics are summarised

Table 3. Toxicity of the 5-FU/LV/α-IF part of the phase I study and toxicity of the phase II study

Dose level 5-FU (mg/m²)	500	750	Phase II
No. of patients	4	7	29
No. of courses	10	36	196
Leucopenia			
Grade 1	3	6	24
Grade 2	0	13	20
Grade 3	2	12	11
Grade 4	0	0	2
Anaemia			
Grade 2	0	0	3
Thrombopenia			
Grade 1	1	8	0
Grade 2	2	0	0
Mucositis			
Grade 1	2	6	60
Grade 2	1	13	35
Grade 3	0	4	16
Grade 4	0	0	1
Nausea/vomiting			
Grade 1	0	3	37
Grade 2	0	4	17
Diarrhoea			
Grade 1	2	6	13
Grade 2	3	2	31
Grade 3	0	2	1
Grade 4	0	0	1

Data indicate number of courses.

in Table 1. A total of 196 courses were given, median of eight per patient. All patients were evaluable for toxicity, 1 patient had early tumour-associated complications and was not evaluable for response. In 28 patients three CR and 12 PR were observed (response rate: 54%, 95% CI, 34 – 72%). Responses occurred in patients with liver (3 CR and 9 PR), abdominal (2) and lung (1) metastases. During the follow-up after completion of chemotherapy, 10 out of 15 responders had progression of tumour and median time to progression after cessation of therapy was 20 weeks (range 8–52+ weeks). 10 patients had SD, 6 of these patients had liver metastases and 4 had intra-abdominal metastases. PD occurred in 3 out of 28 patients, 2 patients had intra-abdominal masses and 1 had liver metastases. Toxicity in this phase II study was moderate and comparable to the 5-FU/LV/α-IF part of the phase I study (Table 3): mucositis grade 1–4 occurred in 112/196 (57%) courses, leucopenia grade 1–4 in 57/196 (29%), and diarrhoea in 46/196 (23%) courses. HFS occurred in 16 patients and alopecia grade 3 in 9 patients. HFS and alopecia were only observed after administration of two or more courses. Neurological toxicity, particularly cerebellar dysfunction, occurred in 3 patients. In 11 patients 25% dose reduction of 5-FU was necessary because of persistent toxicity, total 29 courses. Hospital admission because of severe toxicity was necessary in 2 patients, 1 patient had grade 1 mucositis and another grade 4 diarrhoea. There were no treatment-related deaths. Median survival in this study is now 16 months.

Pharmacokinetics

Pharmacokinetics of the repeated oral dose of LV were performed in 3 patients receiving 5-FU/LV and in 3 patients receiving 5-FU/LV/α-IF. Characteristics of those patients are summarised in Table 4. The mean and standard error of the mean (S.E.M.), concentrations of LV and MTHF during the 3-day courses of 5-FU/LV and 5-FU/LV/α-IF are shown in Figs 1 and 2. Comparison of all peak and trough levels of the patients treated with 5-FU/LV with those treated with 5-FU/LV/α-IF revealed higher serum LV ($P < 0.001$) as well as MTHF concentrations ($P < 0.02$) during 5-FU/LV/α-IF treatment. Patients treated with 5-FU/LV had mean (± S.E.M.) peak serum LV concentration on days 2 and 3 (days of 5-FU infusion) of 1.6 μmol/l (± 0.14 μmol/l) and mean (± S.E.M.) trough serum concentration of 1.07 μmol/l (± 0.04 μmol/l). Mean peak and trough concentrations of serum MTHF during these days were 1.46 μmol/l (± 0.08 μmol/l) and 0.78 μmol/l (± 0.07 μmol/l), respectively. For patients treated with 5-FU/LV/α-IF mean peak and trough serum LV levels on days 2 and 3 were 2.21 μmol/l (± 0.07 μmol/l) and 1.52 μmol/l (± 0.04 μmol/l), respectively. On these days, mean serum MTHF peak and trough levels were 1.60 μmol/l (± 0.07 μmol/l) and 1.04 μmol/l (± 0.05 μmol/l), respectively.

Table 4. Clinical characteristics of patients who underwent pharmacokinetics of repeated oral dose LV

Chemotherapy	Sex	Age (years)	Body surface (m^2)	CR.CL (ml/min/1.73 m^2)
A 5-FU (750 mg/m^2)/LV	F	38	1.68	94
B 5-FU (750 mg/m^2)/LV	F	34	1.68	82
C 5-FU (500 mg/m^2)/LV	M	58	1.92	77
D 5-FU (750 mg/m^2)/LV/α-IF	M	44	1.84	71
E 5-FU (750 mg/m^2)/LV/α-IF	M	54	1.96	71
F 5-FU (750 mg/m^2)/LV/α-IF	M	31	1.96	112

F, female; M, male; CR.CL, creatinine clearance.

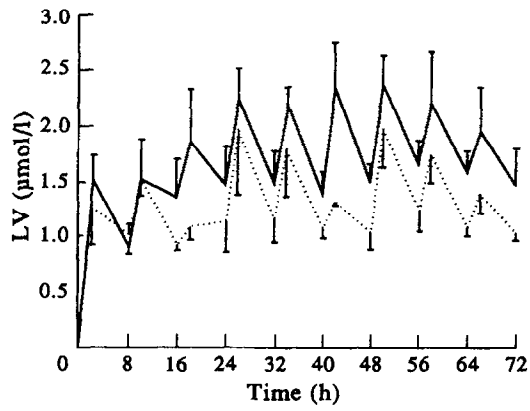

Fig. 1. LV concentrations during the 3-day schedule of repeated (every 8 h) oral dose of 60 mg LV in patients treated with 5-FU-LV (· · · ·) or 5-FU/LV/α-IF (————). Bars are S.E.M. Higher ($P < 0.001$) LV concentrations in patients treated with 5-FU/LV/α-IF.

In order to exclude any possible interpatient variations that could be responsible for the higher serum levels of LV and MTHF in the 5-FU/LV/α-IF group, 3 additional patients scheduled for α-IF treatment received LV 200 mg/m^2/day as a continuous infusion for 68 h. After 20 h of LV infusion seven blood samples were drawn at t = 0.5, 2, 4, 6, 8, 12 and 23 h. Then α-IF (18 × 10^6 U/s) was given, and again seven blood samples were drawn at t = 0.5, 2, 4, 6, 8, 12 and 23 h after α-IF administration. Mean (± S.E.M.) serum LV concentration before α-IF administration was 14.9 μmol/l (± 0.73 μmol/l) and after α-IF administration 17.2 μol/l (± 0.52 μmol/l). For MTHF, mean serum concentrations before and after α-IF were 2.55 μmol/l (± 0.15 μmol/l) and 2.98 μmol/l (± 0.04 μmol/l), respectively (Fig. 3). These values are higher after α-IF administration for LV ($P < 0.05$) as well as for MTHF ($P < 0.001$, two-tailed paired Student's t-test). In these 3 additional patients, urine was collected during the 23 h before and after the α-IF administration. Urine was collected and analysed as previously described [9, 24]. Mean (± S.E.M.) renal

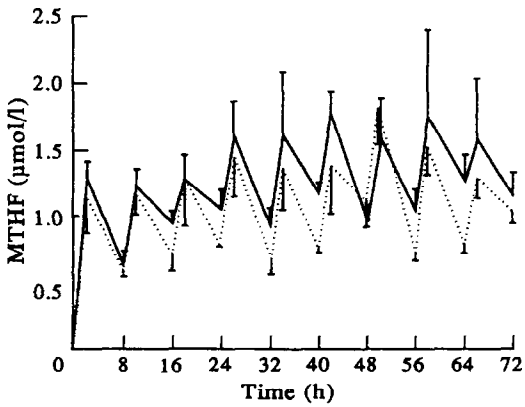

Fig. 2. MTHF concentrations during the 3-day schedule of repeated (every 8 h) oral dose of 60 mg LV in patients treated with 5-FU/LV (· · · ·) or 5-FU/LV/α-IF (————). Bars are S.E.M. Higher ($P < 0.02$) MTHF concentrations in patients treated with 5-FU/LV/α-IF.

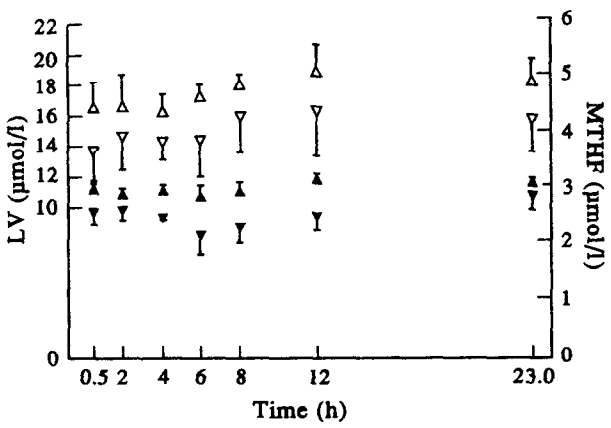

Fig. 3. LV concentrations before (▽) and after (△), and MTHF concentrations before (▼) and after (▲) α-IF administration during continuous infusion of LV. Bars are S.E.M.

clearance [amount excreted/(serum concentration × time)] of total LV before α-IF was 21.6 ml/min (± 4.18 ml/min) and after α-IF 24.6 ml/min (± 2.86 ml/min). Mean renal clearance of MTHF before and after α-IF was 41.72 ml/min (± 11.03 ml/min) and 47.9 ml/min (± 10.7 ml/min), respectively (NS, two-tailed paired Student's *t*-test).

DISCUSSION

The purpose of the phase I study was to determine the optimal dose of bolus injection 5-FU, administered for 2 consecutive days every 2 weeks, when combined with the modulating agents LV and α-IF. These agents were started 1 day before 5-FU infusion and given for 3 consecutive days. The initial dose of 5-FU was based upon a report of Hryniuk *et al.* [25], who stated that the optimal dose of bolus injection 5-FU is probably 600 mg/m²/week. As mentioned above, the optimal dose and route of administration of LV remain controversial. We decided to give an intermediate dose of oral LV and to measure serum levels of LV and MTHF. The α-IF dose was based upon a recent phase I study using a fixed dose of 5-FU and escalating doses of α-IF: the maximum tolerated dose of α-IF was 15–18 × 10^6 U daily [26].

In the present study, an optimal dose of 750 mg/m²/day 5-FU was found for 5-FU/LV, as well as for 5FU/LV/α-IF. Dose-limiting toxicities were gastrointestinal (mucositis, nausea/vomiting, diarrhoea) and leucopenia. α-IF addition to 5-FU/LV led to more fever and fatigue, which are α-IF-related toxicities. However, α-IF addition did not result in a lower optimal dose of 5-FU.

The 5-FU/LV/α-IF part of the phase I study was continued as a phase II study at the optimal dose of 5-FU. The response rate in this part of the study (54%) appears promising. Proof of superiority to 5-FU/LV or 5-FU/α-IF requires large randomised trials. Although the response rate is encouraging, median time to progression of responding patients after completion of chemotherapy was short (20 weeks). The frequently observed, sometimes severe, side-effects indicate that this 5-FU/LV/α-IF schedule can only be given for a maximum of eight to 10 courses. Further intensification by prolonged treatment appeared not to be feasible. It is conceivable that a less toxic maintenance therapy may prolong median time to progression in responders.

Pharmacokinetics of the repeated oral dose (60 mg, every 8 h × 9) LV revealed mean peak and trough concentrations of LV and MTHF on days 2 and 3 (days of 5-FU infusion) sufficiently high enough to modulate *in vitro* 5-FU cytotoxicity [1–4, 27]. This justifies the oral administration of LV when started 1 day before 5-FU bolus injection and this will facilitate outpatient treatment. Higher serum concentrations of LV (Fig. 1) and MTHF (Fig. 2) were found in patients treated with 5-FU/LV/α-IF compared with those treated with 5-FU/LV. The role of α-IF was further analysed in a continuous infusion study of LV. Again, significantly higher LV and MTHF concentrations were found after α-IF. The observation of raised serum levels of reduced folates after both oral and intravenous administration of LV, indicates that other mechanisms than altered absorption from the digestive tract must account for these differences. Renal clearance of LV and MTHF in the continuous infusion study of LV was not changed after α-IF. Thus, as gastrointestinal absorption and renal clearance are not responsible for the higher serum levels, other mechanisms should be looked for. α-IF has not only been associated with altered pharmacokinetics of 5-FU [20, 21], but also of theophylline and antipyrine: clearance of these two drugs is impaired after α-IF administration probably due to inhibition of hepatic oxidative drug metabolism [28, 29]. On the other hand, serum folic acid concentrations are lower after induction of hepatic enzyme systems [30, 31]. Although LV is a reduced folate, unlike folic acid, it cannot be excluded that inhibition of the hepatic oxidation system by α-IF accounts for the higher serum levels of reduced folates. Whether α-IF influences intracellular pools of reduced folates remains to be established.

In conclusion, this 5-FU/LV/α-IF combination seems active in previously untreated patients with metastatic colorectal cancer. Addition of α-IF to LV results in higher serum levels of reduced folates. This interaction has not previously been reported, and it may contribute to the efficacy of this 5-FU/LV/α-IF regimen.

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Phase II Study of Vinorelbine (Navelbine) in Previously Treated Small Cell Lung Cancer Patients

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26 previously treated patients with progressive recurrent small cell lung cancer (SCLC) were given vinorelbine (Navelbine), 30 mg/m² weekly. All patients had responded to first-line chemotherapy and were off therapy for at least 3 months. Partial response was observed in 4 out of 25 eligible patients (16%; 95% confidence interval 4–36%), stable disease in 7 patients and progression in 12 patients. The limiting toxicity was a non-cumulative leucopenia (80%, 32% WHO grade 3–4). Reaction at the site of injection was observed in 5 patients, causing treatment discontinuation in 2 cases. Other non-haematological toxicities were moderate. These results suggest acceptable toxicity and some antitumour activity of vinorelbine in pretreated SCLC patients.
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INTRODUCTION

VINORELBINE (5'-NOR-ANHYDROVINBLASTINE, Navelbine) is a new semisynthetic vinca alkaloid, which was selected for its high affinity to prevent tubulin polymerisation [1]. *In vivo* studies performed on human tumours xenografted in nude mice showed the activity of vinorelbine against different types of lung carcinoma [2]. In most lung cancer xenografts vinorelbine compared favourably with vincristine and vinblastine [2]. Clinical phase I studies showed dose-related leucopenia, while other toxicities were moderate [3, 4]. The recommended dose for phase II studies was 30 mg/m², intravenously (i.v.) weekly. Further clinical studies demonstrated cytotoxic activity of vinorelbine in several tumours, including non-small cell lung cancer [5–7]. Because of these interesting results the EORTC Lung Cancer