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Original paper

Biochemical Modulation of 5-Fluorouracil by Leucovorin with or without Interferon-α-2c in Patients with Advanced Colorectal Cancer: Final Results of a Randomised Phase III Study

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5-Fluorouracil (5-FU) remains the mainstay of treatment for advanced colorectal carcinoma, although response rates are generally less than 20%. Improved therapeutic efficacy has been reported using biochemical modulation of 5-FU by leucovorin (LV) or interferon α (IFN), the combination of 5-FU/LV frequently considered as standard therapy in metastatic colorectal cancer. In an attempt to enhance the cytotoxicity of 5-FU, a prospective randomised trial was initiated to compare 5-FU/LV with 5-FU/LV plus IFN. Patients were randomised to receive either LV, 100 mg/m² intravenously (i.v.), followed by 5-FU, 500 mg/m² as a 1-h i.v. infusion, daily for 4 days, followed by weekly infusions until week 8, or the same regimen of 5-FU/LV plus IFN-α-2c, 30 μg subcutaneously (s.c.), three times weekly. Cycles were repeated after a 2-week rest period. Among 269 enrolled patients, 219 were available for response and 243 for toxicity. An objective tumour response was observed in 38 of 107 (36%) and 28 of 112 (25%) patients in the treatment arms with and without IFN, respectively (difference not significant). There was no significant difference between the two groups in response duration (median 8.4 versus 12.1 months), time to treatment failure (median 6.5 versus 4.9 months), or overall survival (median 10.0 versus 12.6 months). However, patients in the IFN arm experienced significantly more haematological and gastrointestinal toxicity and more frequent alopecia. In conclusion, the addition of IFN to 5-FU/LV in the schedules and doses used in the study did not provide any clinical benefit over 5-FU/LV alone and cannot be recommended for routine use in the treatment of advanced colorectal cancer.

Key words: advanced colorectal cancer, chemotherapy, leucovorin, interferon α
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INTRODUCTION

SINCE 5-FLUOROURACIL (5-FU) was introduced 40 years ago [1], it has remained the mainstay of treatment for patients with advanced colorectal carcinoma. However, response rates of single-agent 5-FU are generally less than 20%, usually approximately 10% in prospective randomised phase III trials [2–4] and with no significant impact on survival.

There have been several attempts to improve the therapeutic effectiveness of 5-FU by the use of modulating drugs such as methotrexate [5, 6], *N*-phosphonoacetyl-L-aspartate (PALA) [7, 8], leucovorin (LV) [9–11] or interferons (IFN) [12–14]. A number of randomised trials has demonstrated

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increased response rates in patients receiving combined treatment of 5-FU and LV compared with 5-FU alone and a survival advantage has been demonstrated in several studies [2–4]. However, whilst an advantage of adding LV to 5-FU to enhance tumour response seems to be established, a survival benefit has not been confirmed in a meta-analysis of nine randomised trials [15].

Based on preclinical observations [16, 17] that IFN and 5-FU have synergistic activity in colon cancer cell lines, IFN has also been extensively investigated in a series of clinical studies [12–14]. Impressive response rates from 26 to 76% [12–14] had been reported in patients treated with 5-FU combined with IFN-α.

In view of data from *in vitro* studies, demonstrating additive or complementary effects of LV and IFN on 5-FU cytotoxicity [18] and preliminary results from clinical investigations with double modulated FU [19, 20], a prospective randomised trial was designed to determine whether IFN can enhance the therapeutic efficacy of FU plus LV in previously untreated patients with recurrent or metastatic colorectal cancer. The objectives of the present study were to compare the clinical activity and toxicity of a combination of FU plus LV with or without IFN α-2c, the endpoint of interest being tumour response and overall survival.

PATIENTS AND METHODS

Eligibility of patients

Seven institutions participated in this multicentre, open randomised trial. Patients (pts) were required to have histologically confirmed recurrent or metastatic colorectal adenocarcinoma, progressive and measurable disease, no brain metastases, no previous systemic treatment for advanced disease, age <75 years, life expectancy >3 months. World Health Organisation (WHO) performance status of ≤ 2, adequate bone marrow [whole blood cells (WBC) $> 3\times10^9$/l and platelets $> 100\times10^9$/l], renal and liver function (serum creatinine concentration <1.5 mg/dl, bilirubin level <2 mg/dl), no concurrent uncontrolled illness (ischaemic heart disease, history of thromboembolic disease or history of other malignancy and no active infection. All patients gave their informed consent. Adjuvant chemoradiotherapy or radiotherapy was allowed, if terminated more than 6 months before this study. The study was approved by the local ethics committees of the participating centres.

Treatment

Each complete course of therapy consisted of 8 weeks of treatment followed by a 2-week rest period. All patients received a loading dose of LV, 100 mg/m^2, given over 15 min intravenously (i.v.), followed immediately by 5-FU, 500 mg/m^2, administered as a 1-h infusion, daily for 4 consecutive days, followed by weekly infusions of the same dose (starting on day 12) until week 8. Patients allocated to IFN received the same schedule and dose of 5-FU/LV, but in addition IFN α-2c (Berofor®; Boehringer-Ingelheim, Vienna, Austria), 30 μg, corresponding to 7×10^6 IU. IFN was self-administered subcutanously (s.c.) three times weekly. Repeated cycles of therapy were administered at 10-week intervals.

Patients who achieved a complete response (CR) received one additional cycle of therapy; those with a partial response (PR) or no change (NC) were treated with two or four further courses, respectively. Patients with progressive disease (PD) were withdrawn from the study. Dose modifications were made based on WHO toxicity criteria [21]. Doses of 5-FU were reduced by 25% for grade 2 myelosuppression; for patients with grade 3 haematological toxicity, stomatitis or diarrhoea, 5-FU was withheld until recovery and than resumed at 75% of the full dose. The IFN dose was reduced by 50% for all grade 3 toxicities, with the exception of ≥ 3 neurological toxicity, in which case IFN was discontinued. Patients with grade 4 toxicities had to be withdrawn from the study.

Response criteria

Patients who completed one course of therapy were assessable for response. Tumour measurement was performed before the initiation of treatment and after each cycle of therapy. It included ultrasonography, X-ray, computed tomography and determination of carcinoembryonic antigen (CEA) or CA 19-9. Tumour measurement followed WHO criteria [21]. CR was defined as the disappearance of all dedectable disease; PR was defined as a reduction of at least 50% of the sum of the products of the two greatest diameters of all measurable lesions, with no new lesions appearing and none progressing, for ≥ 4 weeks. NC was defined as an increase of less than 25% or a decrease of less than 50%. PD was defined as either an increase of $\geq 25\%$ or the appearence of new lesions. Time to progression and duration of PR were determined from the start of therapy to the first documentation of PD, the duration of CR was calculated from the onset of CR to the first documentation of PD. Survival time was measured from the start of treatment until death, or to the last follow-up.

Statistical considerations

Calculation of the sample size was carried out using the program STPAN [22]. The study was designed to detect an improvement in response rates from 30 to 45%; the difference between treatment groups should be detected with a probability of 77%. Using a level of significance of 0.05, 240 patients were required. Differences in tumour response rates and treatment toxicities in the two arms were analysed by means of chi-square tests. Time to progression, response duration and survival probabilities were examined using the method of Kaplan and Meier [23]. For comparison of treatment arms the Mantel test was used; the influence of therapy and other relevant factors on overall survival was analysed by means of Cox regression [24]. Analysis of haematotoxicity was performed using *t*-tests; simultaneous analysis of haematotoxicities was performed by means of multivariate repeated-measures analysis of variance. All mentioned *P* values are the results of two-sided tests; values <0.05 were considered statistically significant.

RESULTS

Patient accrual and demography

From April 1991 to April 1995 a total of 269 patients was enrolled onto this multicentre phase III trial (Figure 1). 26 patients were withdrawn from the study due to ineligibility; since they did not fulfill entry criteria ($n=20$) or refused therapy ($n=6$). The two treatment groups were well matched for pretreatment characteristics (Table 1). Overall, 70% of patients had been diagnosed with colorectal cancer within the previous 6 months, more than two-thirds of patients had liver metastases and no patient had received prior chemotherapy for advanced disease.

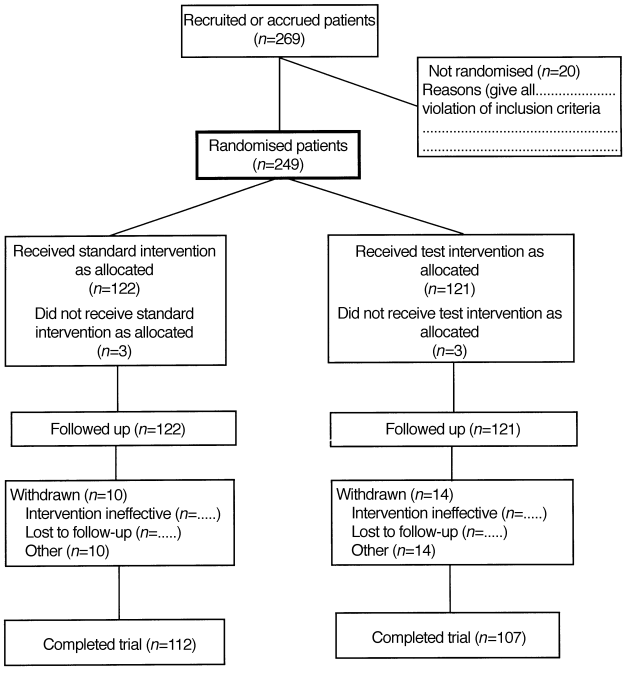

Figure 1. Flow chart of the progress of patients through the trial. (Adapted from Begg C, Cho M, Eastwood S, *et al.* Improving the quality of reporting of randomised controlled trials: the CONSORT statement. *JAMA* 1996, 276, 637–639.)

Objective tumour response

In 12 patients in the 5-FU/LV arm and 12 patients in the 5-FU/LV plus IFN arm, response could not be assessed due to major protocol violations ($n = 3$), refusal of further treatment ($n = 8$), unacceptable toxicity ($n = 6$), other intercurrent medical problems ($n = 5$) and insufficient data ($n = 2$). However, all of these patients were included in the survival analysis on an intention-to-treat basis.

Table 2 lists tumour responses according to treatment of 219 evaluable patients with measurable disease. The overall response rate (CR + PR) was 25% in the 5-FU/LV arm [95% confidence interval (CI) 17.30–34.07%] and 36% (95% CI 26.50–45.35%) in the 5-FU/LV/IFN group, but the difference was not statistically significant ($P = 0.09$). NC was observed in 43% and 48% of patients allocated to 5-FU/LV with or without IFN, respectively. The frequency of response by primary tumour or site of metastases did not differ between the two treatment regimens (data not shown). Evaluation of the response rates in view of delivered dose intensity of IFN showed no significant difference between patients receiving the full IFN dose versus those with any dose reduction during first two treatment cycles (data not shown).

The median duration of objective response was found to be 12.1 months (95% CI 7.37–not defined) and 8.4 months (95% CI 7.24–not defined) for patients allocated to 5-FU/LV and 5-FU/LV and IFN, respectively. This difference was not statistically significant ($P = 0.740$). The median time to progression was 4.9 months (95% CI 4.24–6.12) in the 5-FU/LV arm and 6.5 months (95% CI, 5.26–7.47) in the 5-FU/LV plus IFN group ($P = 0.432$).

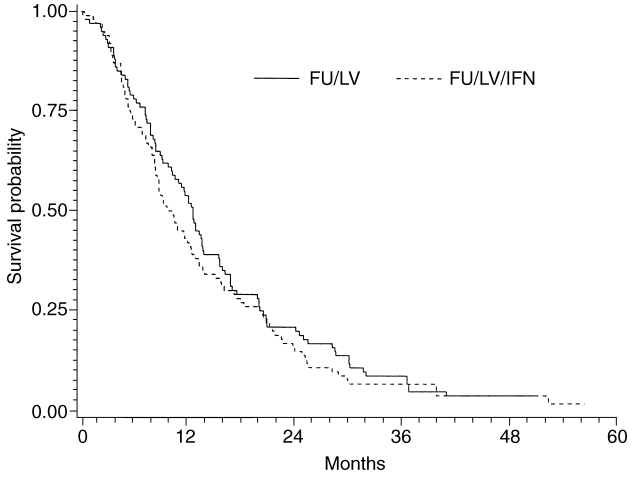

Figure 2. Overall survival by allocated treatment.

Table 1. Pretreatment characteristics

Characteristic	5-FU/LV *n* (%)	5-FU/LV/IFN *n* (%)
Number of patients	124 (100)	119 (100)
Sex		
Male	76 (61)	69 (58)
Female	48 (39)	50 (42)
Median age (range)	61 (34–76 years)	62 (31–76 years)
Performance status		
WHO 0	56 (45)	47 (39)
WHO 1	56 (45)	61 (51)
WHO 2	12 (10)	11 (9)
Primary site		
Colon	75 (60)	73 (61)
Rectum	49 (40)	46 (39)
Weight loss		
$< 10\%$	95 (77)	89 (75)
$\geq 10\%$	29 (23)	30 (25)
Metastatic sites		
Liver only	52 (42)	48 (40)
Liver and other	37 (30)	44 (37)
Lung only	9 (7)	7 (6)
Locoregional	17 (14)	13 (11)
Other	9 (7)	7 (6)
Prior radiotherapy	6 (5)	6 (5)
Prior (adjuvant) chemotherapy	7 (6)	5 (4)
Disease-free interval		
< 6 months	87 (70)	83 (70)
≥ 6 months	37 (30)	36 (30)

5-FU, 5-fluorouracil; LV, leucovorin; IFN, interferon-α; WHO, World Health Organisation.

Table 2. Response rates by treatment regimen

Response	5-FU/LV *n* (%)	5-FU/LV/IFN *n* (%)
Evaluable patients	112 (100)	107 (110)
CR	4 (4)	5 (5)
PR	24 (21)	33 (31)
CR + PR	28 (25)	38 (36)
NC	54 (48)	46 (43)
PD	30 (27)	23 (21)

5-FU, 5-fluorouracil LV, leucovorin; IFN, interferon-α; CR, complete response; PR, partial response; NC, no change; PD, progressive disease.

Table 3. Toxicity

Toxicity	WHO grade	5-FU/LV (%)	5-FU/LV/IFN (%)	*P*-value
Evaluable patients		100.0	100.0	
Leucopenia	1–4	20.3	56.3	< 0.001
	3–4	0.0	1.7	0.241
Thrombocytopenia	1–4	3.3	19.3	< 0.001
	3–4	0.8	1.7	0.620
Anaemia	1–4	25.2	44.4	0.002
	3–4	2.4	5.1	0.324
Nausea	1–4	51.6	62.7	0.083
	3–4	0.8	2.5	0.364
Vomiting	1–4	11.5	29.7	< 0.001
	3–4	0.8	1.7	0.617
Stomatitis	1–4	18.0	33.1	0.008
	3–4	4.9	1.7	0.281
Diarrhoea	1–4	43.4	47.5	0.532
	3–4	8.2	8.5	1.0
Alopecia	1–4	24.6	33.1	0.148
	3–4	1.6	3.4	0.441
Fever	1–4	9.8	55.1	< 0.001
	3–4	0.0	0.0	
Flu-like syndrome, fatigue	1–4	55.7	82.2	< 0.001
	3–4	1.6	3.4	0.441

WHO, World Health Organisation; 5-FU, 5-fluorouracil; LV leucovonin; IFN-interferon-α.

Survival

Figure 2 shows the overall survival of patients according to treatment group. The median survival of patients receiving 5-FU/LV was 12.6 months (95% CI 10.59–13.55), and was 10.0 months (95% CI 8.42–12.24) for those receiving 5-FU/LV plus IFN, the difference being statistically not significant ($P = 0.312$). The influence of other prognostic covariates predictive for survival was analysed by Cox regression. Only performance status was significantly ($P = 0.004$) associated with patient survival, whereas no influence was found for weight loss, site of primary or metastatic disease, disease-free survival, or treatment centre (data not shown).

Toxicity

Toxocity was assessed in 243 patients. In general, toxicity was mild, mainly restricted to WHO grades 1 and 2 (Table 3). Grade 3 or 4 leucopenia and thrombocytopenia occurred in only 0.8% and 4.6% of patients treated, respectively. Overall, the rate of moderate or severe nausea, vomiting, stomatitis and diarrhoea was 2.1%, 1.6%, 3.3%, and 8.7%, respectively, and there was no difference between treatment groups. There were no treatment-related deaths.

As documented in Table 3, haematological toxicity associated with 5-FU/LV was significantly less than that observed with 5-FU/LV plus IFN. There was a significantly higher degree of vomiting, stomatitis, fever, and flu-like symptoms in the treatment arm including IFN. The IFN dose had to be reduced at least once during the first two cycles of treatment in 45% of patients, primarily because of fever and lethargy.

DISCUSSION

In this prospective randomised trial, a non-significant higher response rate could be demonstrated with double-modulated 5-FU compared with 5-FU/LV alone, with no improvement of response duration, time to progression or survival. Subgroup analysis failed to demonstrate any therapeutic advantage of 5-FU/LV/IFN influenced by prognostic covariates. Unfortunately, significantly increased toxicity, particularly haematological and gastrointestinal toxicity, occurred with this regimen compared with the group not given IFN.

These results are disappointing in view of the promising data of unrandomised phase II studies on combined 5-FU plus IFN [6, 12–14] or 5-FU/LV plus IFN [26, 27]. Altogether, there have been 11 randomised trials, comparing 5-FU [28–33] or 5-FU/LV [34–38] with or without IFN. Only one study reported on a small but significant benefit for the IFN-containing regimen [29]. The lack of benefit has been suggested to be the result of significant dose reductions and withdrawals of patients due to adverse events in the IFN group and, therefore, a shorter time on treatment for these patients [25, 28, 31]. Although the optimum dose and schedule of IFN-α for interaction with 5-FU/LV is unknown and there may be a dose–response relationship, there was no evidence of a higher response rate with higher IFN-α dose intensity in the current study. Response rates were 38% and 34% with and without any dose reduction in the first two treatment cycles, respectively. Similar results have been reported in another randomised trial comparing 5-FU/LV and 5-FU/LV/IFN [34]. A non-significant trend towards higher response rates with a lower IFN dose intensity was observed by this group.

One critical point may relate to sequence and timing of the administration of 5-FU and IFN [34]. Preclinical studies suggest that IFN should precede the administration of 5-FU for synergistic cytotoxic effects to occur [16]. One of several possible mechanisms of enhanced cytotoxicity is assumed to be based on increased levels of the anabolic enzyme thymidine phosphorylase, induced by IFN [39]. Thymidine phosphorylase has shown to enhance the sensitivity of colon cancer cell lines to 5-FU and correlates with response to

therapy [40]. In the context of these data, it is possible that enhanced activity of 5-FU would have been better exploited by giving 5-FU concurrently or soon after the administration of IFN. This approach was recommended initially as part of the current trial but it could not be maintained in the regard to the preference of patients, administering IFN at bedtime.

Alternative explanations for the negative clinical results include experimental data on the pharmacokinetics of 5-FU biomodulated by IFN. Combining 5-FU with IFN has been demonstrated to result in a significant decrease in the plasma clearance of 5-FU and a subsequent higher exposure of the tumour to the drug [41]. This effect was abrogated when folinic acid (LV) was added to this regimen, explaining, in part, the lack of clinical benefit in the trials of 5-FU/LV with IFN versus studies with 5-FU/LV alone.

However, virtually all trials have demonstrated a significantly higher rate of adverse events and decreased quality of life in patients who received IFN. In one study, the double modulation arm was stopped early because of equal response rates but statistically increased toxicity compared to 5-FU/LV alone [36].

In conclusion, the results of this and other randomised phase III trials of 5-FU/LV modulated by IFN-α have failed to demonstrate a significant clinical benefit by adding IFN to standard chemotherapy in advanced colorectal cancer. Therefore, interferon-containing regimens cannot be recommended for routine use outside clinical investigation. However, studies of 5-FU/LV/IFN are ongoing in the adjuvant setting of patients with surgically resectable colorectal cancer.

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