13. Wulff HR, Schlichting P. *MEDSTAT Statistical Programme Version 2.1*. Copenhagen, Astra Group, 1988.
14. Lokich JJ, Zamcheck N, Löwenstein M. Sequential carcinoembryonic antigen levels in the therapy of metastatic breast cancer. *Ann Intern Med* 1978, **89**, 902–906.
15. Lokich JJ, Ellenberg S, Gerson B. Criteria for monitoring carcinoembryonic antigen: variability of sequential assays at elevated levels. *J Clin Oncol* 1984, **2**, 181–186.
16. Loprinzi CL, Tormey DC, Rasmussen P, *et al.* Prospective evaluation of carcinoembryonic antigen levels and alternating chemotherapeutic regimens in metastatic breast cancer. *J Clin Oncol* 1986, **4**, 46–56.
17. Hayes DF, Kiang T, Korzun AH, Tondini C, Wood WC, Kufe DW. CA15-3 and CEA spikes during chemotherapy for metastatic breast cancer. *Proc Am Soc Clin Oncol* 1988, **7**, 38.
18. Kiang DT, Greenberg LJ, Kennedy BJ. Tumor marker kinetics in the monitoring of breast cancer. *Cancer* 1990, **65**, 193–199.
19. Kjellgren K. The prognostic significance of consecutive determinations of serum tissue polypeptide antigen (TPA) in human breast cancer. In: Lehman FG, ed. *Carcinoembryonic Proteins*. Amsterdam, Elsevier, 1979, 607–610.
20. Lüthgens M, Schlegel G. Combined use of carcinoembryonic antigen and tissue polypeptide antigen in oncologic therapy and surveillance. *Cancer Detect Prev* 1983, **6**, 51–59.
21. Mughal A, Hortobagyi GN, Fritsche HA, *et al.* Serial plasma carcinoembryonic antigen measurements during treatment of metastatic breast cancer. *JAMA* 1983, **249**, 1881–1886.
22. Vogelsang NJ, Lange PH, Goldman A, Vessela RH, Fraley EE, Kennedy BJ. Acute changes of alfa-fetoprotein and human chorionic gonadotropin during induction chemotherapy of germ cell tumors. *Cancer Res* 1982, **42**, 4855–4861.
23. Wood CB, Ratcliffe JG, Burt RW, Malcolm AJH, Blumgart LH. The clinical significance of the pattern of elevated serum carcinoembryonic antigen (CEA) levels in recurrent colorectal cancer. *Br J Surg* 1980, **67**, 46–48.
24. Sölétormos G, Nielsen D, Schiøler V, Skovsgaard T, Dombernowsky P. Does addition of a new tumor marker CA 549 to CA 15.3 and TPA give further information in monitoring metastatic breast cancer? *Proc Am Assoc Cancer Res* 1990, **31**, 170.
25. Henderson IC, Hayes DF, Come S, Harris JR, Canellos G. New agents and new medical treatments for advanced breast cancer. *Semin Oncol* 1987, **14**, 34–64.
26. Nielsen D, Dombernowsky P, Skovsgaard T, *et al.* Epirubicin or epirubicin and vindesine in advanced breast cancer. A phase III study. *Ann Oncol* 1990, **1**, 275–280.

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Phase II Study of 5-Fluorouracil plus Leucovorin and Interferon alpha 2_b in Advanced Colorectal Cancer

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15 untreated patients with advanced measurable colorectal cancer along with other 29 patients in progression after failing first line chemotherapy with fluoropyrimidines received 5-fluorouracil (5FU) 500 mg/m² given as a weekly bolus at mid-infusion of leucovorin (LV), 500 mg/m² administered intravenously over 2 h and interferon alpha 2_b (IFN) 3×10^6 U given intramuscularly every other day. All patients had their previous chemotherapy at least 4 weeks prior to 5FU-LV-IFN. 5 patients discontinued the three drug regimen due to toxicity (intense weakness, fever and influenza-like symptoms in 4 patients; diarrhoea in 1 patient) however no grade IV toxicity was observed. IFN administration was reduced to twice/weekly in 5 patients due to influenza-like symptoms. 1 complete response and 5 partial responses were observed (13.6% response rate); the complete response was obtained in a patient resistant to 5FU: the response rate was only twice as much in untreated patients (3/15 patients, 20%) compared with that in patients previously treated with fluoropyrimidines (3/29 patients, 10.3%). Therefore, modulation of 5FU with LV plus IFN at the doses and schedules employed in this study may rarely overcome clinical resistance to the fluoropyrimidine and the addition of IFN does not appear to enhance the activity of 5FU plus LV.
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INTRODUCTION

THE LACK of active agents against colorectal cancer has long drawn the attention to the possibility of enhancing the efficacy of 5-fluorouracil (5FU) that remains the only marginally active drug available against this disease. Continuous infusion appears more active than the standard 5 days a month schedule, although efficacy was comparable for the 2 administration schedules in a recent large phase III study [1]. Several randomised comparisons demonstrated that biochemical modulation with either high dose leucovorin (LV) [2–7] or methotrexate [8] produces higher response rates than those obtained with 5FU alone. In addition, the combination of the fluoropyrimidine with immunomodulatory agents such as levamisol [9, 10] and interferon [11] are particularly interesting. The former combination has produced

such remarkable results in the adjuvant setting to be recommended as the standard regimen for patients with resected Duke's C colon carcinoma [12]. 5FU plus Interferon alpha 2_b (IFN) is a newly tested combination producing an impressive 76% response rate in one study on patients with advanced untreated colorectal cancer [11]. Different groups have tried to duplicate these data, but, although very preliminary, their results do not appear to support the initial findings [13, 14]. Toxicity was considerable in all studies. The original regimen is in fact very intensive, with high doses of IFN and a loading course of 5FU followed by high weekly doses of the fluoropyrimidine (750 mg/m^2).

We have designed a less toxic regimen that incorporates IFN at low dose in the combination 5FU plus LV and tested it as first and second line treatment in 44 patients with metastatic colorectal cancer.

PATIENTS AND METHODS

500 mg/m^2 of 5FU were administered as an intravenous bolus 1 h after the beginning of a 2 h infusion of 500 mg/m^2 of LV diluted in 250 ml of normal saline every week. This protocol is a modification of the original RPMI schedule [3] in that (a) no 2 week rest period is planned after the first 6 weeks of treatment and (b) the dose of 5FU is 500 instead of 600 mg/m^2. This treatment plan was chosen on the basis of our previous experience on 73 patients treated according to the original RPMI schedule within a randomised phase III comparison of 5FU vs. 5FU plus LV [7]. Our data showed that the actually delivered 5FU dose intensity, when combined with LV, was 480 mg/m^2/week as confirmed by other authors [15]. The dose of IFN was chosen empirically to be a total of 3×10^6 units given intramuscularly every other day, beginning the first day of 5FU plus LV administration. Treatment was continued until evidence of progression or, in case of complete response (CR), for a minimum of 2 months from establishing the CR state. No dose reduction was planned for LV. In case of cytopenia, diarrhoea or stomatitis, WHO grade II or greater, treatment was withheld until complete recovery and white blood cells (WBC) > 3000/μl and platelets > 100 000/μl.

Partial response (PR) required a 50% reduction in the sum of the products of the maximum perpendicular tumour diameters lasting at least 8 weeks. No new areas of malignant disease should have appeared during the period of tumour regression.

Complete response required total disappearance of all tumours initially observed. Stable disease was defined as less than 50% reduction or less than 25% increase in the sum of the products of maximum perpendicular tumour diameters with no new lesions appearing during at least 2 months of treatment.

The studies on untreated and pretreated patients, were designed according to Simon's two-stage optimal design [16]. For untreated patients, P_0 and P_1 were set at 20% and 40%, respectively, while for pretreated patients a P_0 of 10% and a P_1 of 30% were chosen. Setting alpha error at 0.05 and beta error at 0.20, the combination had to be rejected for untreated patients if 3 or fewer responses were observed among the first 13 patients or if 12 or fewer responses were observed in 43 patients. For previously treated patients, the corresponding stopping rules were < 1 response/10 patients and < 5 responses/29 patients.

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Table 1. Patients' characteristics

Total eligible patients	44
Males	34
Females	10
Median age, years	61
Median ECOG performance status	1
Site of primary: colon	33
Site of primary: rectum	11
Site of metastatic disease	
Liver	27
Pelvis	12
Lung	8
Peritoneum	8
Lymph nodes	3
Bones	3
Prior treatment with 5FU	12
Prior treatment with 5FU + LV	17
No previous treatment	15

RESULTS

Table 1 shows the patients' characteristics along with their first-line treatment. 15 of 44 patients were previously untreated while two thirds of the rest failed prior treatment with 5FU plus LV (600 mg/m^2 given as a weekly intravenous bolus 1 h after the beginning of a 2 h infusion of LV, at the dose of 500 mg/m^2). The remaining patients received 5FU alone, 450 mg/m^2 daily for 5 consecutive days every 4 weeks. There was a minimum of 4 weeks between the end of the first line treatment and the beginning of 5FU plus LV plus IFN.

1 patient was lost to follow up, 3 suffered from disease progression within the first month of therapy (early progression) and one refused treatment continuation for psychological reasons. A total of 44 patients are evaluable for toxicity. No toxic deaths occurred and no grade IV toxicity was reported. However 5 patients discontinued treatment for toxicity: these cases are accounted for by intense weakness, fever and infuenza-like symptoms attributable to IFN in 4 patients and diarrhoea in 1 patient. Mucositis and myelotoxicity were common but mild (Table 2). Conjunctivitis was observed in 5 patients receiving more than 2 months of continuous treatment. The severity of this side effect was not graded, but it was reversible in all patients upon treatment discontinuation.

According to our stopping rules [16], the study on untreated patients was interrupted at the first stage (after the first 15

Table 2. Toxicity according to the WHO criteria

	Grade I	Grade II	Grade III
Leukopenia	5 (11)	16 (36)	1 (2.5)
Thrombocytopenia	5 (11)	2 (5)	0 (0)
Nausea/vomiting	6 (15)	1 (2.5)	2 (5)
Diarrhoea	4 (9)	7 (16)	1 (2.5)
Mucositis	0 (0)	2 (5)	0 (0)

n = 44(%).
Influenza-like symptoms with fever was observed in 22 patients, marked asthenia in 10 patients and conjunctivitis in 5 patients. No grade IV toxicity was observed.

patients), since only three responses were observed, while our investigation on previously treated patients went on to the second stage (29 patients).

On the whole, one complete response and 5 partial responses out of 44 patients were observed (13.6% response rate), lasting 11 months and 2,3,3,5 and 6 months, respectively. The complete response was obtained in a patient resistant to 5FU and the response rate was only twice as much in untreated patients (20%) compared with patients previously treated with fluoropyrimidines (10.3%). Therefore, modulation of 5FU with LV plus IFN may rarely overcome clinical resistance to the fluoropyrimidine but the addition of IFN at this dose does not appear to enhance the activity of 5FU plus LV, given by this schedule, at these doses.

DISCUSSION

The encouraging preliminary results recently reported with the 5FU+IFN combination [11] opened new hopes for the treatment of colorectal cancer.

Several biochemical reasons have been proposed to explain the antitumour activity enhancement of 5FU by IFN [17] and these theories allow speculations to further potentiate the therapeutic effect of 5FU. Such could be the case for LV. For example, the stabilisation of the ternary complex thymidylate synthase—5 fluorodeoxyuridylate—5–10 methylenetetrahydrofolate by high dose LV may be particularly effective in the presence of IFN. This cytokine has in fact been demonstrated to interfere with thymidine salvage [18] and to prevent thymidylate synthase gene amplification occurring shortly after 5FU treatment in cancer cells *in vitro* [19].

Despite these rationales, the initial experience with this combination was not confirmed in subsequent trials employing the same dose and schedules of the two agents [13, 14]. Furthermore two preliminary reports on the combination 5FU plus LV plus IFN, with 5FU given by the 5 days a month schedule and IFN given only for 7 days, describe a 23% [20] and 25% [21] response rate to this three drug combination. Our results obtained with the weekly schedule of the fluoropyrimidine are in keeping with these latter experiences. The lack of life threatening toxicity using low doses IFN and the weekly schedule of 5FU plus LV administration contrasts with the severe toxicity reported using the loading course of 5FU plus high dose IFN [14]. It is thus possible that the antitumour enhancement of 5FU activity occurs only at the maximum tolerated doses of both 5FU and IFN. The addition of LV does not allow such intense course of 5FU to be delivered to patients. This may in turn compromise the value of combining 5FU plus IFN.

1. Lokich JJ, Ahlgren JD, Gullo JJ, *et al.* A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: a mid-Atlantic oncology program study. *J Clin Oncol* 1989, 7, 425–432.
2. Poon MA, O'Connell MJ, Moertel CG, *et al.* Biochemical modulation of fluorouracil: evidence of significant improvement of survival and quality of life in patients with advanced colorectal carcinoma. *J Clin Oncol* 1989, 7, 1407–1418.
3. Petrelli N, Herrera L, Rustum Y, *et al.* A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma. *J Clin Oncol* 1987, 5, 1559–1565.
4. Petrelli N, Douglass HO Jr, Herrera L, *et al.* The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: a prospective randomized phase III trial. *J Clin Oncol* 1989, 7, 1419–1426.
5. Doroshow JH, Bertrand M, Multhauf P, *et al.* Prospective randomized trial comparing 5-FU versus 5-FU and high dose folinic acid for treatment of advanced colorectal cancer. *Proc Amer Soc Clin Oncol* 1987, 6, 96.
6. Erlichman C, Fine S, Wong A, *et al.* A randomized trial of fluorouracil and folinic acid in patients with metastatic colorectal carcinoma. *J Clin Oncol* 1988, 6, 469–475.
7. Sobrero A, Nobile MT, Vidili MG, *et al.* Randomized trial of maximum tolerated dose of weekly bolus 5-fluorouracil alone or combined with high-dose leucovorin in advanced measurable colorectal cancer. In: Pinedo HM, Rustum YM, eds. *Leucovorin Modulation of Fluoropyrimidines: a New Frontier in Cancer Chemotherapy*. London, Royal Society of Medicine Services, 1990.
8. Nordic Gastrointestinal Tumor Adjuvant Therapy Group: Superiority of sequential methotrexate, fluorouracil, and leucovorin to fluorouracil alone in advanced symptomatic colorectal carcinoma: a randomized trial. *J Clin Oncol* 1989, 7, 1437–1446.
9. Laurie JA, Moertel CG, Fleming TR, *et al.* Surgical adjuvant therapy of large bowel carcinoma: an evaluation of levamisole and the combination of levamisole and fluorouracil: The North Central Cancer Treatment Group and the Mayo Clinic. *J Clin Oncol* 1989, 7, 1447–1456.
10. Moertel CG, Fleming TR, Macdonald JS, *et al.* Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. *N Engl J Med* 1990, 321, 352–358.
11. Wadler S, Schwartz EL, Goldman M, *et al.* Fluorouracil and recombinant alfa-2a-Interferon: an active regimen against advanced colorectal carcinoma. *J Clin Oncol* 1989, 7, 1769–1775.
12. Moertel CG. Consensus conference on colorectal cancer. *Educational Booklet of ASCO* 1990, 9, 223–229.
13. Kemeny N, Kelsen D, Derby S, *et al.* Combination 5-fluorouracil (FU) and recombinant alpha Interferon (IFN) in advanced colorectal carcinoma: activity but significant toxicity. *ASCO* 1990, 109.
14. Pazdur R, Ajani JA, Patt YZ. Phase II study of Fluorouracil and recombinant Interferon alfa-2a in previously untreated advanced colorectal carcinoma. *J Clin Oncol* 1990, 8, 2027–2031.
15. Arbuck S. Overview of clinical trials using 5-fluorouracil and leucovorin for the treatment of colorectal cancer. *Cancer* 1989, 63, 1036–1044.
16. Simon R. Optimal two-stage designs for phase II clinical trials. *Controlled Clin Trials* 1989, 10, 1–10.
17. Wadler S, Schwartz EL. Antineoplastic activity of the combination of Interferon and cytotoxic agents against experimental and human malignancies: a review. *Cancer Res* 1990, 50, 3473–3486.
18. Divizia M, Baglioni C. Lack of correlation between thymidine kinase activity and the antiviral or antiproliferative response to Interferon. *Virology* 1984, 133, 216–219.
19. Allegra C. Clinical drug resistance. *Educational booklet of ASCO* 1990, 197–229.
20. Yalavarthi P, Murthy S, Budd GT, *et al.* Phase I/II trial of 5-FU, leucovorin (LV) and rHuIFN alpha-2a in metastatic colorectal cancer: possible decrease in myelosuppression. *ASCO* 1990, 9, 125.
21. Grem JL, Allegra CJ, McAtee N, *et al.* Phase I study of interferon alpha-2a (IFN-A), 5-fluorouracil (5-FU) and high dose leucovorin (LV) in metastatic gastrointestinal cancer. *ASCO* 1990, 9, 70.

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