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Subcutaneous low-dose interleukin-2 and intravenous 5-fluorouracil plus high-dose levofolinic acid as salvage treatment for metastatic colorectal carcinoma

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Thirty-three consecutive patients with recurrent and/or metastatic colorectal carcinoma (CRC) refractory to previous chemotherapy have been treated with levofolinic acid (l-FA) 100 mg/m^2 i.v. over 1 h infusion followed by 5-fluorouracil (5-FU) 600 mg/m^2 i.v. bolus every week for 6 weeks followed by a 2 week interval. Patients also received rIL-2 s.c. at 3 MU daily from day 1 to day 5 of each week for at least four consecutive weeks per cycle. Enrolled patients were divided in two groups: (i) group 1 including patients with progressive tumor refractory to chemotherapy with l-FA + 5-FU given for metastatic disease and (ii) group 2 consisting of patients with diagnosis of metastatic disease within 3 months from the completion of adjuvant chemotherapy with 5-FU + levamisole (LEV) after primary surgery. No objective response was observed in the group of 11 patients with CRC resistant to previous l-FA + 5-FU, thus no further patient with progressive disease after l-FA + 5-FU was included in the trial. In the group of patients pretreated with 5-FU + LEV, four patients experienced a PR with a mean duration of 7.3 months (range ≥4.0–8.6) for an overall response rate of 18% (95% CI 12–26%). A stabilization of disease was observed in five cases (23%) with a mean duration of ≥5.6 months (range ≥2.0–7.0). The remaining 13 patients progressed. No complete responses were achieved. The mean overall survival was ≥9.5 months (range ≥2.0–14.0). Toxicity was generally mild. This study demonstrates that the combination of s.c. rIL-2 and intravenous 5-FU + l-FA on a weekly schedule may be safely given to patients with metastatic CRC on an outpatients basis. The addition of low-dose rIL-2 does not modify the toxicity profile of 5-FU + l-FA, even if IL-2-related side-effects such as systemic symptoms or cardiac abnormalities are to be expected. The clinical activity of the combination is not good, at least in terms of response rate, even if the duration of partial responses may suggest to test rIL-2 in a prospective study with response duration and overall survival as the final endpoints.

Key words: 5-Fluorouracil, interleukin-2, levofolinic acid.

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Introduction

In spite of recent progress achieved in the management of gastro-intestinal malignancies, clinical results of palliative chemotherapy for recurrent and/or metastatic colorectal carcinoma (CRC) are still largely unsatisfactory.[1] Such dismal results have prompted several investigators to explore the role of cytokine therapy in the treatment of advanced malignant tumors.[2]

The use of cytokines in clinical oncology as a part of medical treatment for advanced CRC is not new. In 1989, based on pharmacokinetic data and the remarkable preclinical evidence of a synergism between 5-fluorouracil (5-FU) and interferons (IFNs), Wadler *et al.* reported the use of IFN-α2a in combination with 5-FU in a series of 32 previously untreated patients with an overall response rate of 63%.[3] Further confirmatory studies have demonstrated that this combination is certainly active against advanced CRC even though toxicity was significant and median overall response rate was lower that that previously reported.[4,5]

Recombinant interleukin-2 (rIL-2) has recently become commercially available and has been successfully employed in combination with various cytotoxic agents in the management of advanced melanoma[6,7] and renal cell carcinoma.[8,9] This pleiotrophic cytokine, mainly generated by T helper lymphocytes in response to a variety of mitogens, may play an indirect antineoplastic activity through the activation of natural killer cells, macrophages or via the regulation of production of lymphokines such as IFNs and tumor necrosis factor.[10,11,12,13] In 1987, Rosenberg *et al.*[14] reported that rIL-2 alone or in combination with LAK cells may obtain a nearly 17% overall response rate, and both preclinical[15,16] and

clinical[17] data have suggested a possible increase of rIL-2 activity by some chemotherapeutic drugs. Recombinant IL-2, given as i.v. infusion, has been tested with 5-FU alone or in combination with IFN-α against advanced metastatic CRC, achieving a 30% overall response rate.[18,19] These results, as well as the occurrence of significative toxicity, did not induce oncologists to further explore this field.

The use of s.c. rIL-2 has considerably reduced the incidence and severity of rIL-2-related side-effects while retaining the same antineoplastic activity.[20,21] Moreover, recent studies on the physiology of the rIL-2 receptor and its expression on human T lymphocytes have supported the use of low-dose rIL-2 in the clinical management of human malignant neoplasms.[22]

5-FU remains the most active chemotherapeutic agent against several human gastrointestinal malignances.[1] 5-FU antineoplastic activity against CRC may be significantly enhanced by the addition of exogenous levofolates (l-FA) which strengthen the binding of FdUMP to the 5-FU target enzyme thymidylate synthetase inside neoplastic cells.[23] To date, 5-FU in modulation with l-FA is considered as one of the 'standard' chemotherapeutic regimens for the palliation of recurrent and/or metastatic CRC.[24]

In this paper we report the results of a phase II study of rIL-2 in combination with 5-FU and l-FA on a weekly schedule as salvage chemotherapy for patients with metastatic CRC resistant to previous 5-FU based chemotherapy.

Patients and methods

Thirty-three patients with recurrent and/or metastatic CRC refractory to previous chemotherapy with 5-FU + l-FA or levamisole (LEV) were enrolled into the study. Entry criteria included written informed consent and the presence of measurable disease according to the WHO criteria.[25] Absence of previous chemotherapeutic treatments as adjuvant to surgery or for advanced disease was not a prerequisite. Other elegibility criteria were: absence of severe, uncontrolled cardiovascular, renal, metabolic and neurological diseases, age >18 but ≤ 75 years, life-expectancy >2 months, ECOG performance status ≤ 2; absence of previous immunotherapy with IFNs or rIL-2; WBC $\geq 4000/mm^3$, PTL $\geq 120\,000/mm^2$, Hb ≥ 10 g%; serum total bilirubin <2 mg%; serum creatinine ≤ 1.2 mg%, and BUN ≤ 50 mg%; geographical accessibility in order to guarantee correct follow-up.

Consecutively enrolled patients were divided in two groups: (i) group 1 including patients with progressive tumor refractory to chemotherapy with l-FA + 5-FU given for metastatic disease and (ii) group 2 consisting of patients with diagnosis of metastatic disease within 3 months from the completion of adjuvant chemotherapy with 5-FU + LEV after primary surgery. The trial was designed according to the two-step model of Gehan[26] for the design of clinical trials. Thus for an expected therapeutic effectiveness of 25% and assuming a rejection error of 5%, 11 patients per group had to be enrolled in the first part of the study. If all patients in each group failed to respond no further patients were enrolled in that group. On the contrary, if at least one success was observed in any of the groups, then the trial was continued with 10% required standard error.

The treatment plan was: l-FA 100 mg/m^2 i.v. over 1 h infusion followed by 5-FU 600 mg/m^2 i.v. bolus every week for 6 weeks followed by a 2 week interval; rIL-2 was given s.c. at 3 MU daily from day 1 to day 5 of each week for at least four consecutive weeks per cycle. The chemotherapy schedule was chosen accordingly to our previous experience with a weekly administration.[27] Chemotherapy was delayed by 1 week or until recovery if WBC $<3000/mm^3$ and/or PTL $<100\,000/mm^3$. If at nadir WBC $<2000/mm^3$ and/or PTL $<60\,000/$ mm^3, 5-FU dosage was reduced by one third. In case of severe diarrhea (at least grade 3) or stomatitis, chemotherapy was delayed until recovery and then 5-FU dosage reduced by one third. If greater than grade 2 cardiovascular toxicity, IL-2 was stopped. Six weeks of therapy plus 2 weeks of rest were considered as one cycle. In case of partial objective response or stabilization, chemo-immunotherapy was continued until progression or unacceptable toxicity. In case of progressive disease, treatment was definitively withheld.

At entry all patients were staged with physical examination, chest X-ray, abdominal sonogram, CT scan of the abdomen, ECG, routine hematological tests and serum chemistry, CEA and Ca 19-9. MNR and endoscopy were employed as needed. All patients were staged after at least 8 weeks of therapy employing most of the above-reported techniques.

Responses to chemo-immunotherapy were reported accordingly to the WHO criteria.[25] Briefly, a complete response was defined as the total disappearance of all signs and symptoms of disease for at least 4 weeks; a partial response (PR) was defined as a $\geq 50\%$ reduction in the sum of the

products of the largest perpendicular diameters of all measurable lesions for a least 4 weeks without the appearance of any other lesions. Stable disease (SD) was defined as a $< 50\%$ decrease or $< 25\%$ increase in the size of neoplastic lesions. Progressive disease (PD) indicated a $> 25\%$ increase in the size of pre-existing lesions or the appearance of any new tumoral deposit. The length of both objective responses and survival were calculated from the first day of chemotherapy until progressive disease or death occurred. Results of chemo-immunotherapy are reported as relative rates with their 95% confidence limits (95% CI). Survival analysis was carried out according to the Kaplan–Meyer product limit analysis.

Results

Patient population

The main clinical and demographic characteristics of enrolled patients are depicted in Table 1. Thirty-three patients with advanced CRC recurrent after previous 5-FU-based chemotherapy were enrolled in this phase II trial. There were 25 males and eight females with a mean age of 62 years and a mean performance status (PS) of 1 according to the ECOG criteria. All patients had undergone previous surgery for colon (82%) or rectal (18%) adenocarcinoma. Five patients (15%) had previous external beam radiotherapy adjuvant to rectal amputation. Eleven patients had progressive disease during or shortly after chemotherapy with l-FA plus 5-FU given for advanced disease and 22 patients showed metastatic disease within 3 months from the completion of adjuvant immuno-chemotherapy with 5-FU + LEV given for 1 year. Seven patients had a well-differentiated adenocarcinoma, 12 patients had a moderately differentiated tumor and 14 patients a poorly differentiated neoplasm. Site of tumoral deposits included liver, lungs, lymph nodes and the abdomen.

Table 1. Patients characteristics

No. of enrolled patients	33 (100%)
Age	
mean	62
range	46–75
Sex	
males	25 (76%)
females	08 (24%)
PS	
mean	1
range	0–2
Site of primary tumor	
colon	27 (82%)
rectum	6 (18%)
Histology	
well-differentiated adenocarcinoma	7 (21%)
moderately differentiated adenocarcinoma	12 (37%)
poorly differentiated adenocarcinoma	14 (42%)
Previous treatments	
surgery	32 (100%)
radiotherapy	5 (15%)
chemotherapy	32 (100%)
adjuvant	11 (33%)
advanced	22 (67%)
Sites of disease	
liver	22 (67%)
lymph nodes	10 (30%)
lung	4 (12%)
abdominal mass	12 (36%)

Antineoplastic activity

No objective response was observed in the group of 11 patients with CRC resistant to previous l-FA + 5-FU. Thus, according to Gehan's criteria, no further patient with PD after l-FA + 5-FU was included into the trial. On the other hand, two partial responses were achieved in the group of 11 patients refractory to adjuvant 5-FU + LEV treated with IL-2 + l-FA + 5-FU. Thus a further 12 patients were enrolled in the trial. Among these patients two other PRs were observed.

Overall, in the group of patients pretreated with 5-FU + LEV, four patients experienced a PR with a mean duration of 7.3 months (range ≥ 4.0–8.6) for an overall response rate of 18% (95% CI 12–26%). A stabilization of disease was observed in five cases (23%) with a mean duration of ≥ 5.6 months (range ≥ 2.0–7.0). The remaining 13 patients progressed. No CRs responses were achieved. Among responding patients, two had liver metastases, one had lung metastasis from rectal cancer and one had an abdominal recurrence of left colon adenocarcinoma. The mean overall survival was ≥ 9.5 months (range ≥ 2.0–14.0).

Toxicity

Type and degree of observed side-effects are reported in Table 2. IL-2 did not seem to amplify predictable toxicity of l-FA + 5-FU. Over a total of 57 cycles (1.7 cycles/patients) for a total of 342 weeks of treatment, gastrointestinal toxicity was the most frequent complaint. Grade 1–2 diarrhea was recor-

Table 2. Toxicity

Type of toxicity	WHO scoring system: number of patients (%)			
	Grade 1	Grade 2	Grade 3	Grade 4
Cutaneous	13 (40%)[a]	4 (12%)[b]	0	0
Fever	11 (33%)	3 (9%)	2 (6%)	0
Leukopenia	12 (36%)	5 (15%)	1 (3%)	0
PTL	6 (18%)	1 (3%)	0	0
Anemia	5 (15%)	2 (6%)	0	0
Bilirubin	4 (12%)	1 (3%)	0	0
Vomiting	8 (24%)	12 (36%)	0	0
Diarrhea	6 (18%)	5 (15%)	2 (6%)	0
Stomatitis	4 (12%)	5 (15%)	0	0
Cardiac (rhythm)	02 (06%)	0	0	0
Pulmonary	2 (06%)	1 (3%)	0	0
Alopecia	3 (09%)	1)3%)	0	0
Neurologic	2 (06%)	0	0	0

ded in 11 patients (33%) and grade 3 in two cases (6%). It should be stressed that patients with diarrhea were promptly and vigorously treated with oral loperamide and parenteral octreotide with i.v. fluid repletion. This procedure allowed good control of diarrhea in most cases. Vomiting was seldom observed: grade 2 vomiting was recorded in 12 cases (36%), but no case of grade 3 vomiting was observed. Grade 1–2 stomatitis was recorded in nine patients (27%). No case of grade 3–4 stomatitis was recorded.

Two patients had cardiac abnormalities of the rhythm and a further two patients suffered transient lethargia. Skin side-effects were quite common: 13 patients (40%) had local erythema at the IL-2 injection sites and four (12%) dry desquamation of the skin.

Discussion

Recombinant IL-2 alone[1] or in combination with IFNs[6,8,9] has shown a remarkable antineoplastic activity against several human malignances, such as melanoma and renal cell carcinoma, and has been successfully employed for the palliatiation of neoplastic effusions.[28,29] The possibility of combining IL-2 immunotherapy with several chemotherapeutic agents with interesting results has recently been suggested by several preclinical[15,16] and clinical investigations.[7,8] Some phase I and II trials on the combination of 5-FU plus l-FA and rIL-2 have been carried out with non-univocal results; the response rates ranging from 0 to 33%.[18,19,30] These results along with the significant toxicity related to the i.v. use of high-dose rIL-2 have discouraged investigators from further exploring the role of rIL-2 in the management of advanced CRC.

Our study has evaluated the clinical efficacy and toxicity of s.c. low-dose rIL-2 in combination with l-FA and 5-FU on a weekly schedule in a group of patients with metastatic CRC resistant to previous chemotherapy. The treatment was generally well tolerated and it is safely administered to neoplastic patients on an outpatient basis. However, this combination failed to show any activity in patients with metastatic CRC who showed progression during or shortly after standard chemotherapy with l-FA and 5-FU given as first line chemotherapy for advanced metastatic disease. These data suggest that rIL-2 is not able to further modulate 5-FU antineoplastic activity or to induce responses in patients with progressive CRC pretreated with 5-FU + l-FA. On the other hand, a 18% overall response rate (95% CI 12–26%) was observed in the group of 22 patients with recurrent disease during or shortly after adjuvant chemotherapy with 5-FU and LEV. These results are certainly disappointing, at least in terms of antineoplastic activity, since the observed response rate is not better than that expected from data published in medical literature with the combination of l-FA and 5-FU without rIL-2.[31,32] Thus the addition of rIL-2 seems to add toxicity to the combination of l-FA and 5-FU without any appreciable improvement in response rate. However, the mean durations of objective responses of ≥ 5.6 months is interesting since they have been recorded in patients clearly resistant to previous 5-FU-based chemotherapy. In fact, it should be noted that in previous studies with rIL-2 + 5-FU the median duration of response was 6 months.

While this trial was in progress, Heys *et al.* reported the results of a randomized study of folinic acid + 5-FU with or without rIL-2 in metastatic or unresectable CRC. Although the addition of IL-2 to the combination of l-FA and 5-FU did not increase the objective response rate, there was a trend toward an improvement in survival in patients receiving IL-2.

In conclusion, this study demonstrates that the combination of s.c. rIL-2 and i.v. 5FU + l-FA on a weekly schedule may be safely given to patients with metastatic CRC on an outpatient basis. The addition of low-dose rIL-2 does not modify the toxicity profile of 5-FU + l-FA, even if IL-2-related side-effects such as systemic symptoms or cardiac abnormalities are to be expected. The clinical activity of the combination is not good, at least in terms

of response rate, even if the duration of PRs may suggest to test rIL-2 in a prospective study with response duration and overall survival as the final endpoints.

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