Cancer Chemother Pharmacol (1997) 39: 300–306

ORIGINAL ARTICLE

James F. Cleary · Rhoda Arzoomanian · Donna Alberti
Chris Feierabend · Barry Storer · Pauline Witt
Paul Carbone · George Wilding

A phase I study of 5-fluorouracil, leucovorin and levamisole

Received: 20 October 1995 / Accepted: 16 June 1996

Abstract *Purpose*: The activity of 5-fluorouracil (5-FU) against colon cancer is enhanced by leucovorin and the combination of 5-FU and levamisole has activity in the adjuvant treatment of colonic malignancies. The combination of 5-FU with both leucovorin and levamisole may provide additional benefit in the treatment of colon cancer. *Methods*: A phase I study to assess qualitative and quantitative toxicities of this three-drug combination and to determine a dose for further phase II testing was undertaken. The role of levamisole as an immunomodulator was also assessed. *Results*: A group of 38 patients with incurable metastatic malignancies received 119 cycles of treatment at eight dose levels. 5-FU (375 mg/m^2 per day) and leucovorin (200 mg/m^2 per day) were administered intravenously (days 1–5). Levamisole was administered orally (days 1–3 and 15–17) at doses from 30 to 470 mg/m^2 per day. Patients received both 5FU/leucovorin and 5-FU/leucovorin/levamisole in random order for their initial two cycles. All subsequent treatments were with the three-drug combination. Toxicities included nausea, vomiting, stomatitis, thrombocytopenia and granulocytopenia. Diarrhea was the dose-limiting toxicity at 470 mg/m^2 per day levamisole. The addition of levamisole resulted in more toxicity than 5-FU and leucovorin alone. No clinical responses were seen with this regimen. The addition of levamisole resulted in more immunomodulation than 5-FU and leucovorin alone as evidenced by release of neopterin from monocytes. *Conclusion*: With this schedule and dose of 5-FU and leucovorin, the maximum tolerated dose of levamisole was 354 mg/m^2. However, given the lack of response and the absence of dose-dependent immunomodulation, this may not be the appropriate dose for further phase 11 studies.

Key words 5 Fluorourcil · Leucovorin · Levamisole · Phase 1 · Toxicity

Supported by NIH CM07306 and MOIRR03186

Dr Cleary is supported by a Vincent Fairfax Family Foundation Research Fellowship of the Royal Australasian College of Physicians

J. Cleary (✉) · R. Arzoomanian · D. Alberti · C. Feierabend
B. Storer · P.P. Carbone · G. Wilding
University of Wisconsin Comprehensive Cancer Center,
600 Highland Aoe., Madison, WI, 53792, USA
Tel. (608) 265-8131; Fax (608) 265-8133

P.L. Witt
Medical College of Wisconsin, Milwaukee, WI, 53226, USA

Introduction

5-Fluorouracil (5-FU) has activity against a broad spectrum of solid tumors and as a single agent has been the standard therapy of choice for metastatic colon cancer. 5-FU is a prodrug and is metabolized to fluorodeoxyuridine monophosphate (FdUMP) which is a potent inhibitor of thymidylate synthase, the enzyme that catalyzes the *de novo* production of deoxythymidine monophosphate (dTMP) [20]. Depletion of dTMP within cells leads to reduced levels of thymidine triphosphate (dTTP), one of the four deoxyribonucleotides necessary for DNA synthesis. Despite this documented mode of action, objective response rates to 5-FU in metastatic colon cancer are initially only in the range of 10–20% and little improvement in survival has been found [5, 23]. Methods to increase the antitumor activity of 5-FU have been sought.

Preclinical observations [30] that an excess of intracellular reduced folates may be necessary for optimal inhibition of thymidine synthetase have led to a number of clinical studies examining the activities of 5-FU and leucovorin [11]. Varying combinations and doses of 5-FU and leucovorin have been administered at weekly and monthly intervals and as infusions and bolus injections [1, 4, 15, 16]. It has become accepted

that while the combination of 5-FU and leucovorin may provide an increase in tumor response, it is not without increased toxicity. The role of high- versus low-dose leucovorin is still debated; high-dose leucovorin was chosen for this study as it was standard treatment at this hospital.

As many patients with metastatic colorectal cancer present initially with surgically resectable local disease, the need for adjuvant chemotherapy has been considered. One such regimen considered in this setting is the combination of 5-FU and levamisole. Levamisole has been used as an antihelminthic drug for over 20 years and might prolong the intracellular half-life of metabolites of 5-FU through inhibiton of alkaline phosphatase activity [3]. It has been studied in the adjuvant setting for breast cancer and melanoma with little success, but review of these studies would suggest that higher doses of levamisole alone would result in a better response rate. However, levamisole when combined with 5-FU has been found to be an effective agent in the adjuvant treatment of colon cancer, and a number of cooperative group studies with good statistical design and sufficient power have been reported [14, 18]. The dose and schedule of levamisole used in these studies was chosen arbitrarily from the antihelminthic experience. Based on these studies, the NCI has advised that 5-FU and levamisole combined should be considered standard therapy for Dukes C colon carcinoma [21], although this is now being brought into question [6, 7, 28].

As well as prolonging the half-life of the metabolites of 5-FU, it has also been proposed that levamisole acts as a biological response modifier [25]. Reported lymphocyte immune enhancements include enhanced lymphocyte proliferation to mitogens, enhanced E rosette formation [24] and enhanced T-cell cytotoxicity [10]. Effects on monocyte activation [17] and chemotaxis [22] have also been observed. Others have suggested that levamisole may act through modifying chemotherapy-related myelosuppression, thereby allowing increased dose intensity of therapy [27]. The role of levamisole as a biological modifier can be assessed by measuring serum concentrations of β2 microglobulin and neopterin. β2 microglobulin is noncovalently linked to MHC class 1 and is shed into serum in response to viral and bacterial infections [8, 13], malignancy [2] or exogenous cytokine therapy [26, 29]. Neopterin reflects monocyte activation and is a secreted product of the interferon-induced protein, GTP cyclohydrase [12].

To date, no studies have been performed to evaluate the efficacy of levamisole in combination with 5-FU and leucovorin. However, based on the general agreement that the combination of 5-FU and leucovorin is superior to 5-FU alone and that 5-FU and levamisole has activity in the adjuvant treatment of colon cancer, it was logical to ask if levamisole could further enhance the activity of 5-FU and leucovorin. As levamisole has shown dose-dependent effects on both alkaline phosphatase inhibition and immunomodulatory effects, the aim of this study was to establish the maximum tolerable dose (MTD) of levamisole, that could be administered for 3 days every 2 weeks with an established regimen of 5-FU and leucovorin. Toxicity attributable to levamisole was to be assessed within individual patients by administering the two- and three-drug combinations in a random order for the first two cycles. It was planned to examine the effect of levamisole as an immunomodulator by measuring the effect of the drug on monocyte function.

Materials and methods

Patient selection

Individuals with metastatic, incurable and histologically confirmed malignancy with an anticipated life span of greater than 12 weeks and who had given informed consent according to Institutional and Food and Drug Administration guidelines were eligible. Patients were required to have adequate bone marrow function (WBC $\geq$ 4000/mm^3, platelets $\geq$ 100 000/m^3), hepatic function (bilirubin $\leq$ 1.5 mg%, SGOT $<$ 3 times normal) and renal function (creatinine clearance $\geq$ 45 ml/min or serum creatinine $\leq$ 2.0 mg/dl). Chemotherapy or radiotherapy must have been administered at least 3 weeks prior to entry into this study except in the case of nitrosoureas or mitomycin where a 6-week interval was required. An ECOG performance status of 0, 1 or 2 was expected as was a life expectancy of greater than 12 weeks. Patients were ineligible if they had previously received the drug combination of 5-FU and leucovorin or if they had previously received levamisole for any reason.

Drug dose and formulation

Both 5-FU (500 mg per 10-ml ampoule) and leucovorin (50 mg per 10-ml ampoule with sodium chloride 45 mg) were provided as sterile liquids and diluted to the appropriate volumes by the Cytotoxic Pharmacy of the University of Wisconsin Hospital and Clinics. Throughout the study, the doses of 5-FU and leucovorin were constant at 375 mg/m^2 and 200 mg/m^2, respectively. Levamisole (National Cancer Institute, Bethesda, Md.) was provided as a 50-mg oral tablet and administered at a variable dose.

Drug administration and escalation

Patients were randomized to receive the drugs in the following way: *cycle 1* 5-FU/leucovorin, *cycle 2*, 5FU/leucovorin/levamisole; or *cycle 1*, 5-FU/leucovorin/levamisole, *cycle 2*, 5FU/leucovorin. All patients were to receive the three drugs for cycle 3 and beyond. On days 1–5 of the each cycle, leucovorin was administered as an intravenous infusion over 2 h at a dose of 200 mg/m^2 diluted in 250 ml 5% dextrose in water. Following the completion of the leucovorin infusion, 5-FU was administered as an intravenous bolus at a dose of 375 mg/m^2. Levamisole was administered as a single daily oral dose on days 1, 2 and 3 and again on days 15, 16 and 17 with the dose rounded to the nearest multiple of 50 mg. Each cycle was 28 days and patients must have recovered from the toxicity of the previous cycle prior to further treatment. For individual patients, in whom the preceding cycle had been associated with reversible dose-limiting toxicity (DLT), defined as $\geq$grade 2 diarrhea or $\geq$grade 3 any other toxicity, the dose of 5-FU was reduced by 25% when the patient was next treated.

Dose escalations of levamisole were from the initial dose of 30 mg/m^2 to 60, 100 and 150 mg/m^2 and then by increments of 33% . Escalation to the next dose level was carried out after at least three patients had been evaluated for the 5-FU/leucovorin/levamisole cycle. All patients who received the three-drug combination were considered evaluable for determination of the MTD. Those who received the two-drug combination only were considered evaluable for toxicity. The MTD estimation was based on the best of five schema according to DLT. When DLT occurred in two of five patients or fewer, dose escalation was continued. The occurrence of DLT in three of five patients or more designated the preceding dose as the MTD. If any grade 4 toxicity developed in more than one-third of the patients at a given level, additional patients were entered at the next lowest level to define better the incidence of DLT. If no DLT was observed at that level after a total of six patients had been entered, then entry of additional patients at the next higher dose level was again according to the best of five schema.

Patient follow-up

Patients were assessed prior to treatment and then at 4-weekly intervals. Hematological, hepatic and renal parameters were followed weekly. Clinical measurement of disease was made every 4 weeks while radiological studies to measure disease were carried out every 6–9 weeks. Standard ECOG response criteria, i.e. complete response (CR), partial response (PR), and stable and progressive disease, were used to assess response to treatment.

Activity of levamisole/Immunomodulation studies

The effect of levamisole on the immunological activity of each patient was studied. Blood was collected throughout the first two cycles on days 1 and 29 (pre treatment and 3 h posttreatment) and days 4 and 32 and the serum separated. Polymorphonuclear trafficking was assessed by β2 microglobulin concentrations, and monocyte activation by neopterin concentrations. Serum β2 microglobulin was measured in a quantitative competitive radioimmunoassay (Kabi Pharmacia Diagnostics, Uppsala, Sweden) and neopterin was assayed by radioimmunoassay (ICN Biomedicals, Costa Mesa, Calif.). Analyses of activity were by paired *t*-test after log transformation of the data. The percentage changes were computed from the antilog of the mean differences.

Results

The characteristics of the 38 patients entered into the study are shown in Table 1. Of these, 33 were evaluable for determination of MTD which required eight dose levels ranging from 30 to 470 mg/m^2 of levamisole. A total of 119 treatment cycles were evaluated for toxicity in the 38 patients (Table 2).

Toxicity

The predominant toxicities encountered in this study were stomatitis, nausea, vomiting, diarrhea and decreased white cell count (Tables 3, 4). There did not appear to be a dose-dependent relationship with these toxicities. Of the 38 patients, 27 received both 5-FU/leucovorin and 5-FU/leucovorin/levamisole in the initial two courses of treatment. There was no appreciable difference in the effect of the two treatments on the white cell count (Table 3). At the higher doses of levamisole, there appeared additional toxicity to that seen for 5-FU and leucovorin alone. At levels 6 and 7, only patients receiving the three-drug combination experienced grade 2 and greater nausea and vomiting (Table 4). Not enough patients developed stomatitis at these levels to make a comparison, although one patient who received three drugs at level 8 developed grade 4 stomatitis. Grade 4 diarrhea only occurred in three patients all of whom had received 5-FU, leucovorin and levamisole at dose levels 3, 6 and 7.

Table 1 Characteristics of patients

No. of patients	38
Sex (M:F)	26:12
Age (years)	
Median	65
Range	28–78
Initial performance status (ECOG)	
0	16
1	19
2	3
No. of patients without previous chemotherapy	21
Diagnosis	
Colorectal	15
Unknown	7
Pancreas	4
Breast	3
Lung	2
Adrenal	1
Ovary	1
Esophagus	1
Prostate	2
Stomach	1
Small bowel	1

Although not statistically significant, there were some trends in toxicity evident with increasing doses of levamisole. An increased incidence of thrombocytopenia (grades 1–2) was evident above level 4 of levamisole. Decreased absolute neutrophil counts became evident at level 5 and were seen throughout the subsequent levels. Two cases of fever were seen at levels 4 and 5, but only following the two-drug treatment. Two patients at level 8 had an infection, one receiving the two drug treatment and the other receiving 5-FU, leucovorin and levamisole. The infections were a thrombophlebitis and an infected Hickman catheter.

A grade 3 cardiac toxicity was seen at level 8. The patient was admitted to hospital on day 5 of his third cycle of treatment with atrial fibrillation which was managed with intravenous digoxin. He continued on treatment until the end of the course although the levamisole dose was reduced on day 16 due to nausea and vomiting. He was taken off study after this third cycle. This patient had not previously experienced cardiac arrhythmia but had a past history of ischemic

Table 2 Dose, dose escalation schedule and number of patients and cycles at each dose level (*DLT* dose limiting toxicity)

Level	Levamisole dose (mg/m^2/day)	No. of evaluable patients	No. of evaluable patients with DLT (%)	No. of evaluable cycles
1	30	3	0	8
2	60	4	1 (25%)	13
3	100	5	2 (40%)	21
4	150	3	0	11
5	200	5	2 (40%)	23
6	266	4	1 (25%)	15
7	354	6	1 (17%)	22
8	470	3	2 (66%)	6

Table 3 Hematological toxicity in all cycles administered

Level	Total cycles	Number of courses with grade of toxicity													
		Hemoglobin			Platelet count			Absolute neutophil count				White cell count			
		1	2	3	1	2	3	1	2	3	4	1	2	3	4
1	8 (2)[a]	–	–	–	–	1	–	–	–	–	–	–	2	–	–
2	13 (3)	–	–	–	3	1 (1)	–	–	–	–	–	3	3 (1)	1 (1)	–
3	18 (5)	–	–	–	–	–	–	–	–	–	–	3 (1)	1	1	–
4	11 (4)	–	1 (1)	–	1	–	–	–	–	–	–	3	–	–	–
5	23 (5)	5 (1)	–	–	9 (1)	3 (1)	–	–	–	–	–	4 (1)	4 (1)	3 (1)	–
6	21 (5)	–	–	–	4 (1)	–	–	–	–	2 (1)	1	–	2 (1)	1 (3)	1
7	22 (6)	1	–	–	3	–	–	3 (1)	1	1	–	3 (1)	3 (1)	1	–
8	6 (2)	–	1	–	1	–	–	2	–	1 (1)	1	3 (1)	1	–	–

[a] 5-FU and leucovorin alone

Table 4 Gastrointestinal toxicity in all cycles administered

Level	Total cycles	Number of courses with grade of toxicity													
		Nausea			Vomiting				Stomatitis				Diarrhea			
		1	2	3	1	2	3	4	1	2	3	4	1	2	3	4
1	8 (2)[a]	4 (1)	–	–	–	–	–	–	–	2 (1)	–	–	–	–	–	–
2	13 (3)	3	3 (2)	–	–	2	–	–	4 (1)	4 (1)	1 (1)	–	4 (2)	2 (1)	1	–
3	18 (5)	7 (1)	1	–	1	3 (1)	–	–	3 (1)	7 (1)	3	–	4	8 (2)	–	1
4	11 (4)	5	1 (1)	–	1	2 (1)	–	–	3 (1)	3 (1)	1	–	1	1 (1)	–	–
5	23 (5)	6 (1)	–	2	1	5 (1)			6 (1)	10 (2)	–	–	6 (2)	4 (1)	–	–
6	21 (5)	7 (2)	1	1	6	1	–	1	4 (1)	–	1 (1)	–	2 (1)	1		1
7	22 (6)	6 (1)	3	1	2	4	–	–	4	1	–	–	2	–	–	1
8	6 (2)	2	3 (1)	–	–	5 (1)	–	–	2 (1)	–	–	1	–	1	–	–

[a] 5-FU and leucovorin alone

heart disease and aortic stenosis. Skin toxicity in the form of a "rash" was seen in many patients at the lower levels of the study but was seen in only one individual above level 4. The proportion of patients with skin toxicity was equal among those receiving both the two-drug and the three-drug combinations. Grade 1 alopecia was also seen in levels 2 and 3 of the study but again not at higher levels of dose escalation.

Dose escalation ceased at level 8 (levamisole dose, 470 mg/m^2) due to the occurrence of severe toxicity. One patient developed a grade 4 absolute neutrophil count which was reversible. The second patient at that dose level developed a grade 4 stomatitis which was still present when he died on day 15 following treatment with 5-FU, leucovorin and levamisole. One further patient had no appreciable toxicity at 470 mg/m^2. A total of six patients were then entered at level 7 and only one of six patients experienced DLT, and the MTD for levamisole when combined with 5-FU (375 mg/m^2) and leucovorin (200 mg/m^2) was determined to be 354 mg/m^2.

Clinical response

No CR or PR were seen. Ten patients had stable disease and the remainder had progressive disease on treatment. Half of all colon cancer patients (7/14), nine

Table 5 Changes in plasma concentations of β2 microglobulin and neopterin 3 h after the administration of a single dose of levamisole and after the administration of 4 days of levamisole (*% difference* is difference between no levamisole treatment and levamisole treatment)

	3 hours			4 days		
	Baseline change			Baseline change		
	No levamisole	Levamisole	% Difference	No levamisole	Levamisole	% Difference
β2 microglobulin	−3.3%	−5.2%*	−1.8%	−1.0%	10.4%	11.3%
Neopterin	−28.4%	51.4%*	110.9%	−26.2	116.3%*	193.1%*

* $P \leqslant 0.05$

of whom had had no previous chemotherapy, showed stable disease.

Levamisole immunomodulation activity

Levamisole significantly enhanced the serum concentrations of neopterin from baseline at both 3 h and at 4 days (Table 5). There were no significant changes in serum concentrations of neopterin for the 5-FU and leucovorin treatment without levamisole. There was no significant increase in the serum concentration of β2 microglobulin with either the two- or three-drug treatment regimens.

Discussion

This study's dual objectives were to determine the toxicity of the combination of 5-FU, leucovorin and levamisole and to establish a dose level for further studies of this three-drug combination. An established 5-day regimen of 5-FU (375 mg/m^2 per day) and leucovorin (200 mg/m^2 per day) was used with an escalating dose of levamisole. The predominant toxicities seen were stomatitis and diarrhea. Nausea and vomiting were seen frequently, as were reductions in white cell counts but these were not associated with the development of infection. Toxicities were as previously reported for 5-FU both alone and when combined with leucovorin. In reviewing these toxicities, only those studies that used a similar 5-FU and leucovorin regimen were considered.

In a study of colorectal cancer 130 patients were randomized to receive either bolus 5-FU (370 mg/m^2) alone or bolus 5-FU following leucovorin (200 mg/m^2) on 5 consecutive days with cycles repeated every 28 days [9]. The 5-FU dose was adjusted in subsequent courses to ensure that toxicity was equivalent for both treatment arms. The predominant toxicities were mucositis and diarrhea; some neutropenia was also noted. For the first cycle of treatment, the toxicity of 5-FU and leucovorin combined was greater than for 5-FU alone. Similar toxicity was seen in another study with the administration of 21-day cycles of the varying drug combinations [23]. The same schedule of 5-FU and leucovorin was used in a study that compared six combinations of 5-FU, leucovorin and methotrexate for the treatment of colon cancer. The incidence of leucopenia (<2000/μl) was significantly less in those given 5-FU and leucovorin (19%) compared with those given 5-FU alone (48%, $P < 0.01$). The incidence of diarrhea and stomatitis was increased with two drugs but was only of statistical significance for stomatitis (58 to 76%, $P < 0.01$).

The toxicities seen in our study were also similar to the toxicities documented by Moertel [18] in a study comparing levamisole and 5-FU with levamisole alone. Of the 310 patients who received levamisole alone, 24% experienced nausea, 7% vomiting and 13% diarrhea, though most of these symptoms were not severe. The addition of 5-FU to levamisole resulted in an increase in the percentage of patients who experienced nausea (37%), diarrhea (25%), stomatitis (27%) and leucopenia (38%). Alopecia was a common toxicity with long-term administration as were neurological symptoms which were experienced by 18% (83/457) of the patients and ranged from light headedness and emotional changes to disabling cerebellar ataxia. Few neurological toxicities were seen in our study as these side effects occur more commonly when 5-FU and levamisole are administered over a longer duration than was the case here.

In our study, the addition of levamisole appeared to add to the toxicity of 5-FU and leucovorin. This difference may in fact have been underestimated if patients were not compliant with the self-administration of levamisole, a factor not assessed. It is difficult to make a statistical comparison of the toxicity differences between the two combinations at each treatment level given that fewer cycles of 5-FU and leucovorin treatment were administered. Despite these limitations, severe diarrhea was only seen in those given the three-drug combination and was more frequent with higher doses of levamisole. Dose escalation was terminated because of two cases of grade 4 diarrhea (one irreversible) in patients treated at level 8. Other gastrointestinal toxicities were more prevalent in those receiving the three-drug combination at the higher levamisole dose

Table 6 Comparison of toxicity between 5-FU, leucovorin and levamisole and 5-FU and leucovorin at the MTD

Treatment	Number of courses with grade of toxicity																
	Nausea			Vomiting		Stomatitis		Diarrhea				White cells		Platelets		ANC	
	1	2	3	1	2	1	2	1	2	3	4	1	2	1	2	1	1
5-Fluorouracil/leucovorin/ levamisole ($n = 6$)	3	1	1	–	3	1	1	1	–	–	1	2	1	2	–	1	–
5-Fluorouracil/Leucovorin ($n = 6$)	2	–	–	1	–	1	–	1	–	–	–	1	1	–	–	1	–

levels. In particular the incidence of nausea and vomiting was greater at levels 6 and 7 (Table 3). The incidence of thrombocytopenia appeared directly related to the increasing dose of levamisole as did the fall in the absolute neutrophil count seen at the higher levels of treatment (Table 4) Neither of these toxicities contributed to the determination of the MTD.

To remove the effect of multiple treatments on the occurrence of toxicity, a more detailed comparison of the relative toxicities of the two randomly assigned arms was made by considering only the two initial cycles administered to each patient at the MTD (Table 6). The number of treatments in each arm were small ($n = 6$) and sequential and again did not allow meaningful statistical review. However, the three-drug combination appeared more toxic, with more nausea, vomiting, thrombocytopenia and particularly a grade 4 diarrhea.

Review of the patient data indicated that the MTD could possibly have been established at a lower dose on the basis of two grade 4 toxicities at level 6. At the time, one of these toxicities (a grade 4 granulocytopenia) was considered a laboratory error as it was inconsistent with the previous results from the same patient on preceding days. Treatment was escalated to level 7 (354 mg/m^2 per day) and one of four patients experienced DLT prior to escalation to level 8 (470 mg/m^2 per day). On the basis of DLT and irreversible toxicity at level 8, two further patients had treatment at level 7 without evidence of DLT and level 7 was considered to be the MTD.

The lack of clinical response in this study, which incorporated a standard 5-FU and leucovorin therapy, is surprising. Of the 15 patients with colorectal cancer, 9 had received no prior chemotherapy, 4 had received bolus 5-FU injections weekly and 1 had been treated with 5-FU and mitomycin C. While the study included good performance status patients, there may well have been other disease factors which encouraged the treating physicians to recommend this phase 1 study. Many patients had stable disease for at least four cycles of treatment with one patient progressing after eight cycles of therapy.

The mode of action of levamisole was assessed through the measurement of neopterin concentrations in serum. The changes in neopterin concentrations were consistent with immunomodulation with increasing concentrations released into serum with repeated administration of levamisole over time. The absence of such changes with the use of 5-FU and leucovorin alone confirms levamisole's role in this immunomodulation, most likely monocyte activation. The concentrations of neopterin released did not increase in relation to increasing doses of levamisole.

Given the absence of increased immunomodulation with increasing dose and the lack of tumor response seen in our patients, one must question the need to escalate the dose of levamisole in this situation. It is possible that the addition of a small dose of levamisole combined with 5-FU and leucovorin may provide the same therapeutic effect. Further studies to assess the activity of the combination of 5-FU, leucovorin and levamisole in the treatment of metastatic colorectal cancer are underway and low doses of levamisole together with leucovorin and 5-FU have been chosen for these [19].

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