

# Oxaliplatin in the management of advanced colorectal cancer: Different associations and schedules

## Research Article

Francesco Recchia<sup>1,2\*</sup>, Alisia Cesta<sup>1</sup>, Gaetano Saggio<sup>1</sup>, Giampiero Candeloro<sup>1</sup>, Silvio Rea<sup>2,3</sup>

<sup>1</sup>Unità operativa di Oncologia, Ospedale Civile di Avezzano,

<sup>2</sup>Fondazione Carlo Ferri, Monterotondo, Roma,

<sup>3</sup>Oncologia chirurgica, Università degli studi de L'Aquila. Italy

\*Correspondence: Prof Francesco Recchia, MD., Via Rossetti 1, 67056 Luco dei Marsi (AQ), Italy, Tel, 0863-499250; Fax, 0863-499388; E-mail: frecchia1946@libero.it

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**Abbreviations:** 13-cis retinoic acid, (RA); 5-fluorouracil, (5FU); alanine aminotransferase, (ALT); aspartate aminotransferase, (AST); combination of CPT-11, bolus 5-FU and LV, (FOLFIRI); combination of L-OHP with LV and infusional 5-FU, (FOLFOX); confidence intervals, (CI); vascular endothelia growth factor, (VEGF); interleukin-2, (IL-2); irinotecan, (CPT-11); leucovorin, (LV); metastatic colorectal cancer, (MCC); minimal residual disease, (MRD); oxaliplatin, (L-OHP); upper limit of normal, (ULN)

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## Summary

Since the introduction into clinical practice of irinotecan (CPT-11) and oxaliplatin (L-OHP) for the treatment of metastatic colorectal cancer (MCC), we have adopted several therapeutical strategies in order to improve response rate and decrease the toxicity profile. In a phase II study the administration of L-OHP was fractionated over two days and combined with leucovorin (LV) and 5-fluorouracil (5FU) in bolus and continuous infusion (FOLFOX regimen) for the treatment of 46 patients with MCC, pretreated with CPT-11 and 5FU/LV (FOLFIRI regimen). A 32.6% response rate was obtained with an overall median survival of 12.2 months. In a further phase II study conducted with the same drug combination administered to 54 chemotherapy-naïve patients, a 50% response rate and overall survival of 19.2 months were achieved. As the FOLFOX and FOLFIRI regimens have been shown to be the two most active regimens in the treatment of MCC, an additional study was carried out in 26 untreated patients, alternating FOLFOX and FOLFIRI regimens, with the aim of further reducing toxicity and avoiding the emergence of chemotherapy resistant cellular clones. With this strategy the response rate was increased to 69%. Finally, in order to prolong responses, a selected group of 20 patients, after surgical resection of metastases from MCC and 12 courses of fractionated FOLFOX regimen, was treated with a therapy including interleukin-2 (IL-2) and 13-cis retinoic acid (RA), aimed to decrease the vascular endothelial growth factor (VEGF). After a median follow-up of 20 months, a statistically significant decrease of VEGF was observed, while median time to progression and overall survival were not reached yet (5-year survival rate 52%). In conclusion, different associations and schedules may improve the clinical outcome of patients with MCC treated with L-OHP-based chemotherapy.

## I. Introduction

Colorectal carcinoma is the second most common cancer in the Western World. In Europe 90,000 patients die from this disease each year (Black et al, 1997). In Italy each year, nearly 20,000 new patients develop colorectal cancer with a 55% mortality rate (Bonadonna et al, 1999). In the last 4 decades, 5-fluorouracil (5-FU) has been the most important drug in the treatment of metastatic

colorectal cancer (MCC). Modulation of 5-FU action with leucovorin (LV) has increased response rates in the treatment of this disease, but unfortunately the duration of response has not improved (Advanced colorectal cancer meta-analysis project, 1992). Additional progress has been accomplished by changing the schedule of 5-FU administration. In fact, 5-FU when given in a bolus preferentially inhibits RNA synthesis, while as a

continuous infusion it inhibits thymidilate synthase and DNA synthesis (Sobrero et al, 1997). Based on these principles, the introduction into clinical practice of a bimonthly schedule combining LV with 5-FU bolus and continuous infusion (the “de Gramont” regimen), has improved response rates and decreased toxicity profiles, but has still not improved survival (de Gramont et al, 1997). Irinotecan (CPT-11) and oxaliplatin (L-OHP) are 2 new drugs that have been recently introduced into clinical practice. The combination of CPT-11, bolus 5-FU and LV (IFL) has been the most widely used regimen for the treatment of patients with MCC in the United States of America and Canada (Saltz et al, 2000). The combination of L-OHP with bolus LV and 5FU and infusional 5-FU (FOLFOX) was approved in 1999, in Europe, as first-line treatment of MCC, following the results of a study that showed improved response compared with a regimen containing LV and 5-FU alone (de Gramont et al, 2000). Combining CPT-11 with the de Gramont regimen (FOLFIRI) resulted also in increased response rate, time to progression and overall survival with respect to the de Gramont regimen alone (Douillard et al, 2000). Recently published trials have ascertained the superiority of FOLFOX with respect to FOLFIRI, both in the adjuvant and in metastatic disease chemotherapy setting (Goldberg et al, 2003, 2004). Furthermore, the FOLFOX regimen has been shown to be superior to both the FOLFIRI and CPT-11/L-OHP combinations, both in terms of time to progression and overall survival, with a comparable toxicity profile (Goldberg et al, 2004). While both CPT-11 and L-OHP have contributed substantially to the improvement of survival of patients with MCC, here we report on four studies conducted with L-OHP- containing regimens, but with different schedules and associations, aimed at improving response rates and decreasing the toxicity profiles in patients with MCC.

## II. Patients and Methods

### A. Patient eligibility

Patients were required to have histologically confirmed, colorectal adenocarcinoma, a measurable lesion >2 cm. Patients enrolled in the first phase II study were treated with a FOLFIRI-like regimen as first-line chemotherapy, while those entered in the second phase II study were chemotherapy naïve. Patients exposed to radiation therapy for rectal cancer were included, if the measurable lesion was outside the irradiated field. Other inclusion criteria were, adequate hematological (neutrophils >2 x 10<sup>9</sup>/L, platelets >100 x 10<sup>9</sup>/L, hemoglobin >10 g/dL, hematocrit >30%), hepatic [total bilirubin level of <1.5 mg/dL, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) < 3 times the upper limit of normal (ULN)] and cardiac functions. Patients with additional malignancies, other than curatively treated skin and cervical cancer or with active cardiovascular disease, were excluded. All patients were required to sign a consent form approved by the Ethical Committee of the Civilian Hospital of Avezzano, in adherence with provisions set forth in the Helsinki Agreement.

### B. Treatment and follow-up evaluation

Following the initial staging procedures, a central venous line catheter was positioned into the subclavian vein under local anaesthesia. Chemotherapy was administered on an outpatient basis for 2 consecutive days and repeated every 2 weeks if

toxicity permitted. Treatment, preceded by administration of an hydroxytryptamine-3 antagonist and dexamethasone, consisted of L-OHP 50 mg/m<sup>2</sup> in 250 ml of 5% dextrose in water, LV 200 mg/m<sup>2</sup> in a 2-hour I.V. infusion, followed by 5-FU 400 mg/m<sup>2</sup> bolus and 5-FU 600 mg/m<sup>2</sup> in a 22-hour continuous infusion, over two consecutive days. Elastomeric pumps were used for the infusion. Patients were assessed for toxicity before each cycle of chemotherapy according to WHO Criteria. Peripheral neuropathy was graded using the oxaliplatin-specific scale (Caussanel et al, 1990). Chemotherapy was delayed until recovery, if neutrophil and platelet counts were <1.5 x 10<sup>9</sup>/L and <100 x 10<sup>9</sup>/L, respectively or for significant non-hematological toxicity. In case of grade 2 hematological toxicity or diarrhea the doses of all drugs were reduced by 10%. In case of grade 3 or 4 neutropenia, mucositis or diarrhea the 5-FU dose was reduced by 20%. The dose of L-OHP was lowered by 20% in case of grade 3 thrombocytopenia or grade 3 diarrhea and by 50% in case of grade 4 thrombocytopenia. If grade 3 neurotoxicity occurred, the doses of L-OHP were reduced by 20% and stopped in case of grade 4. Patients who continued to be treated with the protocol chemotherapy beyond 6 months, were allowed to prolong the interval between chemotherapy cycles from 2 to 3 weeks, in order to permit full recovery of treatment-induced toxicities. Patients were removed from the study in case of progressive disease, unacceptable toxicity, consent withdrawal or disease stability after 1 year.

### C. Evaluation

Prior to treatment a complete history was taken and a physical examination was performed, weight was recorded and complete differential blood count, serum bilirubin, creatinine, albumin, alkaline phosphatase, transaminases, lactic dehydrogenase, and carcinoembryonic antigen were determined. Initial radiological investigations included chest X-ray and computed tomography of abdomen and pelvis and an electrocardiogram. An X-ray skeletal survey was performed when abnormal areas of uptake were observed in bone scans; CT scanning was used to evaluate hepatic lesions. Blood counts were repeated weekly, serum biochemistry was determined before each course of treatment and CEA and radiological investigations were repeated every 4 courses of chemotherapy (2 months). Follow-up visits were performed monthly. Treatment endpoints were patients' response rate and survival. Objective responses were evaluated according to WHO criteria (Miller et al, 1981).

### D. Statistical methods

For the response rate, exact binomial 95% confidence intervals (CI) were calculated. Survival and time to progression were calculated from the date of protocol entry to the time of progression or death, and both were assessed using the Kaplan and Meier product-limit method (Kaplan and Meier, 1958). Patients who underwent metastasectomy were not censored for progression-free survival. Logrank test was used to compare survival curves.

## III. Results

### A. Patients' characteristics

The studies comprise 146 consecutive patients with metastatic colorectal carcinoma evaluated and treated at the Civilian hospital of Avezzano. The patients median age was 60 years, 77 patients had been treated with a 5-FU/LV adjuvant chemotherapy before developing metastatic disease, while 69 patients had metastatic disease at the diagnosis. Twenty patients were operated for metastasectomy, and 15 patients underwent resection of

metastatic disease after administration of the FOLFOX regimen. Patients' characteristics are included in **Table 1**.

**B. Fractionation of FOLFOX over two days in first and second line chemotherapy for metastatic colorectal carcinoma**

*In vitro* and clinical studies have shown that L-OHP as a single agent has demonstrated superior activity compared to cisplatin or carboplatin (Raymond et al, 1997), while in a combination with 5-FU has a greater than additive effect on colorectal cancer cell lines (Raymond et al, 1998). Moreover, synergism in the action of L-OHP, 5-FU and LV has been demonstrated, irrespective of sequence of administration (Fischel et al, 1998). We therefore conducted 2 phase II studies administering this combination of drugs (FOLFOX regimen), to chemotherapy naïve (Recchia et al, 2004a) and to pretreated MCC patients (Recchia et al, 2003a). L-OHP doses were fractionated over two days and administered with 5-FU/LV bolus and continuous infusion with the aim of increasing the activity of this regimen and decreasing toxicity. Neurotoxicity, which may occur as acute neurosensory toxicity involving hands, feet and larynx and as chronic sensory neuropathy with loss of

effect of the doses administered and by the peak plasma concentrations of L-OHP (Extra et al, 1998). In order to further decrease toxicity, chemotherapy was preceded by the administration of 2400 mg of reduced glutathione (GSH), 4 meq Mg SO<sub>4</sub> and 10 meq KCl. L-OHP was administered, 50 mg/m<sup>2</sup> and LV 200 mg/m<sup>2</sup> in a 2-hour I.V. infusion, followed by 5-FU 400 mg m<sup>2</sup> bolus and 5-FU 600 mg/m<sup>2</sup> in a 22-hour continuous infusion, on days 1 and 2 every 2 weeks (De Gramont regimen). In the first-line chemotherapy study including 54 patients (Recchia et al, 2004a) we obtained an overall response rate of 50% (95% CI, 36% to 64%), with a median time to progression and overall survival of 10.3 and 19.2 months, respectively (**Figure 1**), whereas in the study of 46 patients pretreated with the FOLFIRI regimen (Recchia et al., 2003a), the response rate to salvage chemotherapy was 32.6% (95% CI, 19.5% to 48,06%). Median time to progression and overall survival were 6.4 months (range 3.1-31+), and 12.2 months (range 3.7-31.1+), respectively (**Figure 2**).

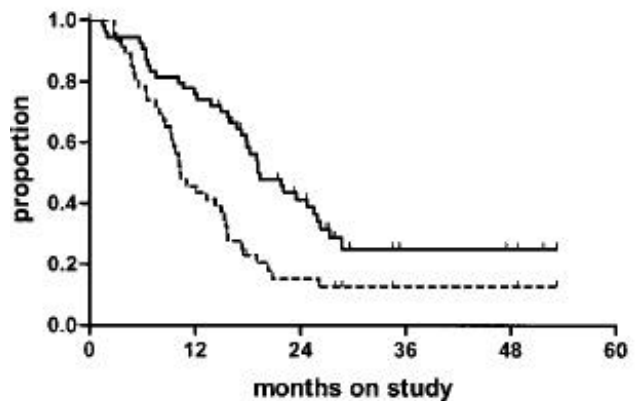
Overall survival for the 100 patients enrolled in these phase-II studies, from the start of chemotherapy for metastatic disease was 22.5 months (**Figure 3**). The toxicity profile of these 2 studies was particularly favorable. In chemotherapy naïve patients, grade 2-3 leukopenia was observed in 31% of patients, while only 10% of patients developed peripheral neuropathy. Grade 4 thrombocytopenia was observed in 1 patient. Eighty-five percent of patients had no nausea or vomiting. Grade 1-4 diarrhea occurred in 11% of patients. Treatment was delayed in 39 courses of chemotherapy (8%) and the doses were reduced in 24 (5%).

**Table 1.** Characteristics of patients

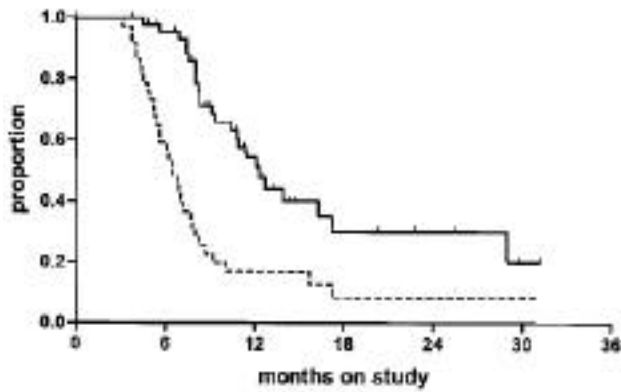
Characteristics	No.	%
No of patients	146	100
<b>Age, years</b>		
Median	60	
Range	35-79	
<b>Sex</b>		
Males	95	65
Females	51	35
<b>Performance status (ECOG)</b>		
0-1	115	79
2	26	18
3	5	3
<b>Site of primary disease</b>		
Colon	102	70
Rectum	44	30
<b>Metastatic disease at diagnosis</b>	74	51
<b>Metastatic sites</b>		
Liver	92	42
Lung	31	14
Abdomen	51	23
Bone	13	6
Nodes	14	7
Peritoneal carcinomatosis	12	5
Brain	6	3

35 patients had 2 metastatic sites, and 16 patients had 3 or more metastatic sites

sensation, dysesthesias in the distal extremities and functional impairment, is one of the most important dose-limiting toxicities of L-OHP, caused by the cumulative

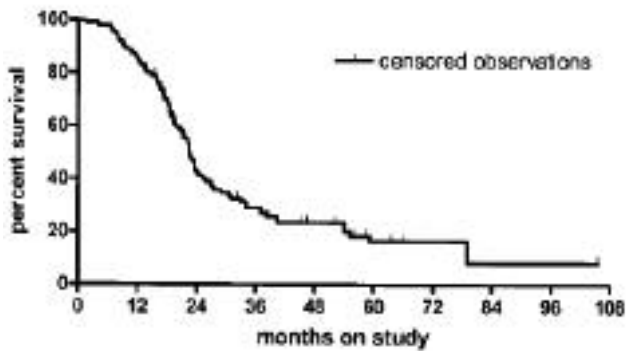


**Figure 1.** --- Time to progression: events 41 (89%), censored 5 (11%), median time to progression: 6.4 months (range 3.1-31+) Overall survival: events 25 (54%), censored 21 (46%), median overall survival 12.2 months (range 1-31.1+)



**Figure 2.** --- Time to progression: events 47 (87%), censored 7 (13%), median time to progression: 10.3 months (range 2.8-53.2+)

Overall survival: events 36 (67%), censored 18 (33%), median overall survival 19.2 months (range 1.5-53.2+)



**Figure 3.** Events 79 (79%), censored 21 (21%), overall median survival 22.5 months (range 1.5-100+)

In patients pretreated with chemotherapy, grade 2-3 leukopenia was observed in 34% of patients, while only 10% of patients developed grade 2-3 peripheral neuropathy. Grade 4 thrombocytopenia was low and was only observed in 1 patient. Allergic cutaneous reactions were observed in 4 patients after a cumulative median dose of oxaliplatin of 800 mg/m<sup>2</sup> (range 450-1000 mg/m<sup>2</sup>).

In these two studies we have shown not only that overall survival was comparable to and even better than the survival obtained in other trials in which L-OHP was administered in one day, but the neurotoxicity profile was particularly favorable.

### C. Alternation of fractionated FOLFOX and FOLFIRI in first line chemotherapy for metastatic colorectal carcinoma

The high failure rates encountered in the chemotherapy of some cancers suggest that drug resistance is a common phenomenon. Since L-OHP and CPT-11 are non-cross resistant their combined use may diminish emergence of resistant neoplastic clones and may be associated with enhanced anti-neoplastic activity with a lower toxicity profile. Based on this hypothesis, rapid cyclic alternating chemotherapy has been suggested as a favourable treatment modality in several cancer types

(Goldie and Coldman, 1979). In a further attempt to improve responses obtained in the 2 previously reported multicenter phase II studies where fractionated CPT-11 had been used as first- (Recchia et al, 2004a) or second-line chemotherapy in MCC (Recchia et al, 2004a), we performed a further study in which both regimens FOLFOX and FOLFIRI were alternated in each patient with aim of decreasing the chances of developing resistance to the drugs and decrease the toxicity profile.

This trial included 26 patients of whom 14 were female; 9 patients had a median disease-free survival of 14 months, while 17 patients had stage IV disease at the diagnosis. Patients received FOLFOX as the first course of chemotherapy, as previously described. After 14 days patients were treated with the FOLFIRI regimen in which CPT-11, 90 mg/m<sup>2</sup> and LV 200 mg/m<sup>2</sup> were administered in a 2-hour I.V. infusion, followed by 5-FU 400 mg m<sup>2</sup> bolus and 5-FU 600 mg/m<sup>2</sup> in a 22-hour continuous infusion, on days 1 and 2 every 2 weeks. A response rate of 69% was observed, while disease stability occurred in 11% of patients, signifying that 80% of patients had a benefit from chemotherapy. After a median follow up of 12 months median time to progression had not been reached yet, while 77% of patients were still alive. Toxicity was exceptionally low with no grade 3 or 4 hematologic or neurologic toxicity.

### D. Immunotherapy for patients radically treated with surgery and chemotherapy for metastatic recurrent colorectal cancer

A high percentage of patients with solid tumors achieve a complete clinical remission after initial treatment. Unfortunately, the majority of them finally relapse due to “minimal residual disease” (MRD) represented by residual tumor cells detectable only by the most sensitive methods (Mathè et al, 1986). The low efficiency of the immune system, induced by surgical and chemotherapeutic treatments will prevent the eradication of these cell clones by cell-mediated immunity (Finke et al, 1999). Such immune dysfunction is usually worsened by cytotoxic therapy (Mackall et al, 1994). Performance status, disease extent, weight loss and, independently from other factors, lymphocytopenia (Riesco, 1970; Stanley, 1980; Lavin et al, 1982) are negative prognostic factors. Chemotherapy may damage interleukin-2 (IL-2) cell-mediated immune function for prolonged periods of time (Mackall et al, 1994; Wise et al, 1988). Vascular endothelial growth factor (VEGF), a diffusible glycoprotein produced by normal and neoplastic cells, is an important regulator of physiologic and pathologic angiogenesis (Ferrara N et al, 2003). Preclinical and clinical studies have shown that a murine monoclonal anti-human antibody against VEGF can inhibit the growth of human tumor xenofrats and an humanized variant of this antibody in combination with irinotecan-based chemotherapy resulted in statistically significant and clinically meaningful improvement in survival among patients with metastatic colorectal cancer (Kim KJ et al, 1993, Hurwitz H et al., 2004). IL-2, defined as the hormone of the immune response, has pleiotropic activities on cell-mediated and humoral immunity. IL-2

induces T cell proliferation, enhances the generation of cytotoxic T lymphocytes and activates T and B cells. In addition, IL-2 amplifies the tumoricidal activity of NK cells (Smith, 1988). The IL-2-receptor interaction (Cantrell et al, 1984) is augmented through a paracrine route, that involves IFN- $\gamma$  function (Smith, 1988). The induction of endogenous LAK cell activity and production of tumor inhibitory cytokines (Pavletic et al, 1993) may constitute the primary cytotoxic activity of IL-2. Another important aspect is the inhibition of angiogenic activity by IL-2 through the induction of IFN- $\gamma$ , which in turn, induces p-10, a protein with potent antiangiogenic activity (Keane et al, 1999). Some chemotherapy-refractory tumors such renal cell carcinoma and melanoma, have shown responses to high-dose IL-2 (Fyfe et al, 1995). Nevertheless, high-dose intravenous IL-2 has been shown to have a high toxicity through the mechanism of vascular leak syndrome with hypotension, oliguria, respiratory distress, cardiac arrhythmias and mental status change (Fyfe et al, 1995).

Murine studies have shown that IL-2 has a higher efficacy, when tumor burden is low (Charak et al, 1991). Consequently it may be preferable to administer of IL-2 after surgical cytoreduction and after having achieved the maximum response to chemotherapy (Belldegrun et al, 2000). In this setting the action of IL-2 could be very effective on cancer cells damaged by chemotherapy (Mitchell, 1992). IL-2 could be, indeed, the optimal cytokine to restore the cell-mediated immune function in cancer patients after successful chemotherapy. Unfortunately the high toxicity of I.V. IL-2 prevents its universal adoption in this category of patients. However, this objective could be achieved by the subcutaneous administration of lower-dose IL-2, known to be active with a minor toxicity profile, not only in cancer patients (Lindemann et al, 1993), but also in HIV-immunodeficient patients (Davej et al, 1997).

Another class of biological agents, capable of enhancing IL-2 function are retinoids that share several synergistic effects with IL-2. In fact, retinoids boost both IL-2 receptors and T-helper cells and co-operate with IL-2 in augmenting IFN- $\gamma$  and IL-2 production by human peripheral monocytes (Prabhala et al, 1991). IL-2 cultured with RA produces a synergistic increase in IFN- $\gamma$  production (4 to 90 fold), while anti-IL-2 antibodies abrogate this effect (Prabhala et al, 1991). Finally, retinoids inhibit the proliferation of various cell lines, inducing differentiation and apoptosis. We have previously reported that a 0.5 mg/Kg dose of 13-cis-retinoic acid (RA) on a 5-day/week schedule was very well tolerated in patients with advanced non-small cell lung cancer (Recchia et al, 1999; Recchia et al, 2000). In a phase 1B study, associating IL-2 administered subcutaneously with RA administered orally for prolonged periods of time, we determined that the optimal biological dose (OBD) for a phase II study was  $1.8 \times 10^6$  IU and 0.5 mg/Kg for 5 days/week respectively, for 2 cycles of 3 weeks/month, for up to 2 years (Recchia et al, 2001). This regimen was easily administered, well tolerated and improved both total lymphocyte count and CD4/CD8 ratio in patients with tumor response or stabilization after

standard chemotherapy. Moreover, it induced a complete response in 2/18 patients treated with long-term maintenance therapy. In a further phase II randomized study we have shown that the combination of IL-2 and RA could decrease, significantly, the VEGF in the same category of patients (Recchia et al, 2004b).

In an attempt to apply the aforementioned findings to clinical practice, we conducted a study in a selected group of 20 patients with MCC (Recchia et al, 2004c), with the objective to verify whether the combination of IL-2 and RA could improve the outcome of patients operated for colorectal cancer recurrence (8 liver, 12 pelvis + peritoneum). Twenty patients with a median age of 66 years were entered into the study from September 1998 to September 2001. Fifty-five percent of patients were males, performance status 0 in 65% of patients and 1 in the remaining 35%. Eight patients had synchronous metastases, while 12 patients had a median disease-free survival of 14 months and had received a 5-FU/LV based adjuvant chemotherapy. After curative resection of metastases patients were treated for 6 months with the fractionated FOLFOX regimen as previously described. After chemotherapy patients received, subcutaneously, IL-2,  $1.8 \times 10^6$  I.U. plus RA 0.5 mg/Kg, orally, for 5 days/week for 2 consecutive cycles of 3 weeks, with a 1-week rest, for 1 year.

This therapy was continued on alternate weeks for one more year. The third year the patients continued with an alternate schedule or as necessary according to the immune competence and to VEGF value. Patients were monitored every 2 months by determination of VEGF, CD4/CD8 ratio, NK and tumor markers. Responses were assessed every 4 months by the CT scan or MR scan. The toxicity of IL-2 and RA was low. With a combination of surgery and chemotherapy, a 75% response rate was obtained (95% C.I., 51%-91%). After a median follow-up of 20 months, there was an improvement of CD4/CD8, NK and a statistically significant decrease of VEGF (**Figure 4**), while median time to progression and overall survival were not reached yet (5-year survival rate 52%). Compared with 40 patients well matched for all characteristics, IL-2 and RA therapy improved the DFS and overall survival after curative resection of metastases from colorectal cancer (**Figure 5**). A phase III randomized trial has been started.

#### IV. Discussion

Despite the progress that has been made in the adjuvant treatment of colorectal cancer, approximately half of the patients develop metastatic disease (Boring et al, 1992). The low 5-year survival rate of patients with MCC, shows that this is a relatively chemo-resistant disease; in fact, both MDR1 and GSH S-transferase genes are hyper expressed in colorectal carcinoma cells (Shen et al, 1997). However, with a series of therapeutical strategies it is possible to improve the clinical outcome of these patients.

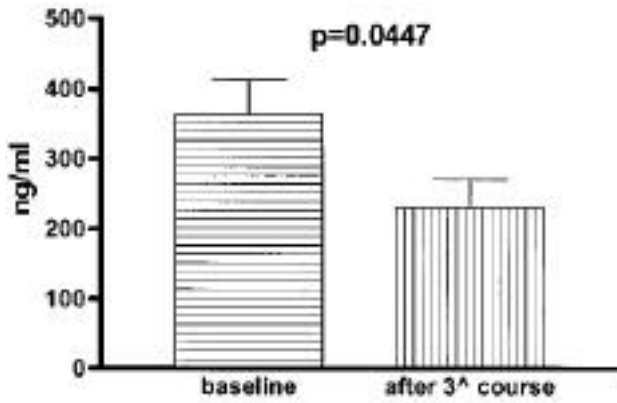


Figure 4. Vascular endothelial growth factor

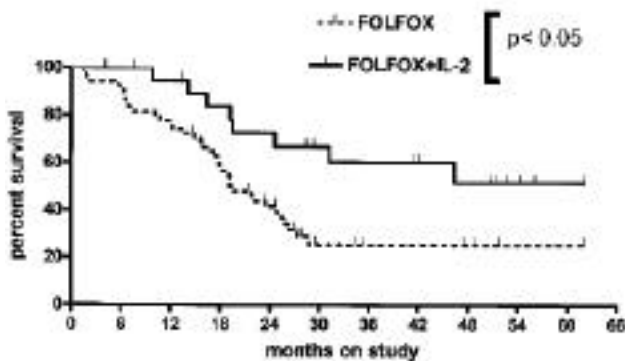


Figure 5. Overall survival of FOLFOX alone versus FOLFOX plus interleukine-2 and 13-cis-retinoic acid

In fact, the overall response rate and overall survival, in the aforementioned studies has been progressively increasing. From a 32.6% of response rate and overall survival of 12.2 months for the pretreated MCC patients, we improved the response rate of 50% and an overall survival of 19.2 months for patients treated with FOLFOX in first-line chemotherapy (range 3.7-31.2+). For the entire cohort of 100 patients treated with all three drugs, the median survival was 22.5 months with a 5-year survival rate of 18% (Figure 3). The response rate was further increased to 69% in the protocol alternating FOLFOX and FOLFIRI regimens. In this study median disease-free survival and median overall survival have not been reached yet, after a median follow-up of 12 months. A major step forward was obtained for a selected group of 20 patients that presented with metastatic, resectable, colorectal carcinoma. In these patients after resection of metastases and chemotherapy, the biological therapy produced a prolonged disease-free survival and a 5-year overall survival of 52%. In this cohort of patients some immunological parameters were monitored. With respect to baseline values, there was a statistically significant improvement of CD4+/CD8+ ratio, NK and lymphocyte number and a statistically significant decrease of VEGF (Figure 4). Treatment compliance for this regimen was good, with the median relative dose-intensity delivered for

L-OHP, LV and 5-FU being 92 %, 92%, and 94% respectively. These results are further supported by the fact that only 8% of cycles were delayed, the dose was reduced in only 5% and treatment was maintained over a considerable length of time with a median of 9 cycles administered (range 4-21).

One of the major toxicities encountered with the administration of L-OHP is neurotoxicity (Table 2). Recent data indicate that L-OHP may act on specific isoforms of the voltage gated sodium (Na<sup>+</sup>) channel to increase the excitability of sensory neurons, an action inhibited by the Na<sup>+</sup> channel blocker carbamazepine (Gamelin et al, 2002). In our studies (Recchia et al, 2003a, 2004a) grade 2 or 3 neurotoxicity was observed in 10% of patients. Such a low rate of neuropathy, as compared with the data in literature, was most likely due to the low daily dose of L-OHP and to the prior administration of 2400 mg of GSH, KCl (10 meq) and MgSO<sub>4</sub> (4 meq). In fact, the mean cumulative dose of the L-OHP administered to each patient was 900 mg (range 400-1700). Moreover it has been demonstrated that the addition of GSH to the chemotherapy does not reduce the clinical activity of L-OHP (Cascinu et al, 2002). All studies employing L-OHP in the treatment of advanced colorectal cancer have reported varying degrees of neurological toxicity with grade 3 toxicity increasing sharply with increasing L-OHP dosage. In 2 studies where the dose of L-OHP was 130 mg/m<sup>2</sup>, 89% of patients showed some form of neurological toxicity (Machover et al, 1996). In the study that lead to approval, in Europe, of L-OHP combined with LV and infusional 5-FU as first-line treatment of MCC, L-OHP was administered on day 1 at the dose of 85 mg/m<sup>2</sup>. Grade 1,2 or 3 neurological toxicity was observed in 68% of patients (de Gramont et al, 2000). Haematological toxicity was also reported; however, it reached grade 4 in only 16% of patients. Stomatitis and diarrhea were in the same range as that reported with other regimens, while severe nausea-vomiting occurred less frequently (4% of patients) as compared to CPT-11 based regimens. Grade 3 alopecia was reported in 29% of patients.

Recently published results of international studies have firmly established the role of a combination of L-OHP with LV/5-FU regimen in the treatment of MCC. One study in particular has shown the superiority of the FOLFOX regimen compared both with the CPT-11/LV/5-FU and the CPT-11/L-OHP regimens in terms of time to progression and overall survival, with a comparable toxicity profile (Goldberg et al, 2004). In this study, paresthesias grade 3 or greater, were observed in 18% of patients, whereas quality of life did not vary with respect to the three chemotherapy schedules. These data confirm that FOLFOX should be considered as a reference regimen in the first-line treatment of MCC.

Considering the decreased toxicity profile with the fractionated administration of L-OHP, an activity comparable to that observed in other studies using standard regimens and an acceptable safety profile, the fractionated bimonthly L-OHP, 5-FU/LV may be considered as an attractive treatment for patients with MCC. In the adjuvant setting, the characteristics of the fractionated FOLFOX regimen are very appealing, due to

**Table 2.** Toxicity according to WHO criteria

	WHO grade										Total		
	0		1		2		3		4		No.	%	
	No.	%	No.	%	No.	%	No.	%	No.	%			
<b>Hematologic</b>													
Leucopenia	49	34	46	31	41	28	10	7	16	11	146	100	
Neutropenia	59	40	14	10	24	16	33	23	0	0	146	100	
Thrombocytopenia	103	71	24	16	16	11	3	2	0	0	146	100	
Anemia	85	58	48	33	13	9	0	0	0	0	146	100	
Infection	134	92	3	2	6	4	3	2					
<b>Gastrointestinal</b>									0	0	146	100	
Oral	110	75	23	15	7	6	6	4	0	0	146	100	
Nausea & vomiting	121	83	14	10	6	4	5	3	0	0	146	100	
Diarrhea	123	84	9	6	7	5	7	5	0	0	146	100	
Hepatic	137	93	8	6	1	1	0	0	0	0	146	100	
<b>Neurotoxicity</b>	121	83	16	11	5	4	4	2	0	0	146	100	
<b>Renal</b>	141	96	3	2	1	1	1	1	0	0	146	100	
<b>Allergy</b>	132	90	0	0	3	2	11	8	0	0	146	100	
<b>Cutaneous</b>											146	100	
Alopecia	33	23	23	15	48	33	42	29	0	0	146	100	
skin	139	95	6	4	1	1	0	0	0	0	146	100	

the fact that high neurological toxicity should not be tolerated in non-metastatic patients. In conclusion, a 3.5 month increase in overall survival of patients with MCC has been obtained from chemotherapy with 5FU/LV and L-OHP or CPT-11. Furthermore, a major step forward has been achieved in our trials with the use of biological response modifiers IL-2 and RA. One of the major factors responsible for the increase of disease-free survival and overall survival could be the statistically significant improvement of lymphocyte, NK and CD4+/CD8+ ratio. In fact, a parallel increase in lymphocyte count and response has been described in patients with non-small-cell lung cancer (Lissoni et al, 1999). Moreover the statistically significant decrease of VEGF observed in our studies, could have had a role in the inhibition of the angiogenic switch. The strategies described in the chemotherapeutic regimens reported here have contributed to an overall improvement in the outcome of patients with MCC.

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From left to right: dott. Giampiero Candeloro, dott. Gaetano Saggio, dott. Francesco Recchia, dott. Alisia Cesta

