Oxaliplatin Combined with 5-Fluorouracil and Methotrexate in Advanced Colorectal Cancer

M.G. ZAMPINO[1], K. LORIZZO[1], A. ROCCA[1], M. LOCATELLI[1], L. ZORZINO[2], S. MANZONI[1], C. MAZZETTA[3], N. FAZIO[1], R. BIFFI[5] and F. DE BRAUD[1]

Divisions of [1]Medical Oncology, [2]Pathology and Laboratory Medicine, [3]Epidemiology and Biostatistics, [4]Diagnostic Radiology and [5]General Surgery, European Institute of Oncology, Milan, Italy

Abstract. *Background: A promising regimen including 5-Fluorouracil, methotrexate and oxaliplatin is reported. Patients and Methods: Patients with untreated measurable metastatic disease received bolus 5-Fluorouracil (600 mg/m^2) on days 2 and 16, modulated by methotrexate (200 mg/m^2) 24 h earlier, alternated with 4 weeks of continuous infusion of 5-Fluorouracil (200 mg/m^2/daily) plus oxaliplatin (130 mg/m^2) on days 29 and 56, followed by 2 weeks of rest. Serum vascular endothelial growth factor (VEGF) was analyzed at baseline and before every cycle. Results: Fifty-eight patients were enrolled. Objective remissions were reported in 45.6% (95% CI=34.3%, 57.3%). The median progression-free survival was 7.8 months and the median overall survival was 19.4 months. No grade 4 toxicity was reported, except for one case of diarrhea. The serum VEGF evaluated in 23 patients showed a decreasing trend during therapy. Conclusion: The regimen was active, well tolerated and may be a possible option in patients not suitable for radical surgery.*

Fifty percent of patients with colorectal cancer treated by surgery relapse and, in most cases, medical treatment represents a palliative approach (1).

After the "fluorouracil-only" era, a new generation of chemotherapeutic agents have become available, such as oxaliplatin (OX) and irinotecan (2). These drugs, combined with fluoropyrimidines, have improved the objective response rate, from 20-30%, obtained by 5-Fluorouracil (FU) plus folinic acid (L) (3) and/or methotrexate (M) (4), to 40-50% (5-6). As second-line treatment, oxaliplatin combinations achieve remission in 17-23% of cases (7, 8). These results have produced an increase in median survival from the historical 9-12 months to 12-20 months and contributed to changing the standard treatment for advanced colorectal cancer (ACC) (5-9). The concept of tumor vascularization as target therapy was also investigated and bevacizumab, a recombinant humanized monoclonal antibody anti-vascular endothelial growth factor (VEGF), has recently been approved in combination with L and FU as a first-line strategy (10).

Before the advent of these new drugs, a sequential regimen, involving the administration of bolus FU, modulated by M and continuous infusion FU modulated by L, was described as an effective and low-cost chemotherapy (11). Based on these findings, we proposed combining OX to this regimen with the aim of increasing responses and also to evaluate toxicity and survival in patients with inoperable ACC.

Baseline values and temporal trends of circulating VEGF levels during treatment were also evaluated by looking for a correlation with clinical characteristics and response to treatment.

Patients and Methods

Patients. The selection criteria were: pathologically-confirmed inoperable ACC with bi-dimensionally measurable disease, ≥1 cm; no prior chemotherapy for metastatic disease and ≥6 months after the end of adjuvant therapy; ECOG performance status ≤2; age between 18 and 75 years; life expectancy ≥3 months; adequate marrow (absolute granulocytes ≥1,500/mm^3, platelets ≥100,000/mm^3 and Hb ≥10 g/dl), renal (serum creatinine ≤1.25 x ULN) and hepatic functions (bilirubin ≤1.25 x ULN and SGOT/SGPT ≤2 x ULN or ≤4 x ULN in case of hepatic metastases); instrumental and biochemical evaluation within 28 and 7 days from treatment start, respectively. Patients with any serious concomitant illness were excluded. The protocol was approved by the Ethics Committee and signed informed consent was obtained from every patient.

Study design. This was a phase II, non-randomized, mono-institutional study to assess the efficacy and toxicity of the experimental regimen on patients with ACC. Over 10 weeks, one course of treatment was administrered as follows: M 200 mg/m^2 *i.v.*

Correspondence to: Maria Giulia Zampino, MD, Division of Medical Oncology, European Institute of Milan, 20141 Milan, Italy. Tel: +39257489460-482, Fax: +39257489457, e-mail: maria.zampino@ieo.it

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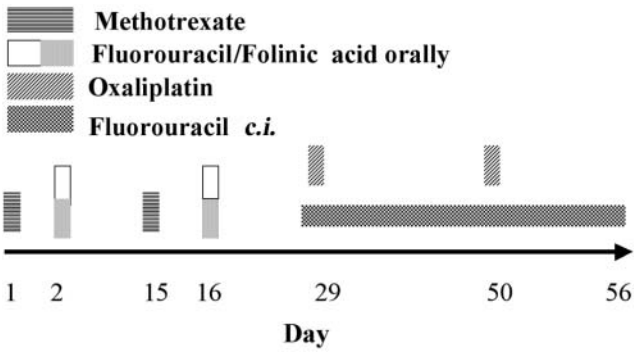

Figure 1. *Drug regimen design.*

days 1 and 15; FU 600 mg/m^2 *i.v.* days 2 and 16; L rescue 15 mg orally q 6 hours six times, just following FU administration (24 h after M); OX 130 mg/m^2 *i.v.* days 29 and 50; FU 200 mg/m^2/day as continuous *i.v.* infusion (*c.i.*) on days 29-56 (Figure 1).

FU was infused through a totally implanted venous catheter connected to a portable external pump (CADD-1, Pharmacia or Baxter elastomeres). The patients were assessed at baseline and every 2 weeks for clinical and laboratory toxicities. Toxicity was graded according to the NCI-CTC scale, version 2.

In the event of granulocytes <1,500/mm^3, platelets <100,000/mm^3 and/or grade ≥2 non-hematological side-effects, treatment was delayed until recovery; if improvement did not occur within 2 weeks, treatment was discontinued. In the case of grades 3 and 4 diarrhea, mucositis or hematological toxicity, all drugs were withheld until recovery to grades 1 or 2 and thereafter were reduced by 25% and 50%, respectively. In the event of OX-related persistent paresthesia, the treatment was delayed for 1 or 2 weeks until recovery to grade ≤1, and the OX dose was subsequently reduced by 25%. If grade 3 peripheral neuropathy was reported, OX was discontinued. Finally, FU as bolus and *c.i.* was reduced by 25% and 50% for moderate (grade 2) or severe hand-foot syndrome (grade 3), respectively.

The baseline evaluation included a full medical history, physical examination, hematological assessments, chest X-ray, ECG and cardiological examination. Bi-dimensional tumor measurement with CT scan or MRI was performed at baseline and repeated before each cycle. Blood samples for VEGF were also collected at the same times. During treatment, all patients had a complete blood count and renal and hepatic functions were determined every 2 weeks.

The VEGF assay was performed by means of a quantitative sandwich enzyme immunoassay technique (Quantikine Human VEGF, R&D Systems, Inc., Minneapolis, MN, USA).

Response was classified according to the WHO/UICC criteria (12). In case of a complete response (CR), the patients received a second cycle and, if the response was confirmed, they stopped treatment. In the case of partial response (PR), the therapy was continued for a maximum of four cycles. In case of stable disease (SD), the patients received another cycle of treatment and then, if stabilization continued, maintenance with *c.i.* FU was proposed. Treatment was stopped in the event of progressive disease (PD), unacceptable toxicity or patient refusal.

Statistical analysis. This was a two-stage phase II clinical trial, taking into account both toxicity and response, using methodology in Bryant and Day (13). A response rate ≤0.3 was considered to be unacceptable, but a response rate ≥0.5 acceptable; unacceptable toxicity was defined as ≥40% of patients with a toxicity of grades 3-4 and acceptable toxicity as ≤20% of patients with grade ≥2 side-effects. Significance levels of 10% for treatment and toxic effects and a 90% power were adopted. From a total of 57 subjects, 23 had to be accrued during stage one and 34 during stage two. If fewer than 22 responses or more than 29 cases of grade ≥2 toxicity were observed by the end of the trial, then no further investigation was warranted.

Binomial distribution was used to calculate the exact confidence intervals (CI) for responses and toxicity.

The association between factors was tested using the Chi-square test or Fisher's exact test. Overall survival (OS) and time-to-progression (TTP) were estimated using the Kaplan-Meier method, with time calculated from the beginning of chemotherapy to death or last follow-up (OS), and from the beginning of chemotherapy to first progression or last follow-up (TTP).

The best global response was defined as the best response obtained from the beginning of treatment to progression.

The distribution of baseline serum VEGF, its possible association with other baseline clinical characteristics and its time trends were evaluated. The logarithmic scale was used to normalize the distributions. In order to distinguish between variability among patients and variability over time within patients, the trends were assessed using a linear mixed effects model (14, 15), employing a restricted maximum likelihood estimation and a general positive-definite covariance matrix. All the analyses were done using SPLUS (16).

Results

From November 1999 to August 2002, 59 patients were enrolled in the study. One patient was considered ineligible because of a performance status of 3; he died without receiving any treatment and was not evaluated; another patient was evaluable for toxicity, but not for response, only the first dose (day one) of therapy having been administered. The main characteristics of the patients are listed in Table I.

The median chemotherapy duration was 4.3 months [1.7, 9.2]. Twenty-three patients (40%) received one cycle, 14 (24%) two cycles, 20 (35%) three cycles, while only one patient received four cycles.

Response. Among the 57 patients assessable for response, three CR, (5.3%, 95%CI=[1.4, 13.0]) 23 PR (40.3%, [29.4, 52.1]), 14 SD (24.6%, [15.5, 35.7]) and 17 PD (29.8%, [20.0, 41.3]) were observed. The overall response rate (CR+PR) was 45.6% (95% CI=[34.3%, 57.3%]). Forty-four percent (24/54) objective remissions in liver, 43% (6/14) in lung, 17% (2/12) in lymph nodes and 20% (1/5) in the primary tumor were obtained. Eight patients underwent surgical resection of liver metastases after a median time of 6.5 months from the beginning of chemotherapy. The median progression-free survival (PFS) was 7.8 months (95% CI=[6.4, 10.1]).

Table I. *Characteristics of the patients.*

Assessable for toxicity (No.)	58
Assessable for response (No.)	57
Age: median [min, max]	60 [28, 75]
Gender	F=23 M=35
PS: N (%)	
0/ 1/ 2	35 (60%) /20 (34%) / 3 (6%)
Site: No. (%)	
Colon / rectum	46 (79%) /12 (21%)
Stage at diagnosis: N (%)	
II / III / IV	2 (3%) / 10 (17%) / 46 (80%)
Prior adjuvant therapy	
FU/L	9 (16%)
No. of metastatic sites: N (%)	
1	31 (54%)
2	17 (29%)
≥3	10 (17%)
Sites of metastasis	
Liver only	31 (54%)
Liver and other	23 (40%)
Other (lungs, nodes, peritoneum, pelvis)	4 (6%)
Median baseline serum value (range):	
CEA ng/ml	184 (2-4361)
VEGF pg/ml	2966 (131-19470)

At the end of chemotherapy, the patients were followed for a median of 14.3 months (range: 2.8-39.8) and, over this period, a total of 49 PD and 33 deaths was recorded. In 42 cases, irinotecan-containing regimens were administered and two patients received hepatic intra-arterial chemotherapy. The median survival time was 19.4 months (95%CI=[15.3, 24.2]) and, after 2 years, the proportion surviving was 31.4% (95%CI=[18.9, 52.3]). No significant association between response, site and number of metastatic lesions was identified.

The main patient characteristics were not correlated with a significant change in risk of disease progression or death, while the number of metastatic sites was an important predictor of survival (the median OS with one metastatic site was 22 months, *versus* 15 months with two or more, $p<0.05$).

Evaluation of circulating VEGF. Twenty-three cases were evaluable for serum VEGF. The only significant observation was a lower baseline level of serum VEGF for rectal tumors ($p=0.03$).

The individual time trends of VEGF, measured at baseline, 2, 4 and 6 months, were analyzed. A strong variation across subjects was observed, although on average there was a decreasing pattern; the estimated percentage variations according to the linear mixed effects model showed a percentage reduction (95% CI) of –24% (–41%, –5%), –32% (–53%, –3%) and –50% (–69%, –20%) after 2, 4 and 6 months of treatment, respectively.

Toxicity. The regimen was feasible and generally well tolerated. One patient was withdrawn from the study for a grade 3 hyper-creatininemia, which occurred after the first dose of M, but recovered completely after hydration. The patient was considered assessable only for toxicity. The grade 2/3 toxicity was as follows: neutropenia in 5.2% [95% CI: 1.1-14.4] of patients, anemia 5.2% [1.1-14.4], diarrhea 15.5% [7.3-27.4], nausea 17.2% [8.6-29.4], vomiting 15.5% [7.3-27.4], stomatitis 12.1% [5.0-23.3] and hepatic toxicity 8.6% [2.9-19.0]. Asthenia was mild in 12.1% and moderate in 3.4% [0.4-11.9] of the patients. Sixty-seven percent [53.7-79.0] of the patients suffered from peripheral neuropathy, with grade 2 observed in 13.8% [6.1-25.4]. Grade 1 hand-and-foot syndrome was reported in only two patients. One patient, with subclavian vein thrombosis, due to a central venous catheter, required hospitalization; subsequently, treatment was regularly administered, concomitantly with anticoagulant therapy. No patient deaths occurred from treatment-related causes.

Discussion

The results of this phase II study demonstrated that the regimen containing OX, administered concomitantly with infusional FU and sequentially combined with M and bolus FU, was a well-tolerated first-line treatment.

The toxicity was mild and the only grade 4 event was a case of diarrhea. Grade 2 neurotoxicity occurred in only 14% of cases, while 53% of patients experienced grade 1. This favorable safety profile is probably due to our alternating schedule, with administration of OX only in the second part of each treatment course. The results in terms of response (46%, 95% CI=[34.3%, 57.3%]), median PFS (7.8 months, 95% CI=[6.4, 10.1]) and OS (19.4 months, 95%CI=[15.3, 24.2]) were encouraging, albeit slightly inferior to those reported for other OX-containing regimens. It is noteworthy that, in our series, 46% of the patients had two or more metastatic sites, a known unfavorable prognostic factor, while 80% presented metastatic disease at diagnosis. Nonetheless, eight out of 31 patients with liver-only involvement achieved shrinkage allowing radical surgical resection, while 73% of cases survived long enough to receive a subsequent irinotecan line of therapy.

Results from other phase II studies suggested increasing efficacy with bi-weekly OX dose-escalation from 85 to 100 and 130 mg/m^2, with objective remissions of 51%, 58% and 64%, respectively (5, 17, 18). The high response rate would make regimens with higher dose-intensity the option of choice as neo-adjuvant treatment for metastatic conditions and for locally advanced rectal cancer (19). The only phase III study, comparing 85 and 130 mg/m^2 dose-levels of OX, showed no significant difference in response rate, nor any impact on survival (17, 18).

Furthermore, when therapy is planned in a palliative setting, high doses of OX often determine early neurotoxicity, which may necessitate discontinuation of treatment. This aspect was investigated in the OPTIMOX Trial with a "stop and go" strategy (18). Our sequential regimen mimics the intermittent strategy of OX administration, which seems a suitable approach for long-term palliation.

An alternative strategy, aimed at improving response without increasing the dose-intensity and neurotoxicity of OX, is the "all and now" use of triple-agent combinations with FU, OX and irinotecan. The promising results reported in phase II studies await confirmation in phase III trials (20, 21).

The VEGF values showed an overall decreasing trend over time during the therapy, as has been previously reported both for colorectal cancer (22) and other tumor types (23). The reasons for this are not clear, but the effects of chemotherapeutic agents on platelets, a major source of serum VEGF, and on tumor angiogenesis may be postulated.

In conclusion, our regimen was active and well tolerated and may be proposed as palliative therapy for patients with metastatic disease not suitable for surgery. The regimen may also be considered in combination with new anti-angiogenic drugs.

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