Biweekly oxaliplatin plus irinotecan and folinic acid-modulated 5-fluorouracil: a phase II study in pretreated patients with metastatic colorectal cancer

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Oxaliplatin (OXA) and irinotecan (IRI) are active drugs for metastatic colorectal cancer, their toxicity profiles are not overlapping, and both drugs have shown at least additivity with folinic acid-modulated 5-fluorouracil (5FU). We carried out this phase II study to assess the activity and toxicity of a biweekly regimen including OXA plus IRI on day 1, and levo-folinic acid (LFA) plus 5FU on day 2 (OXIRIFAFU) in pretreated patients with metastatic colorectal cancer. Forty-one patients, all previously treated with adjuvant and/or palliative 5FU-based chemotherapy (16 of them already exposed to IRI, OXA or both), were enrolled into this trial. On the basis of sensitivity to previous treatment, 19 patients were considered as chemo-resistant and 14 patients as chemo-refractory. OXA 110 mg/m^2 (over 2 h) and IRI 175 mg/m^2 (over 1 h) were delivered on day 1, followed by LFA 250 mg/m^2 (2-h infusion) plus 5FU 800 mg/m^2 as intravenous bolus on day 2. Cycles were repeated every 2 weeks. A total of 348 cycles were delivered, with a median of nine cycles per patient (range, 1–12 cycles per patient). Five complete and 13 partial responses were reported on 40 assessable patients, giving a response rate of 45% [95% confidence interval (CI), 29–62%]; eight of 19 (42%) resistant patients and five of 14 (36%) refractory patients achieved a major response, which was also obtained in four of eight (50%) patients pretreated with IRI and in three of eight (38%) patients pretreated with OXA. Grade 3 or higher neutropenia occurred in 68% of patients, but febrile neutropenia or infections affected only seven (17%) patients. No episodes of grade 3 or higher thrombocytopenia or anemia were recorded. Occurrence of severe non-hematologic toxicities by patients were: diarrhea, 34%; vomiting, 17%; peripheral cumulative neuropathy, 15%; stomatitis, 10%; acute cholinergic syndrome, 7%. Actually delivered dose intensities of all three drugs resulted in about two-thirds of the planned ones. After a follow-up of 39 months, median progression-free survival was 7.5 months. Median overall survival was 14.4 (95% CI, 10.4–18.4) months from the start of OXIRIFAFU and 25.3 (95% CI, 18.1–32.5) months from the diagnosis of metastatic disease. This OXIRIFAFU triplet regimen was highly effective in resistant/refractory colorectal cancer patients. A slight dose reduction of all cytotoxic drugs could be advisable in order to improve the tolerability of this regimen without jeopardizing its activity. *Anti-Cancer Drugs* **17:985–992 © 2006 Lippincott Williams & Wilkins.**

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Introduction

The front-line treatment for patients with metastatic colorectal cancer is still based on folinic acid (FA) modulated 5-fluorouracil (5FU), alone or in combination with either oxaliplatin (OXA) or irinotecan (IRI) [1,2]. Both combinations have shown superior activity (i.e. significantly higher response rate and longer progression-free survival) when compared with 5FU alone, although only IRI-including regimens also improved the overall survival [3–7]. Moreover, it appeared from retrospective analyses that patients who were able to receive all three active drugs during the course of their disease experienced a longer survival [8,9]. This observation represented the rationale for assessing triplet regimens in front-line. Indeed, several investigators have demonstrated the feasibility and activity of different triplet combinations in phase I–II studies [10–13].

On the other hand, the management of patients already exposed to cytotoxic therapy is still debated. While patients who have received a 5FU-based regimen after primary surgery may be treated again with this drug at the time of recurrence, provided that a long time interval (more than 6 months) from the end of adjuvant treatment

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had elapsed, there is still uncertainty on how to manage patients suffering from an early relapse or those previously treated with a combination regimen in the adjuvant setting. For these patients, there is an urgent need of investigating new active cytotoxic regimens.

In a previous phase I study, we have already identified the recommended doses of OXA plus IRI delivered on day 1, and levo-folinic acid (LFA)-modulated bolus 5FU on day 2, for a biweekly combination regimen [13]. This schedule of administration was selected on the ground of some in-vitro experiments on the HT29 human colon cancer cell line, demonstrating a better growth-inhibitory effect when OXA shortly preceded the exposure to SN-38 (the active metabolite of IRI) [14]. On the other hand, the assessment of the interaction between SN38 and 5FU on different colon cancer cell lines, either sensitive (SNU-C4) or resistant (SW620 and HT29) to 5FU, has shown that a sequential (with SN38 before 5FU) rather than a simultaneous exposure produced a synergistic or at least additive effect in all cancer cell lines [15]. This observation was also confirmed by in-vivo studies [16]. A similar schedule-dependent interaction has been observed for OXA followed by 5FU: this sequence was more cytotoxic than the reverse one against the HT29 and LoVo colon cancer cell lines, either sensitive or resistant to 5FU [17]. In addition, a short rather than long exposure to 5FU after a previous exposure to OXA was shown to be more cytotoxic in several colon cancer cell lines [18].

Moreover, an in-vitro study on two human colon cancer cell lines (SW620 and WIDR) reported the interaction of different simultaneous exposures to OXA, SN38 and 5FU modulated by FA (FUFA) [19]. In this study, the OXA + FUFA combination was always synergistic, the OXA + SN38 combination was either additive (when SN38 was applied after OXA) or antagonistic (when SN38 was applied first), SN38 + FUFA was always antagonistic, while the triple exposure (OXA + SN38 + FUFA) was additive. Furthermore, the relative contribution of each drug to the overall cytotoxicity of the triplet combination was analyzed. From this analysis, the greatest contribution was derived from OXA, while SN38 appeared to bring a relatively modest addition to the combination effect.

The encouraging evidence of activity of this regimen that we observed in the dose-finding trial [13] prompted us to further investigate the safety and activity of this combination in patients already exposed to chemotherapy.

Patients and methods

Patient selection

Patients with histologically proven recurrent or metastatic colorectal carcinoma were included in this study. At least one bidimensionally measurable lesion should be present. All patients should have received at least one 5FU-based regimen, either in the adjuvant or in the palliative setting. Previous exposure to OXA or to IRI was allowed. According to previous drugs exposure, disease status for each patient was classified as chemo-resistant (relapse within 6 months from the end of adjuvant chemotherapy or disease progression within 3 months from the discontinuation of palliative chemotherapy) or chemo-refractory (relapse during adjuvant chemotherapy or disease progression during palliative chemotherapy).

Other eligibility criteria were: age between 18 and 75 years; performance status ≤ 2 of the Eastern Cooperative Oncology Group scale; life expectancy > 3 months; discontinuation of previous chemotherapy for at least 1 month; normal bone marrow reserve, with an absolute neutrophil count $\geq 2000/\mu l$ and a platelet count $\geq 100\,000/\mu l$; and adequate hepatic (bilirubin serum level < 1.5 mg/dl; ASAT and ALAT $< 2 \times$ upper normal limit), and renal function (creatinine clearance > 60 ml/min). The study protocol was approved by the Independent Ethical Committee of the National Tumour Institute of Naples. All patients were informed of the investigational nature of this study and each patient provided written consent before registration.

Administration of treatment

OXA 110 mg/m^2 (intravenously over at least 2 h) and IRI 175 mg/m^2 (intravenously over 1 h) were delivered on day 1. LFA 250 mg/m^2 (intravenously over 2 h), followed by 5FU 800 mg/m^2 intravenous bolus, was administered on day 2. The initial dosage of all three cytotoxic drugs was reduced by 25% in elderly patients, in patients previously exposed to pelvic radiotherapy or in patients who had already suffered from severe hematologic and/or non-hematologic toxicity on previous adjuvant/palliative chemotherapy. Patients received a standard anti-emetic premedication, with HT_3-receptor antagonists and steroids, on the first day of each cycle. Systematic prophylaxis for early cholinergic symptoms due to IRI was not performed. Similarly, no prophylaxis was given for the occurrence of late diarrhea. Patients, however, were carefully instructed to take loperamide orally as soon as the first stool modification occurred. Granulocyte colony-stimulating factors were not permitted unless in the presence of febrile neutropenia.

Treatment was delivered every 2 weeks until a major response was achieved; in this case, at least eight courses, and a maximum of 12 cycles were planned. Therapy was discontinued in the case of documented disease progression, occurrence of disease complications, unacceptable toxicity, patient's refusal or when it was believed to be for the patient's best interest.

Recycling rules and doses reduction

Courses were repeated every 2 weeks in the presence of an absolute neutrophil count $\geq 1500/\mu l$ and a platelet count $\geq 100\,000/\mu l$, and recovery of any extra-hematologic

toxicity. Otherwise, treatment was postponed for 1 or 2 weeks until recovery. If recovery required more than 2 weeks, the patient went off study. In the presence of World Health Organization (WHO) grade 4 hematologic toxicity, or in the presence of grade ≥ 3 non-hematologic toxicity, the subsequent cycles were administered, after recovery of side-effects, with a 25% dose reduction of all cytotoxic drugs. In the cases of grade 3 neurotoxicity, OXA was reduced by 25%; if there was no recovery at the time of recycling after this dose reduction, OXA delivery was discontinued.

Evaluation of toxicity

For the assessment of acute hematologic toxicity, blood cell count was performed weekly, and two times a week in the case of grade 4 toxicity. Blood cell count with absolute neutrophil count and platelet count, and a full biochemistry profile (serum dosage of bilirubin, ASAT and ALAT, γ-glutamyltranspeptidase, alkaline phosphatase, lactic dehydrogenase, albumin and total protein, urea and creatinine, and urinalysis) was performed at each cycle. Patients were checked to detect signs of neurotoxicity before initial treatment and at every cycle thereafter. The acute toxicity was classified according to WHO toxicity criteria [20]. Neurologic toxicity was graded according to a specific Lévi scale [21].

Evaluation of activity

Initial staging work-up included history and physical examination, routine biochemistry, blood cell count, carcinoembryonic antigen serum level determination, chest X-ray and abdominal ultrasound scan. Bidimensionally measurable indicator lesions were assessed by computed topographic scan, or magnetic nuclear resonance imaging. Subjective symptoms, body weight, physical examination and performance status were recorded before each treatment cycle. All initially abnormal tests were repeated after every four cycles. Responses were classified according to standard WHO criteria [20]. To classify for response, the reduction of tumor burden was confirmed on two consecutive assessments, 2 months apart. Duration of response was measured from the first time it was documented to the date of recurrence or last follow-up.

Evaluation of progression-free and overall survival

Progression-free survival was calculated from the date of initial OXIRIFAFU therapy to the date or progression, death or last follow-up. Overall survival was calculated both from the date of the first documentation of metastatic disease, and from the start of OXIRIFAFU treatment, to the date of death or last-follow-up. Survival curves were estimated with the Kaplan and Meier method [22].

Definition of the sample size

The study population was defined according to the Simon two-stage minimax design [23]. A 20% response rate was the minimum activity of interest for rejecting this regimen, while a 40% response rate was the alternative hypothesis. Therefore, at least five responses on the first 18 patients and at least 10 responses on 33 patients were required to accept this hypothesis with a 0.05 α error.

Results

Patient characteristics

From January 2001 to October 2005, 41 patients entered this trial (Table 1). Most patients had already received adjuvant (73%) treatment and/or one (59%) or two (12%) lines of palliative chemotherapy. According to previous drugs exposure, 33 (80%) patients were classified chemo-resistant (19 patients) or chemo-refractory (14 patients). Eight patients had already received an OXA-based regimen and eight patients an IRI-containing treatment. Almost half the number of the patients has two or more sites of disease and the liver was the most common site of metastasis. One patient was enrolled in this study without evidence of disease after surgical resection of liver metastasis.

Acute toxicity

A total of 348 cycles were delivered, with a median of nine courses per patient (range, 1–12 courses per

Table 1 Main patient characteristics

Characteristics	Number	Percentage
Total patients	41	100
Males	23	56
Females	18	44
Median age (range) (years)	56 (30–74)	
Primary site		
Colon	28	68
Rectum	13	32
Previous surgery	40	98
Previous adjuvant 5FU	27	66
Previous adjuvant FOLFIRI	3	7
Previous one line of palliative CT	24	59
Previous two lines of palliative CT	5	12
Previous Irinotecan exposure	8	20
Previous Oxaliplatin exposure	8	20
Chemo-sensitive	8	20
Chemo-resistant	19	46
Chemo-refractory	14	34
Performance status		
0	22	54
1–2	19	46
Weight loss	8	20
Disease-related pain	17	41
Number of disease sites[a]		
1	23	56
2	13	32
3+	4	10
Site of disease		
Liver	25	61
Lung	13	32
Nodal	9	22
Peritoneal	7	17
Local recurrence	6	15
Bone	2	5
CEA basal value >5 ng/ml	28	68
CEA basal value $\geq$ 100 ng/ml	13	32

[a]One patient was disease-free after liver resection. CT, computed tomography; CEA, carcinoembryonic antigen.

patient). Twenty-six (63%) patients received at least eight cycles and nine (22%) patients received 12 cycles. Thirty patients discontinued their treatment according to the protocol's rules, four patients went off study because of toxicity or refusal (two patients each) and four patients for disease complications. In three cases, the decision to interrupt the treatment early was taken by the attending physician.

No toxic death occurred. Table 2 reports the worst hematologic toxicity registered for each patient during treatment. Severe neutropenia affected 28 (68%) patients. In detail, grade 3 neutropenia was detected in 12 (29%) patients and grade 4 neutropenia in 16 (39%) patients. Febrile neutropenia or infection, however, affected only seven patients in all. It is relevant to note that no severe anemia or thrombocytopenia occurred in any patient during treatment. Main non-hematologic toxicity is shown in Table 3. Diarrhea was the most common side-effect, affecting 80% of patients; it was severe in 14 (34%) patients, and required hospitalization and rehydration in five cases. Some gastric disturbance was reported by 28 (68%) of patients, but it was severe in only seven (17%) patients.

OXA-induced neuropathy occurred in 23 (56%) patients and it was severe in six (15%) patients. As expected, this toxicity was dose-related, because it affected four of 18 (22%) patients receiving a cumulative dosage of OXA exceeding 800 mg/m^2. Three additional patients suffered from acute paresthesia or laringospasm during an OXA infusion, but this side-effect was subsequently prevented by prolonging the infusion over 6 h.

According to protocol rules, the dosage of at least one of the cytotoxic drugs was reduced in 28 of 41 (68%) patients, from initial (eight patients) or subsequent cycles (20 patients); therefore, the median absolute (and relative) dose intensity was 34 mg/m^2/week (62%) for OXA, 54 mg/m^2/week (61%) for IRI and 248 mg/m^2/week (62%) for 5FU.

Table 2 Hematologic toxicity (WHO grade) by patients ($n=41$)

Toxicity	Number of patients				Percentage
	1	2	3	4	3+4
Neutropenia	2	5	12	16	68
Febrile neutropenia/ infection	0	6	1	0	2
Platelets	5	5	0	0	0
Anemia	11	5	0	0	0

WHO, World Health Organization.

Table 3 Non-hematologic toxicity (WHO grade) by patients ($n=41$)

Toxicity	Number of patients				Percentage
	1	2	3	4	3+4
Nausea/vomiting	14	7	5	2	17
Diarrhea	10	9	9	5	34
Stomatitis	5	0	2	2	10
Alopecia	6	3	10	0	24
Chronic neuropathy[a]	13	4	6	0	15
Cholinergic syndrome	0	3	3	0	7
Acute dysesthesia	1	1	1	0	3
Hepatic	3	0	0	0	0

[a]According to the Lévi's scale.
WHO, World Health Organization.

Table 4 Activity reported with OXIRIFAFU

Responses	Number	Percentage
Complete response	5	13
Partial response	13[a]	32
Minor response	6[b]	15
Stable disease	6	15
Progression	10	25
Assessable patients	40[c]	100

[a]One patient was rendered disease-free after liver resection.
[b]Three of six patients showed a 50% or greater drop of baseline abnormal CEA value.
[c]No evidence of disease in one patient at baseline.

Activity

Among 40 patients with evidence of disease, five complete remissions and 13 partial remissions were achieved, giving an overall response rate of 45% [95% confidence interval (CI), 29–62%] according to an intent-to-treat analysis (Table 4). In all but one case, patients achieving a complete remission had their disease confined to one site only, which was the liver in four cases. One additional patient, considered in partial remission after eight cycles, was rendered disease-free by liver resection of residual metastatic deposits. Major responses were documented after a median time from initial therapy of 3 months (range, 2–8 months) and they had a median length of 7.9 months (range, 2.2–21.4 months).

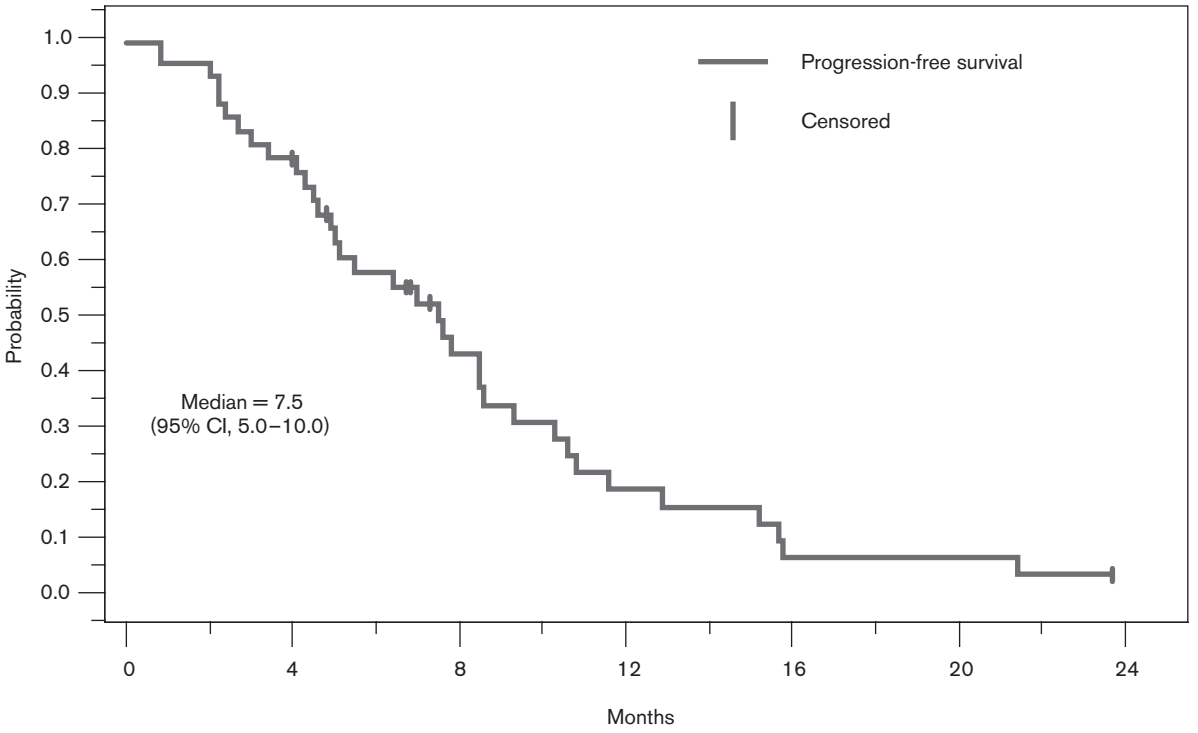
Six patients showed a tumor shrinkage that did not qualify for a major response; however, three of these patients also showed a 50% or greater drop of their initially abnormal serum carcinoembryonic antigen value. Six patients achieved a sustained stable disease for 6 months or more (Table 4).

Activity of this regimen appeared not related with the sensitivity to the previous cytotoxic drug exposure. Indeed, eight of 19 (42%) chemo-resistant and five of 14 (36%) chemo-refractory patients achieved a major response. Of note, four of eight patients previously exposed to IRI and three of eight already treated with OXA subsequently showed a response to OXIRIFAFU. No substantial difference of activity was seen between patients with only one disease site (48%) and patients with two or more disease sites (41%).

Follow-up

After a median follow-up of 39 months, 35 patients progressed and 26 died. The estimated median progression-free survival was 7.5 months (95% CI, 5.0–10.0

Fig. 1



Progression-free survival.

months) (Fig. 1). Median overall survival was 14.4 months (95% CI, 10.4–18.4 months) from the commencement of OXIRIFAFU (Fig. 2), while it was 25.3 months (95% CI, 18.1–32.5 months) from the documentation of the metastatic disease (Fig. 3).

Discussion

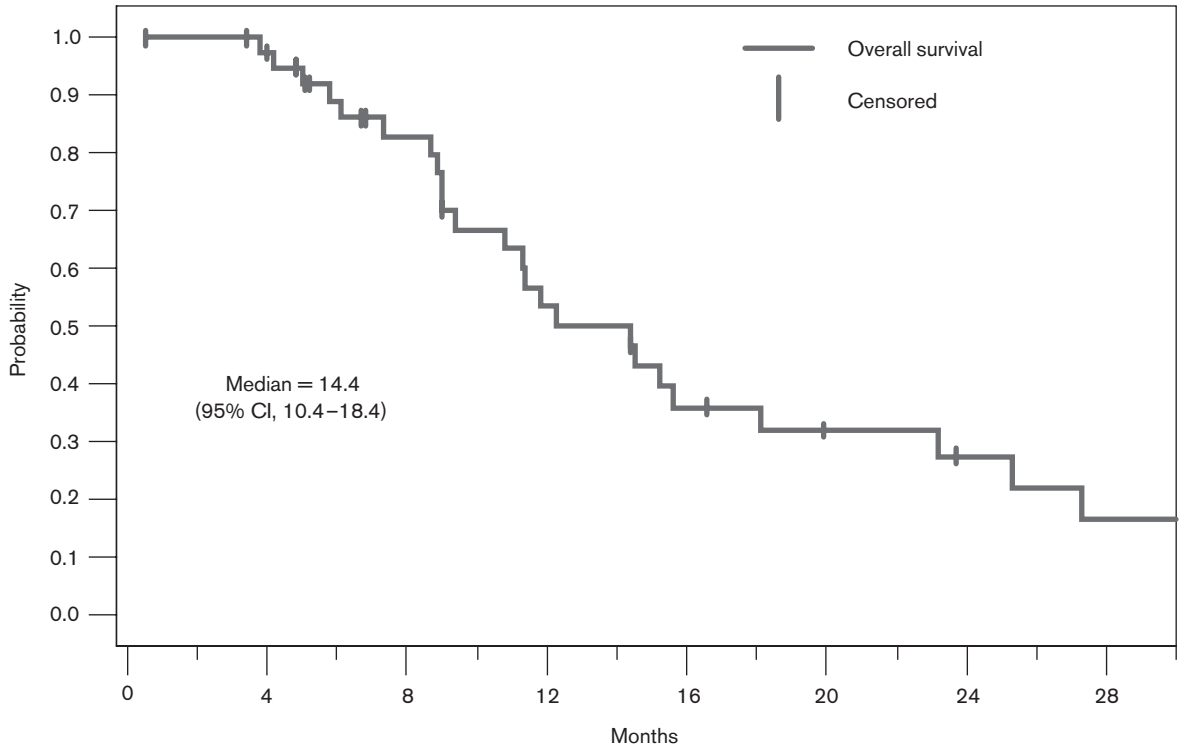
The prognosis of patients with metastatic colorectal cancer is invariably poor, because only palliative treatments are available for this disease [1,2]. Some new cytotoxic and biologic drugs, however, have recently entered into the clinical practice for treating these patients [2]. Namely, IRI and OXA, both associated with 5FU, have shown a high activity in the first-line treatment [3–7], and patients receiving all these active drugs during the course of their disease have shown a prolonged survival [8,9]. A substantial proportion of metastatic patients are, however, unable to receive the three drugs sequentially, mainly due to a worsening of clinical status after the front-line treatment that precludes the delivery of further cytotoxic chemotherapy.

This observation has prompted some investigators to assess the feasibility of an up-front triplet regimens in phase I–II studies [10–12,24]. In all these trials, short-term infusional 5FU has been used. In detail, Masi *et al.* [10] delivered OXA 100 mg/m^2, IRI 175 mg/m^2 and leucovorin 200 mg/m^2 followed by a 48-h infusion of 5FU 3800 mg/m^2. An exciting 67% response rate has been reported on mainly chemo-naive patients. A grade 4 neutropenia, however, was registered in 55% of patients, 12% of whom had also a febrile neutropenia. In addition, grade 3 diarrhea affected 21% of patients. Similarly, Calvo *et al.* [11] reported a 69% response rate on 26 patients treated with a 1-day regimen including OXA 120 mg/m^2, IRI 250 mg/m^2, 5FA 500 mg/m^2 and FU 2600 mg/m^2 infused over 24 h. Moreover, the combination of IRI 150 mg/m^2 on day 1, OXA 65 mg/m^2 on day 2, followed by an leucororin-modulated 5FU bolus plus 22-h infusion for 2 consecutive days (LV5FU2) every 2 weeks has been assessed by Souglakos *et al.* [12] on 31 patients previously unexposed to palliative chemotherapy, achieving a 58% response rate.

The impact on survival of an up-front triple combination, however, is still unclear. Indeed, while a significant survival prolongation was achieved in one study comparing a triplet (FOLFOXIRI) with a doublet (FOLFIRI) regimen [25], another trial reported absolutely identical survival outcome for patients treated either with doublet or with triplet therapy [26].

Our trial is the only one among those assessing the efficacy of a triplet combination that incorporated an

Fig. 2



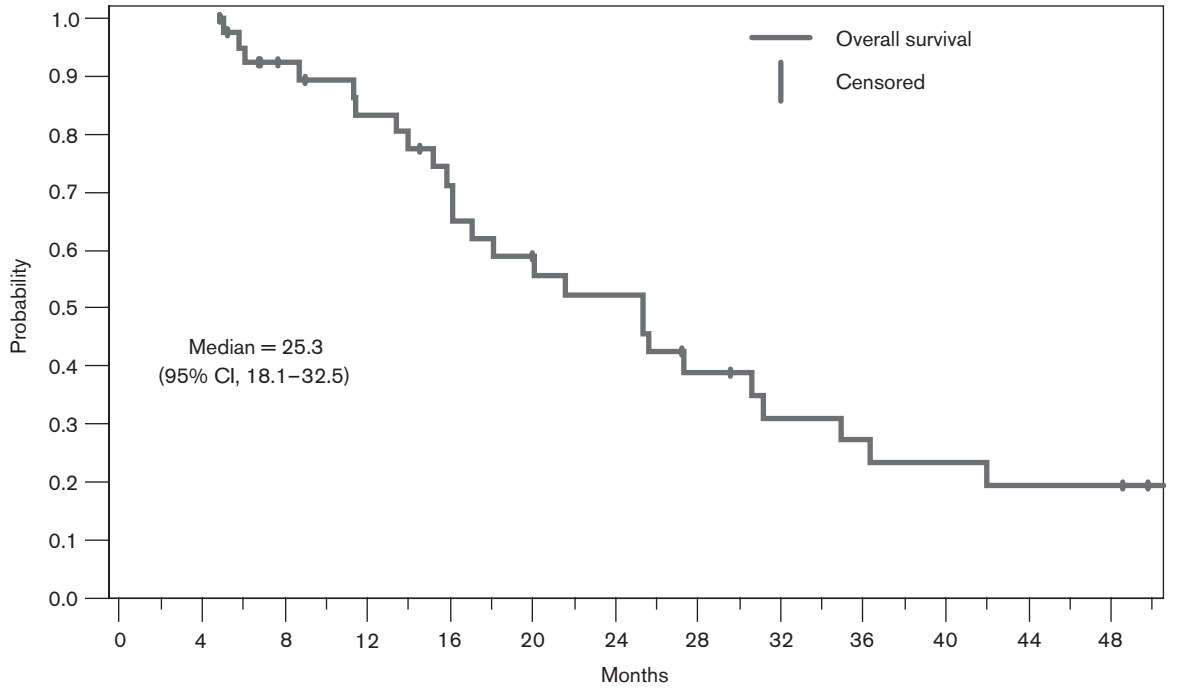
Overall survival from the start of OXIRIFAFU.

intravenous bolus of 5FU. The choice of this schedule was supported by the already mentioned in-vitro evidence [14–18] and also by our own previous dose-finding trial [13]. The results of the present phase II study confirm that all three cytotoxic drugs can be safely combined in close sequence in a 2-day cycle. Severe diarrhea was the main non-hematologic toxicity of this treatment, affecting 34% of treated patients. This figure, however, is comparable to that reported by Souglakos *et al.* [26] (28%) and only slightly greater than that observed by Falcone *et al.* [25] (19%). On the contrary, grade 3 or higher neutropenia affected more patients in our experience (68%) than in the above-mentioned trials (35 and 46%, respectively). This side-effect, however, was often a mere laboratory finding, detected by the weekly blood cell counts performed in our trial, and it usually had no clinically meaningful consequences. Indeed, febrile neutropenia and/or infection occurred in seven patients in all.

On the other hand, the cautious approach we observed for preventing, or the dose reduction we applied after the occurrence of severe toxicity, translated in a dose intensity of all three drugs corresponding to about 60% of the planned ones. It should be noted, however, that the actual dose intensity of OXA was only moderately lower than that usually achieved when this drug is combined with bolus or infusional 5FU [7,27]. Moreover, our regimen was very active despite this dose reduction. This finding confirms the in-vitro observation, which highlighted the greatest contribution of OXA to the overall cytotoxicity of the triplet combination [19], and suggests that a slight dose reduction of this regimen in further clinical evaluation could not jeopardize its activity.

The achievement of a major response in 18 (45%) patients, associated with a minor shrinkage or disease stabilization reported in 12 (30%) further patients, demonstrated that this regimen was highly effective in metastatic colorectal cancer. Of interest was the response rate observed in patients affected by a disease poorly sensitive to previous chemotherapy and in those already exposed to one or two components of the combination. Therefore, this regimen deserves to be further explored in this subset of patients. The duration of responses and the progression-free survival was also unusually long in this trial, confirming that a good disease control was achieved with the treatment on study. The median survival time was not negligible, because it was 14.4 months from the start of the present treatment and it was

Fig. 3



Overall survival from the documentation of metastatic disease.

in excess of 2 years from the date the metastatic disease was initially documented. This observation is an indirect confirmation that patients treated with all available cytotoxic drugs may obtain an unusually long overall survival.

In conclusion, the toxicity profile of our OXIRIFAFU regimen was acceptable and its activity was impressive in a population of heavily pretreated patients. Therefore, we believe it worthwhile to further investigate the effectiveness of this regimen in metastatic colorectal cancer patients, i.e. in patients with early recurrence after adjuvant or palliative FU-based chemotherapy.

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