

First Clinical Experience of Orally Active Epidermal Growth Factor Receptor Inhibitor Combined With Simplified FOLFOX6 as First-Line Treatment for Metastatic Colorectal Cancer

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BACKGROUND. Gefitinib, an orally active inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinase, combined with chemotherapy, has shown efficacy as second-line treatment for advanced colorectal cancer (CRC). Gefitinib combined with FOLFOX6 (oxaliplatin plus folinic acid and 5-fluorouracil) was tested as a first-line therapy.

METHODS. Patients with metastatic EGFR-positive CRC received gefitinib at a dose of 250 mg/day combined with simplified FOLFOX6. Gefitinib was continued as maintenance treatment in nonprogressing patients. Responses were assessed by Response Evaluation Criteria in Solid Tumors (RECIST) criteria and adverse events were assessed with the National Cancer Institute Common Toxicity Criteria (NCI-CTC) scale.

RESULTS. A total of 56 patients were recruited. There were 26 men and 30 women, with a median age of 57.5 years. The Eastern Cooperative Oncology Group (ECOG) performance status was as follows: 0 in 39 patients, 1 in 12 patients, and 2 in 5 patients. Thirty-nine patients (69.6%) had stage IV disease at diagnosis, 92.9% had liver involvement, and 46.4% had ≥ 2 metastatic sites. All patients were evaluated for safety, and 53 were evaluated for response: 40 patients (71.4%; 95% confidence interval [95% CI], 57.8%–82.6%) had complete or partial responses, and 11 patients (19.6%) had stable disease. Median time to progression was 7 months (range, 2.1–33.0 months; 95% CI, 6.2–9.0 months). Radical surgery or thermoablation of metastatic sites was performed in 14 patients (25%). NCI-CTC grade 3–4 events occurred in 36 patients (64.3%): diarrhea in 9 patients (16.1%), and hematologic toxicity in 13 patients (23.2%). Four patients (7.1%) were withdrawn for drug-related adverse events.

CONCLUSIONS. The regimen has shown promising efficacy with manageable toxicity as a first-line treatment for patients with advanced CRC. *Cancer* 2007;110:752–8. © 2007 American Cancer Society.

KEYWORDS: advanced colorectal cancer, epidermal growth factor receptor, gefitinib, oxaliplatin.

Although the combination oxaliplatin, irinotecan, and leucovorin-modulated 5-fluorouracil has improved outcomes for patients metastatic colorectal cancer (CRC), objective responses are obtained in only 40% to 50% of patients, and the median survival is 14 to 18 months. The identification of molecular targets implicated in cancer cell proliferation, such as the epidermal growth factor receptor (EGFR), has opened up the possibility of improving outcomes for metastatic CRC, particularly when combined with chemotherapy.

Two clinical approaches have been used to target the EGFR signaling pathway: monoclonal antibodies against the extracellular domain of the receptor that prevent ligand binding, and small molecule inhibitors of the adenosine triphosphate binding that inhibit tyrosine kinase and hence receptor autophosphorylation. Tyrosine phosphorylation makes docking sites on the EGFR available for proteins that link the receptor to a cascade of downstream biochemical reactions such as the *ras-raf-MAPK-fos* pathway, which stimulates cell growth.¹ EGFR is overexpressed in several cancers, including up to 80% of CRCs; EGFR overexpression is associated with aggressive disease and poor prognosis.²

Gefitinib (ZD1839, Iressa; AstraZeneca Pharmaceuticals, Wilmington, Del) is an orally active, low molecular weight competitive inhibitor of tyrosine kinase domain on EGFR. Its key preclinical features include good tolerability and the ability, at nanomolar concentrations, to delay growth and cause regression in a wide range of tumor xenografts.³ Interestingly, although gefitinib has demonstrated dose-dependent antitumor activity, the level of EGFR expression does not appear to predict tumor response.³

EGFR expression may be up-regulated by chemotherapeutic agents through the induction of apoptosis and coadministration of gefitinib with a variety of cytotoxic drugs has been markedly shown to enhance their efficacy by mechanisms unrelated to levels of EGFR expression.⁴ In particular, gefitinib has induced supra-additive growth inhibition and has enhanced apoptotic cell death when combined with several agents, including oxaliplatin and 5-fluorouracil.⁵

During phase 1 trials, gefitinib was responsible for a number of cases of prolonged disease stabilization in colorectal cancer patients at a maximum tolerated dose of 600 mg/day. However, 225 mg/day was found to be sufficient to produce minimum biologic effects.⁶⁻⁸

In a recent phase 2 study conducted in patients with pretreated CRC who were not selected for EGFR status, a combination of gefitinib plus FOLFOX4 (oxaliplatin plus folinic acid and 5-fluorouracil) resulted in 33% partial remissions and a median survival of 12 months, with relatively manageable toxicity; these results appeared to be better than those reported by chemotherapy alone in a similar population.⁹

The present multicenter phase 2 trial was conducted on patients with metastatic CRC who had not received treatment for their metastatic disease. The aim was to assess the efficacy of gefitinib combined with a simplified FOLFOX6 regimen, followed by gefitinib maintenance monotherapy in cases that did not

progress. We included only patients whose cancers overexpressed EGFR. Most previous studies were conducted in patients with EGFR-positive cancers or in unselected populations and to our knowledge few data are available on the effects of gefitinib on EGFR-negative cancers. We believe gefitinib should have more effect on EGFR-positive cancers.²

MATERIALS AND METHODS

Patient Selection

This was a multicenter, open-label, noncomparative, phase 2 trial in patients with newly diagnosed or recurrent metastatic CRC who had received no prior systemic chemotherapy for metastatic disease and had at least 20% (score 1+) cancer cells positive for EGFR. Eligible patients had histologically confirmed adenocarcinoma of colon or rectum with radiographic evidence of synchronous or metachronous metastatic disease. Material from least 1 metastatic site was examined histologically and EGFR expression determined. All specimens were reviewed by a single laboratory.

Other inclusion criteria included the ability to take and retain oral medication, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, age ≥ 18 years, measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST), absolute neutrophil count $\geq 1.5 \times 10^9/L$, platelet count $\geq 100 \times 10^9/L$, serum creatinine ≤ 1.5 times upper normal limit (UNL), total bilirubin $\leq 1.5 \times UNL$, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 2.5 \times UNL$ (or $\leq 5 \times UNL$ if liver metastases was present), and a life expectancy > 3 months. All patients signed an informed consent form approved by local ethical committees.

EGFR Determinations

Four ultrathin paraffin-embedded slides per specimen (primary or metastatic cancer) were incubated for 1 hour at room temperature with 50 $\mu L/mL$ of commercially available (clone 31G7; DBA, Milan, Italy) mouse monoclonal antibody against the peptide backbone of the extracellular domain of EGFR. This was followed by incubation with detection kit (Dako EnVision Plus-HRP; Dako, Glostrup, Denmark) according to the manufacturer's instructions. The slides were assessed for EGFR by a single observer (G.P.) experienced in tumor pathology and unaware of patient identity. The intensity (weak, moderate, and strong) and pattern (incomplete or complete) of membrane staining and the percentage of positive cells (scanning at least 1000 cells in representative fields) were recorded. Only cells with membrane labeling were considered. A 4-point

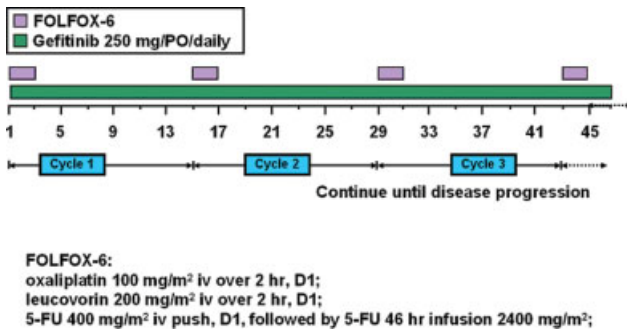


FIGURE 1. Study design. PO indicates orally; iv, intravenously; D1, Day 1; 5-FU, 5-fluorouracil.

score (0–3) that combined staining intensity and staining pattern was assigned to each specimen, using the Dako system formerly used for the HerceptTest. At least 20% of cells (score 1) had to be positive.

Serum EGFR (extracellular binding domain) levels were determined at baseline and at each patient assessment by quantitative enzyme-linked immunoadsorbent assay using a monoclonal capture antibody (Oncogene Science; Bayer, Cambridge, UK).

Study Design

Informed consent and medical history were obtained, disease extent assessed, and a tumor biopsy taken for histology and EGFR determination in the 3 weeks before treatment initiation. Physical examination, ECOG performance status, assessment of concurrent illness/treatments, hematology, biochemistry, urinalysis, heart evaluation with electrocardiogram, blood sample for soluble EGFR, and tumor markers were obtained in the week before treatment.

Patients received gefitinib every day (250 mg orally) starting on Day 1. FOLFOX6 was administered on Day 1 and repeated 14 days later (Fig. 1); it was administered on an outpatient basis via a central venous catheter connected to a single-use elastomer pump.

Clinical evaluation and blood tests were repeated before each cycle; tumor assessment, tumor markers, and soluble EGFR evaluation were performed every 2 months. Patients who responded after 4 courses continued the treatment; if the response was confirmed after 8 courses, gefitinib maintenance therapy (without chemotherapy) was given. The treatment was withdrawn for disease progression, unacceptable toxicity, or withdrawal of consent.

Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) scale (version 2.0). Treatment was delayed for toxicity grade ≥ 2 (except nausea or vomiting) until recovery. If treat-

ment was delayed >3 weeks, the patient was withdrawn from the study. If toxicity recovered to grade <2 from grade 3 or grade 4, chemotherapy continued with a 25% or 50% dose reduction, respectively. For laryngopharyngeal dysesthesia the next oxaliplatin dose was administered as a 6-hour infusion. If grade 3 and 4 allergy occurred the patient was withdrawn.

Statistical Analysis

An initial cohort of 5 patients was treated with gefitinib and FOLFOX6 as detailed above and in Figure 1; after 2 cycles (4 weeks) a full safety evaluation was conducted. In the absence of excessive toxicity, recruitment continued. The Fleming¹⁰ method was used to calculate the number of patients required to estimate the response rate. It was found that 56 patients were sufficient to give at least a 90% probability of rejecting a response rate of 50% if the true response was $>70%$ (clinically significant), assuming an exact 5% 1-sided significance test. The hypothesis that the response rate was $\leq 50%$ could be rejected if ≥ 40 responses were observed in 56 patients.

For all endpoints an intention-to-treat analysis was performed on all enrolled patients who began treatment. All other data reported are based on the safety population. The primary endpoint of the study was gefitinib activity in combination with FOLFOX6 estimated as the overall response rate (complete response [CR] plus partial response [PR]) at trial closure 6 months after the last patient's first dose of gefitinib.

Secondary endpoints were objective response rate 4 months after the initiation of treatment, the efficacy of gefitinib as maintenance therapy in patients with no disease progression after completion of combination therapy, disease control rate (CR, PR, and stable disease [SD]), progression-free survival (PFS), overall survival (OS), and the safety profile of gefitinib plus FOLFOX6.

We also assessed baseline and over-time EGFR serum levels for their ability to predict response. The former was assessed by a logistic regression model and the latter by Cox regression model with time-dependent covariate.¹¹ The results are reported as the mean \pm standard deviation.

RESULTS

Between January 2003 and December 2004, 56 patients with a median age of 57.5 years (range, 33–76 years) were enrolled in the trial from 4 treatment centers. Baseline characteristics are shown in Table 1. Thirty-nine patients (69.6%) had synchronous metastatic disease. The most common site of metastases

TABLE 1
Baseline Characteristics of Patients

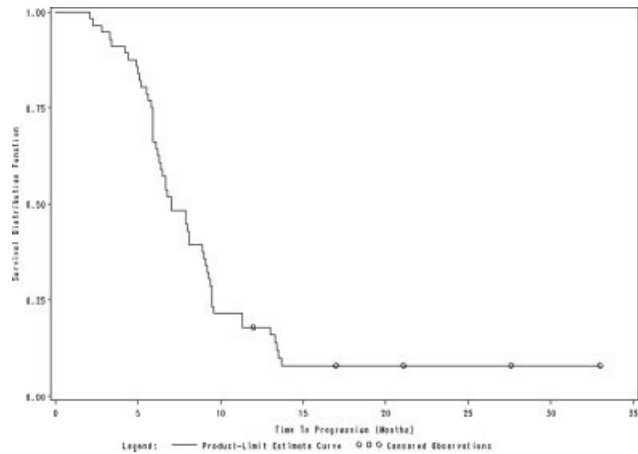
	No.	Percentage
Patients enrolled	56	100.0
Men/women	26/30	43.4/53.6
Median age (range), y	57.5 (33–76)	
ECOG performance status		
0	39	69.6
1	12	21.4
2	5	8.9
Site of primary tumor		
Colon	46	82.1
Rectum	8	14.3
Colon and rectum	2	3.6
No. of metastatic sites		
1	30	53.6
2	14	25.0
>2	12	21.4
Metastatic site		
Liver	52	92.9
Liver only	28	50.0
Lung	17	30.4
Cancer still at the primary site	12	21.4
Metastatic disease		
Synchronous	39	69.64
Metachronous	17	30.36
Median dimension of the lesion at its longest axis (range), cm	8.65 (1–54.7)	
Previous adjuvant chemotherapy	12	21.4

ECOG indicates Eastern Cooperative Oncology Group.

was the liver (92.9%). Four patients had peritoneal effusion.

All patients were evaluable for toxicity and 53 (94.6%) for response. The patients excluded from response assessment had intestinal occlusion (2 patients) or diarrhea due to dihydropyrimidine dehydrogenase (DPD) deficiency (1 patient), which developed before the first assessment. A median of 8 courses (range, 1–12 courses) was given. One patient who achieved late PR (after 8 cycles) received a further 4 cycles. Of the 425 chemotherapy cycles given, 284 (66.8%) were administered at full dose, 35 (8.2%) at reduced dose, 75 (17.7%) at delayed full dose, and 31 (7.3%) at delayed reduced dose. Overall, 90.5% of the planned oxaliplatin dose (mean, 90.5 mg/m²), 90.7% of the planned 5-fluorouracil bolus (mean 362.7 mg/m²), 88.8% of the planned 5-fluorouracil infusion (mean 2130.8 mg/m²), and 90.6% of the planned folinic acid bolus (mean 181.2 mg/m²) were given. Forty-one patients (73.2%) received gefitinib maintenance monotherapy for a median of 16.1 weeks (range, 3.7–46.4).

The median duration of treatment (combination therapy plus gefitinib maintenance) was 29 weeks

**FIGURE 2.** Kaplan-Meier representation of time to disease progression.

(range, 1–141 weeks). CR was obtained in 3 patients (5.36%), PR in 37 patients (66.1%), and SD in 11 patients (19.6%). Disease control (CR, PR, or SD) was obtained in 51 patients (91.1%; 95% confidence interval [95% CI], 80.4–97%), CR or PR was obtained in 40 patients (71.4%; 95% CI, 57.8–82.6%), and disease progression occurred in 2 patients (3.6%).

Twelve patients did not receive gefitinib monotherapy: 5 because of disease progression at second assessment after 8 courses, 5 because of adverse events, 1 because of DPD deficiency, and another because of surgery to remove liver metastases. Of the 41 patients who received gefitinib monotherapy, 23 still had disease control (2 with CR, 14 with PR, and 7 with SD) at Week 8 (first tumor assessment), 9 patients (2 with CR, 6 with PR, and 1 with SD) had disease control at Week 16 (second assessment), and 5 patients (2 with CR, 2 with PR, and 1 with SD) had disease control at Week 24 (third assessment).

Fourteen patients received local treatments: liver surgery in 11 (78.5%), radiofrequency in 2 (14.29%), and liver surgery plus radiofrequency in 1 (7.14%). Median time to progression was 7 months (range, 2.1–33 months; 95% CI, 6.2–9 months) (Fig. 2). After a median follow-up of 15.6 months (range, 2.2–33.3 months), 35 patients (62.5%) were alive. The median survival required by the power analysis had not been reached at the time of the present evaluation; 81.8% were alive at 1 year and 59.9% were alive at 2 years (Fig. 3).

Gastrointestinal toxicity (nausea, diarrhea, vomiting) occurred most frequently, followed by neurologic toxicity (paresthesia attributable to oxaliplatin), hematologic toxicity (leukopenia and neutropenia), fatigue, and dermatologic toxicity (including folliculitis, dry skin, and skin rash) (Tables 2 and 3). Dermatologic toxicity is a typical side effect of gefitinib. Most toxicity

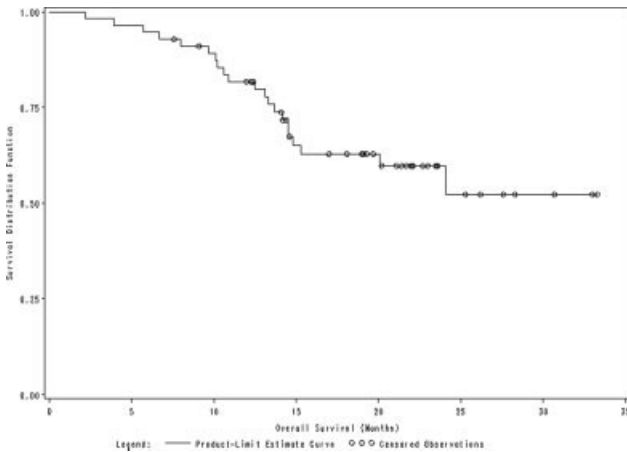


FIGURE 3. Kaplan-Meier representation of overall survival.

TABLE 2
Drug-Related Adverse Events

Site	NCI-CTC grade 1-2		NCI-CTC grade 3-4		Total	
	No. of patients	%	No. of patients	%	No. of patients	%
Gastrointestinal	53	94.6	17	30.4	53	94.6
Skin	46	82.1	0	—	46	82.1
Neurologic	44	78.6	0	—	44	78.6
Blood/bone marrow	20	35.7	13	23.2	28	50.0
Liver	4	7.1	2	3.6	5	8.9
Ocular/visual	4	7.1	0	—	4	7.1
Cardiovascular	2	3.6	0	—	2	3.6
Hemorrhage	2	3.6	0	—	2	3.6

NCI-CTC indicates National Cancer Institute Common Toxicity Criteria.

was NCI-CTC grade 1 or 2; 9.04% of events were grade 3 to 4, occurring in 30 patients (53.6%) (Table 3).

Twenty-two serious adverse events occurred in 11 patients (19.6%): 5 were attributable to both gefitinib and chemotherapy (2 episodes of diarrhea, 1 episode of dehydration, and 1 episode of stomatitis in 1 patient and 1 episode of hypokalemia in another patient) and 5 most likely due to chemotherapy only (2 episodes of abdominal pain and 1 episode of vomiting in 1 patient 1 high transaminase episode in another patient, and 1 episode of infective pneumonitis in another). Twelve serious adverse events were considered not to be drug related: 2 intestinal occlusions, 2 intestinal subocclusions, 1 venous thrombosis in a patient with a port-a-cath, 1 bone fracture, 1 melena, 1 duodenitis, 1 case of ascites, 1 episode of abdominal pain, 1 perforation of the sigmoid colon, and 1 atrial fibrillation.

Four patients (7.1%) withdrew from treatment because of a drug-related adverse event: 1 patient

TABLE 3
Frequency of NCI-CTC Grade 3-4 Adverse Events

Grade 3-4 adverse event (CTC)	No. of patients	% of patients
Neutropenia	10	17.9
Diarrhea	9	16.1
Leukopenia	4	7.1
Mucositis	4	7.1
Intestinal occlusion/subocclusion	4	7.1
Nausea	3	5.4
Abdominal pain	3	5.4
Anemia	3	5.4
Hypokaliemia	3	5.4
Vomiting	2	3.6
Asthenia	2	3.6
Fatigue	2	3.6
Intestinal perforation	2	3.6
Transaminase elevation	2	3.6
Anorexia	1	1.8
Ascites	1	1.8
Atrial fibrillation	1	1.8
Deep vein thrombosis	1	1.8
Dyspnea	1	1.8
Fever	1	1.8
Hyponatremia	1	1.8
Melena	1	1.8
Pneumonitis	1	1.8
Stomatitis	1	1.8

NCI-CTC indicates National Cancer Institute Common Toxicity Criteria.

had grade 4 stomatitis, grade 4 diarrhea, and grade 2 dehydration; another had grade 2 diarrhea; another patient had grade 2 heartburn and anorexia; and another patient had grade 3 pneumonitis. Five patients (8.9%) withdrew from treatment for adverse events considered not to be drug related: 3 with intestinal occlusions, 1 with gastroduodenitis, and 1 after intestinal perforation due the insertion of a prosthesis.

Soluble (serum) EGFR was determined at baseline and 2 and 4 months from initiation of treatment in 42, 28, and 24 patients, respectively, and also at 6 and 8 months after beginning treatment (during monotherapy) in 16 and 2 patients, respectively. To assess the association between serum EGFR levels over time and response, patients were divided into responders (CR or PR) and nonresponders (SD or PD). Responders had higher EGFR values over time than nonresponders. This difference was evident at baseline (49.4 ± 6.2 ng/mL vs 42.4 ± 8.4 ng/mL; Wald Test $P = .038$). At 6 months, responders had EGFR titers (50.2 ± 7.8 ng/mL) that were similar to those at baseline, whereas nonresponders had much lower levels than at baseline (36.3 ± 11.5 ng/mL). Thus, time trends for EGFR titers appear to differ between responders and nonresponders; however, differences

beyond 4 months were never significant because of the small number of determinations available.

DISCUSSION

The most striking finding of this study, which to our knowledge is first phase 2 assessment of gefitinib plus oxaliplatin-containing chemotherapy as first-line treatment for patients with newly diagnosed or recurrent metastatic CRC, is that an overall response (CR + PR) was obtained in 71.4% of evaluable patients, the majority of whom had synchronous metastatic disease at diagnosis.

In phase 1 trials gefitinib was found associated with manageable toxicities such as acneiform skin rash, nausea, and diarrhea. Chronic administration of the drug does not worsen the safety profile, and supports continuous once daily administration to counter the continuous oncogenic signaling through this receptor.^{6-9,12} In pooled data from 3 phase 2 trials of single-agent gefitinib, 10% of patients with advanced nonsmall-cell lung cancer (NSCLC) that had progressed despite prior chemotherapy achieved objective responses.^{13,14} Subsequent attempts to evaluate the single-agent efficacy of gefitinib in patients with progressive CRC proved ineffective.¹⁵

Gefitinib combined with folinic acid and 5-fluorouracil has not been reported to demonstrate cumulative toxicity or significant pharmacokinetic interaction. Gefitinib has also been evaluated with FOLFOX with uncertain advantage.^{5,6,8}

A phase 2 study investigating gefitinib at a dose of 500 mg together with second-cycle FOLFOX4 in 27 patients pretreated for metastatic CRC⁹ found that 33% of patients who progressed to treatment with 5-fluorouracil and irinotecan had a partial remission. This was a higher response rate than reported with FOLFOX4 alone in previous studies. Grade 3 to 4 toxicities included neutropenia (48%), diarrhea (48%), nausea (22%), and vomiting (15%).

EGFR-targeted agents are mainly used in patients whose cancers overexpressed EGFR. However, no clear correlation between EGFR overexpression and response has been found. Nevertheless, immunohistochemical findings are subject to interobserver variability and evaluations are often performed after the slides have been archived for many months, so that protein degradation and loss of sensitivity is likely.¹⁶ Furthermore, immunohistochemical evaluations are often conducted on material from the primary site, whereas chemotherapy-treated metastatic disease often presents a different EGFR expression pattern.

In the current study, we used the relatively low gefitinib dose of 250 mg/day, taking our lead from the

results of randomized phase 2 studies in NSCLC that showed that the lower dose was safer than and had the same efficacy as 500 mg.¹³ The choice of FOLFOX6 was supported by initial investigations indicating a positive correlation between oxaliplatin dose intensity and response rate; however, the recent literature has failed to confirm these results. As first-line treatments, simplified FOLFOX6 and FOLFOX4 are characterized by similar objective response rates, notwithstanding the higher dose of platinum in FOLFOX6.^{17,18}

Twenty-five percent of our patients received local treatments for liver metastases, mainly because the treatment was associated with sufficient shrinkage of metastatic disease to render these treatment worthwhile. By contrast, our data regarding gefitinib as maintenance therapy do not indicate that it is particularly useful; the median time to progression in our patients was 7 months (range, 2.1-33 months; 95% CI, 6.2-9 months) compared with 8 to 9 months obtained with chemotherapy alone in historical series.^{17,18}

We found that the side effects of the combined regimen were similar to those obtained with other first-line oxaliplatin-containing regimens, with the marked exception of cutaneous side effects; however, these effects were mild and easier to manage than those associated with the use of cetuximab.^{19,20} PFS and OS were apparently similar to those reported in other trials on nonpretreated metastatic CRC patients.^{17,18}

It is interesting to compare our finding with those obtained with the monoclonal antibodies cetuximab and panitumumab against EGFR.^{19,21} Phase 2 trials of cetuximab in patients with pretreated advanced CRC produced a 10% response rate when used alone and a 23% response rate when used in combination with irinotecan in patients refractory to irinotecan.^{19,20} Cetuximab plus oxaliplatin or irinotecan-containing chemotherapy as first-line treatments are being investigated in clinical trials.

Although we had insufficient data to assess the prognostic utility of soluble EGFR titers, our observations do suggest that high levels of circulating EGFR may indicate better outcome. We are investigating the relation with other biologic variables to outcomes in patients receiving gefitinib.

Conclusions

The combination of gefitinib and oxaliplatin-containing chemotherapy shows promising first-line efficacy and manageable toxicity in patients with EGFR-positive advanced CRC. Further investigations concerning the 'crosstalk' of EGFR pathways should be conducted with the aim to propose tailored therapies.

REFERENCES

1. Olayioye MA, Neve RM, Lane HA, et al. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J*. 2000;19:3159–3167.
2. Woodburn JR. The epidermal growth factor receptor and its inhibition in cancer therapy. *Pharmacol Ther*. 1999;82:241–250.
3. Wakeling AE, Guy SP, Woodburn JR, et al. ZD1839: an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. *Cancer Res*. 2002;62:5749–5754.
4. Sirotnak FM, Zakowski MF, Miller VA, et al. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. *Clin Cancer Res*. 2000;6:4885–4892.
5. Xu JM, Azzariti A, Colucci G, et al. The effect of gefitinib (Iressa, ZD1839) in combination with oxaliplatin is schedule-dependent in colon cancer cell lines. *Cancer Chemother Pharmacol*. 2003;2:442–448.
6. Baselga J, Rischin D, Ranson M, et al. Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. *J Clin Oncol*. 2002;20:4292–4302.
7. Herbst RS. Targeted therapy using novel agents in the treatment of non-small-cell lung cancer. *Clin Lung Cancer*. 2002;3(Suppl 1):S30–38.
8. Kuo T, Fisher GA. Current status of small-molecule tyrosine kinase inhibitors targeting epidermal growth factor receptor in colorectal cancer. *Clin Colorectal Cancer*. 2005;5(Suppl 2):S62–70.
9. Kuo T, Cho CD, Halsey J, et al. Phase II study of gefitinib, fluorouracil, leucovorin, and oxaliplatin therapy in previously treated patients with metastatic colorectal cancer. *J Clin Oncol*. 2005;23:5613–5619.
10. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics*. 1979;35:549–556.
11. Cox DR. Regression models and life-tables (with discussion). *J R Stat Soc B*. 1972;34:187–220.
12. Nakagawa K, Tamura T, Negoro S, et al. Phase I pharmacokinetic trial of the selective oral epidermal growth factor receptor tyrosine kinase inhibitor gefitinib ('Iressa', ZD1839) in Japanese patients with solid malignant tumors. *Ann Oncol*. 2003;14:922–930.
13. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA*. 2003;290:2149–2158.
14. Cohen MH, Williams GA, Sridhara R, et al. FDA drug approval summary: gefitinib (ZD1839) (Iressa) tablets. *Oncologist*. 2003;8:303–306.
15. Rothenberg ML, LaFleur B, Levy DE, et al. Randomized phase II trial of the clinical and biological effects of two dose levels of gefitinib in patients with recurrent colorectal adenocarcinoma. *J Clin Oncol*. 2005;23:9265–9274.
16. Atkins D, Reiffen KA, Tegtmeier CL, et al. Immunohistochemical detection of EGFR in paraffin-embedded tumor tissues: variation in staining intensity due to choice of fixative and storage time of tissue sections. *J Histochem Cytochem*. 2004;52:893–901.
17. Tournigand C, Andre T, Achille E, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol*. 2004;22:229–237.
18. De Gramont A, Figuer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol*. 2000;18:2938–2947.
19. Saltz LB, Meropol NJ, Loehrer PJ SR, et al. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol*. 2004;22:1201–1208.
20. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med*. 2004;351:337–345.
21. Gibson TB, Ranganathan A, Grothey A. Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody in metastatic colorectal cancer. *Clin Colorectal Cancer*. 2006;6:29–31.