

Phase 1b Dose Escalation Study of Erlotinib in Combination with Infusional 5-Fluorouracil, Leucovorin, and Oxaliplatin in Patients with Advanced Solid Tumors

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Abstract Purpose: Erlotinib (Tarceva) is a potent epidermal growth factor receptor (HER1) inhibitor. Infusional 5-fluorouracil (5-FU), leucovorin, and oxaliplatin (FOLFOX) is a standard therapy for colorectal cancer. This trial assessed the maximum tolerated dose (MTD), safety, preliminary efficacy, and pharmacokinetics of erlotinib combined with FOLFOX.

Experimental Design: Patients with advanced solid tumors were sequentially enrolled into three cohorts (cohort 1: 100 mg/d erlotinib, 65 mg/m² oxaliplatin, 200 mg/m² leucovorin, 400 mg/m² bolus 5-FU, and 400 mg/m² continuous infusion 5-FU; cohort 2: oxaliplatin increased to 85 mg/m² and 5-FU infusion increased to 600 mg/m²; and cohort 3: erlotinib increased to 150 mg/d).

Results: Thirty-two patients were enrolled (23 with colorectal cancer): no dose-limiting toxicities (DLT) were observed in cohort 1. In cohort 2, two of nine patients experienced a DLT (both diarrhea). In cohort 3, two of nine patients had a DLT (diarrhea and staphylococcal septicemia). Cohort 3 determined the MTD cohort and expanded to 17 patients in total. The most common adverse events were diarrhea, nausea, stomatitis, and rash (primarily mild/moderate). No pharmacokinetics interactions were observed. One patient (colorectal cancer) had a complete response, seven patients had a partial response, and nine had stable disease.

Conclusions: The MTD was defined as follows: 150 mg/d erlotinib, 85 mg/m² oxaliplatin; 200 mg/m² leucovorin, 400 mg/m² bolus 5-FU, and 600 mg/m² infusion 5-FU. At the MTD, the combination was well tolerated and showed antitumor activity, warranting further investigation in patients with advanced colorectal cancer and other solid tumors.

During the past decade, advances in our understanding of the mechanisms underlying tumor cell progression have led to the development of several new anticancer agents that act on specific pharmacologic targets. These agents have the potential to improve patient outcomes without compromising tolerability. One therapeutic target is the human epidermal growth factor receptor (EGFR/HER1), known to play an important role in the proliferation and differentiation of normal cells (1). Dysregulation of HER1/EGFR-activated intracellular signaling is also involved in tumor cell progression (2, 3). HER1/EGFR is

overexpressed or dysregulated in many human cancers (4), including colorectal cancer (5), and in some cancers confers a poor prognosis (4, 6).

Targeting HER1/EGFR therefore represents a rational approach to treating many cancers, and several novel agents that inhibit HER1/EGFR are being investigated (2, 3). The anti-HER1/EGFR monoclonal antibody, cetuximab, has shown the feasibility of HER1/EGFR-specific agents in colorectal cancer (7, 8). Erlotinib is a highly potent, orally active, reversible inhibitor of the tyrosine-kinase domain of HER1/EGFR. Promising results from preclinical studies (9–11) led to investigation in phase I and II trials, which revealed that erlotinib monotherapy was active against a wide range of solid tumors (12–15), including colorectal cancer (16, 17). In a randomized, placebo-controlled, phase III trial in patients with advanced/refractory non-small cell lung cancer (NSCLC), erlotinib monotherapy showed statistically and clinically significant improvement in overall survival and progression-free survival as well as in time to deterioration of patient-reported symptoms (18). Based on these data, erlotinib was approved in the United States and many other countries for the second/third-line treatment of advanced NSCLC.

The combination of erlotinib and chemotherapy has also been investigated. Two randomized, phase III trials of erlotinib in combination with chemotherapy in first-line NSCLC failed to meet their primary end point of improved survival (19, 20). However, in both trials, patients who had never smoked

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experienced a marked survival benefit (19, 21). In a phase III, randomized trial in pancreatic cancer (PA.3), erlotinib plus gemcitabine significantly prolonged survival and progression-free survival compared with gemcitabine alone (22), which led to approval of erlotinib in the United States for the treatment of advanced pancreatic cancer in combination with gemcitabine.

Oxaliplatin, a platinum analogue with proven activity against colorectal cancer and other solid tumors (23–25) has, when added to 5-fluorouracil (5-FU) and leucovorin (folinic acid), achieved superior response rates and longer progression-free survival compared with 5-FU/leucovorin alone, both in the first-line (26) and the second-line treatment of colorectal cancer (27). The combination of oxaliplatin with infusional 5-FU/leucovorin (FOLFOX) is now established as the backbone of treatment for patients with metastatic colorectal cancer (mCRC).

Combining oxaliplatin-based regimens with an agent, such as erlotinib, with a complementary mechanism of action and clinical profile, is a promising approach that may further improve patient outcomes. This potential has recently been proven clinically using the anti-vascular endothelial growth factor monoclonal antibody, bevacizumab, which improves outcomes when added to first- and second-line FOLFOX therapy for mCRC (28, 29). The present study was initiated to determine the maximum tolerated dose (MTD) of erlotinib in combination with FOLFOX in patients with advanced solid tumors, such as colorectal cancer, for whom the combination might be appropriate. Patients with other solid tumors without additional chemotherapeutic options were also included. Secondary objectives were to evaluate pharmacokinetics, safety, and preliminary antitumor activity.

Patients and Methods

Patients. Patients with advanced solid tumors, such as colorectal cancer, with measurable or nonmeasurable disease, ≥ 18 years of age, an

Eastern Cooperative Oncology Group performance status of 0 to 1, and a life expectancy ≥ 12 weeks were eligible for the trial. A minimum of 4 weeks (6 weeks for nitrosurea or mitomycin) since previous chemotherapy and/or radiotherapy was required.

Exclusion criteria included the following: any previous treatment with HER-targeted therapy or 5-FU/oxaliplatin; more than one previous chemotherapy regimen for metastatic disease, previous severe reaction to fluoropyrimidine therapy, or known dihydropyrimidine dehydrogenase deficiency; and peripheral neuropathy grade 2 or higher on the National Cancer Institute-Common Toxicity Criteria scale version 2.0. Patients with significant abnormalities of the surface of the eye and central nervous system metastases were also excluded.

Trial design and treatment. This was a phase 1b, open-label, dose escalation study conducted in three centers. A minimum of six patients were to be enrolled into each of three dosage cohorts (described in Fig. 1). If fewer than two patients had a dose-limiting toxicity (DLT) after all patients in that cohort had completed one cycle of treatment, patients were recruited to the next dosage cohort. If two patients experienced a DLT, the cohort was expanded to nine patients. When the additional patients in the cohort had received one cycle of treatment, a decision was made whether to dose escalate, depending on the incidence of DLTs. The MTD was defined as the dose below the dosage cohort where more than one third of treated patients had DLTs in the first two treatment cycles. Cohort 3, which used the known MTD of erlotinib monotherapy and the standard FOLFOX4 regimen, was considered to be the MTD if no more than one third of patients experienced DLTs in the first two cycles. For confirmation of the MTD, the appropriate cohort was expanded to include at least 12 patients with colorectal cancer.

Due to overlapping toxicity profiles (diarrhea and dermatologic effects), the starting dose for erlotinib (100 mg/d) was lower than the MTD established for monotherapy (150 mg/d; ref. 30); initial doses of oxaliplatin and 5-FU (cohort 1; Fig. 1) were also lower than the standard or recommended combined doses (26, 27), but the FOLFOX4 regimen was used in cohorts 2 and 3.

Study drugs were administered as follows: oxaliplatin i.v. infusion (day 1), leucovorin continuous infusion over 120 min (days 1 and 2), 5-FU i.v. bolus over 15 min followed by a continuous infusion over 22 h (days 1 and 2), and erlotinib once daily oral dose (given before

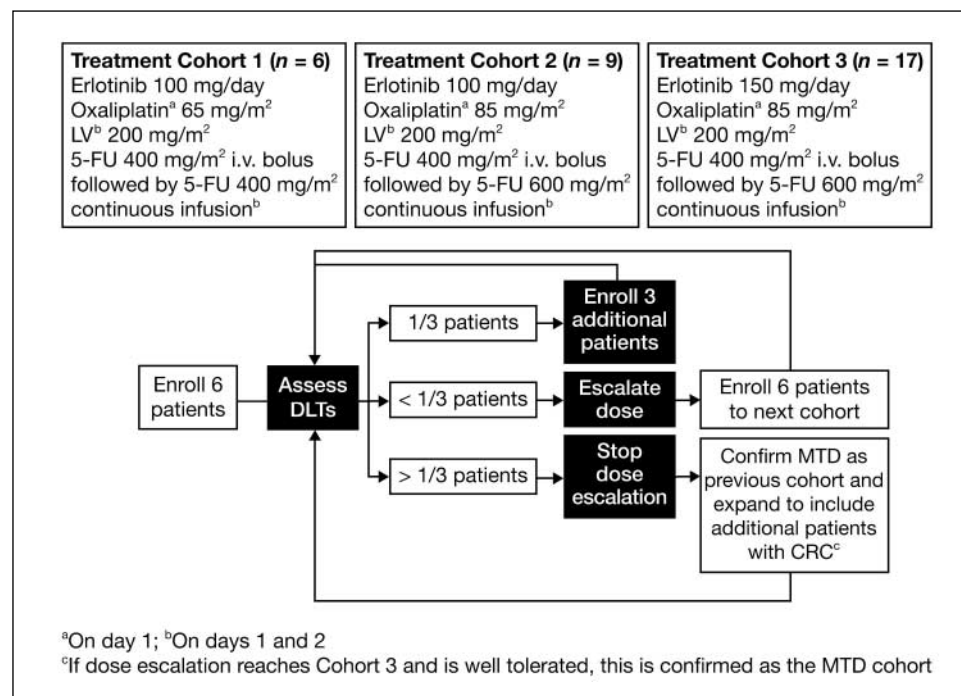


Fig. 1. Erlotinib in combination with FOLFOX4: study schema.

chemotherapy on days 1 and 2; for pharmacokinetics measurement purposes, in the first cycle only, erlotinib was started on day 3). The treatment cycle was repeated every 2 weeks. Dose modifications for toxicity were allowed. Patients received up to 12 cycles of combination treatment. Patients who experienced a tumor response or stable disease after 24 weeks were allowed to continue on the extension phase of the study and received combination treatment until disease progression or unacceptable toxicity.

The study was conducted according to the latest version of the Declaration of Helsinki and in compliance with the laws and regulations of the countries, in which the research was conducted. The study adhered to the Guideline for Good Clinical Practice and the protocol and amendments were submitted to Independent Ethics Committees. Written informed consent was obtained from all patients.

Study assessments. Baseline assessments included full clinical evaluation, laboratory assessments, and electrocardiogram. After initiation of treatment, physical examinations, vital signs, weight, performance status, and blood chemistry were assessed every 2 weeks. Hematology assessments were made weekly, and electrocardiogram was done when a patient withdrew/completed the study, and as needed.

Safety was assessed by the incidence and severity of adverse events using the National Cancer Institute-Common Toxicity Criteria version 2.0 and changes in laboratory values. Preexisting conditions that worsened during the course of the study were also reported as adverse events.

Tumor assessments were done at the end of every three cycles (6 weeks) and response was evaluated according to the Response Evaluation Criteria in Solid Tumors. A patient was defined as a responder if they had a complete response or partial response for 4 consecutive weeks at any time during treatment.

Where possible, a tumor tissue sample was provided within 3 months of enrollment for HER1/EGFR assessment. HER1/EGFR expression was assessed by immunohistochemistry using the antibody H11 (DAKO, Glostrup, Denmark) and graded by staining intensity (0, 1+, 2+, and 3+). A score of '0' was assigned for staining in <10% of tumor cells.

Dose-limiting toxicities. A DLT was defined as any of the following: grade 4 neutropenia for >5 days, thrombocytopenia ($\leq 25,000/\text{mm}^3$ thrombocytes or bleeding episodes requiring platelet transfusions), febrile neutropenia (defined as absolute neutrophil count $< 1.0 \times 10^9/\text{L}$ and temperature of $\geq 38.5^\circ\text{C}$), and any nonhematologic toxicity \geq grade 3 (excluding alopecia, inadequately treated diarrhea and vomiting).

Routine prophylactic treatment for DLTs (e.g., loperamide for diarrhea) was allowed, provided no dose modification of study drug was required. Criteria for dose modification or interruption were specified for different drugs depending on the nature and severity of toxicity.

Pharmacokinetic analyses. Blood samples were collected on days 1, 2, 3, 14, 15, 16, and 17. An additional sample was taken in the event of a serious adverse event or DLT. At designated time points, samples were collected for pharmacokinetics assessments of erlotinib, 5-FU, oxaliplatin, α -1 acid glycoprotein and protein binding.

Measurements of erlotinib and its metabolite OSI-420 were done using a validated and specific liquid chromatography-tandem mass spectrometry assay (MDS Pharma Services, Inc., St. Laurent, Quebec, Canada). Protein binding was assessed by equilibrium dialysis (MDS Pharma Services). α -1 Acid glycoprotein measurement was done by CRL-Medinet (Lenexa, KS) using a turbidometric immunoassay. 5-FU measurement was done by Advion BioSciences, Inc. (Ithaca, NY) using liquid chromatography-tandem mass spectrometry and oxaliplatin (total and free platinum) was measured by MDS Pharma Services (Montreal, Quebec, Canada) using an atomic absorption method.

Plasma concentration versus time data were analyzed by non-compartmental methods (WinNonlin version 4.0.1, Pharsight Corp., Mountain View, CA). Model-independent pharmacokinetic variables, as well as fraction unbound for erlotinib, were compared for each drug alone and in combination. The following pharmacokinetics

variables were evaluated for erlotinib, oxaliplatin, 5-FU, and certain metabolites: maximum observed concentration (C_{max}), time to peak concentration (T_{max}), area under the curve, and half-life. Results were summarized using data tabulations, descriptive statistics, and graphical presentations.

Results

Patient characteristics and treatment. A total of 32 patients with advanced solid tumors were recruited at three centers, between August 2002 and March 2004 (Table 1). Twenty-three (72%) patients had colorectal cancer.

All patients received at least two cycles of erlotinib in combination with FOLFOX. Overall exposure to trial medication was similar across the three cohorts. Erlotinib exposure ranged from 40 to 137 (cohort 1), 40 to 235 (cohort 2), and 12 to 235 days (cohort 3).

DLTs and MTD. No DLTs occurred in the six patients enrolled into cohort 1. Therefore, six patients were enrolled into cohort 2. During the first cycle of treatment, two of six patients experienced dose-limiting grade 3 diarrhea. Cohort 2 was expanded to a total of nine patients. No further DLTs were seen among the additional three patients during the first two cycles of treatment.

Subsequently, six patients were enrolled into cohort 3. Two patients had DLTs during the first two cycles of treatment. These were grade 3 diarrhea and staphylococcal sepsis, the latter resulting in death. A further three patients enrolled at this dose level did not experience DLTs. The dose used in cohort 3 (standard doses of FOLFOX4 and erlotinib) was therefore defined as the MTD according to the protocol (Fig. 1). The cohort was expanded to include an additional eight patients with colorectal cancer. In this expanded cohort, three of the eight additional patients had grade 3 toxicities during cycles 1 or 2. These included one case of grade 3 diarrhea and two of grade 3 anorexia (one with concurrent grade 3 asthenia).

Safety and tolerability. All patients in the study experienced at least one adverse event, but the majority of adverse events were mild to moderate in severity (grade 1 or 2). Overall, the most frequently reported adverse events were gastrointestinal and skin/s.c. disorders (91% and 88%, respectively). Respiratory, thoracic, and mediastinal disorders; infections and infestations; and metabolism and nutrition disorders were all more common with higher doses. The incidence of grade 3 or 4 adverse events was greater at higher doses. Only one patient in cohort 1 had grade 3 toxicity, whereas in cohorts 2 and 3, 56% and 76% of patients, respectively, experienced grade 3/4 events. Although not necessarily related to treatment, the most common grade 3/4 events were general disorders (such as asthenia), blood and lymphatic system disorders, and gastrointestinal disorders.

The incidence of adverse events considered to be related to the trial treatment was similar across the three dosage cohorts: 77%, 69%, and 80% in cohorts 1, 2, and 3, respectively. The most common treatment-related adverse events were diarrhea, nausea, stomatitis, and rash (Table 2).

There were four deaths during the study. One patient died as a result of progressive disease and three patients had fatal adverse events. Only one of the fatal adverse events was thought to be treatment related (staphylococcal sepsis); of the other two patients, one died from septic shock and one had a tumor hemorrhage.

Table 1. Baseline patient and disease characteristics

Characteristics	Cohort 1	Cohort 2	Cohort 3
Patients	6	9	17
Females/males	2/4	2/7	8/9
Age (y)			
Median (range)	65.5 (61-77)	64.0 (50-75)	65.0 (52-76)
Eastern Cooperative Oncology Group performance status (%)			
0	3 (50)	6 (67)	9 (53)
1	3 (50)	3 (33)	8 (47)
Prior therapy (%)			
Chemotherapy	3 (50)	8 (89)	12 (71)
Surgery	4 (67)	9 (100)	17 (100)
Radiotherapy	4 (67)	1 (11)	3 (18)
Primary tumor (%)			
Colorectal	1 (17)	8 (89)	14 (82)
Other*	5 (83)	1 (11)	3 (18)
Time from diagnosis (mo)			
Median (range)	9.60 (1.8-82.4) [†]	19.10 (1.1-59.2) [‡]	13.50 (2.0-71.8) [§]
Stage of disease (%)			
Local/regional	2 (33)	0	1 (6)
Metastatic	4 (67)	9 (100)	16 (94)

*Including penis carcinoma (two), NSCLC (one), melanoma (one), endometrial carcinoma (one), nasal adenocarcinoma (one), parotid gland carcinoma (one) urethral carcinoma (one), and unknown (one).

[†] Five evaluable patients.

[‡] Eight evaluable patients.

[§] Fifteen evaluable patients.

In total, 22 patients withdrew from the study. One patient withdrew due to adverse events (nausea and fatigue) considered possibly related to treatment. Twenty-one patients withdrew for reasons other than adverse events, including progressive disease ($n = 11$), protocol violation ($n = 1$), withdrawal of consent ($n = 8$), or other reasons ($n = 1$).

Pharmacokinetics. The pharmacokinetics profile for erlotinib is summarized in Table 3. There were no clear differences between the pharmacokinetics data collected from patients receiving erlotinib alone (cycle 1, day 14) and with concomitant FOLFOX4 (cycle 2, day 1). The mean plasma concentration-time curves for erlotinib, 5-FU, and total oxaliplatin (cohort 3) are shown in Fig. 2A, B, and C, respectively. These data show that erlotinib exposure is unaffected by concomitant administration of FOLFOX4. Likewise, 5-FU and total oxaliplatin exposure are unaffected by administration of erlotinib. Similarly, the free drug concentration of oxaliplatin did not seem to be affected by the presence of erlotinib (data not shown). The plasma concentrations of erlotinib metabolites (OSI-420 and OSI-413) during combination therapy were also consistent with those observed during erlotinib administration alone (data not shown).

Possible correlations between the level of erlotinib exposure (C_{max} or area under the curve) and particular clinically significant adverse events, such as rash and diarrhea, were evaluated for each patient in the first four study cycles (~2 months) of erlotinib therapy. No apparent relationship between the level of exposure and the intensity of adverse events was found. Erlotinib has been associated with elevated alanine aminotransferase and bilirubin. In this study, there was no apparent relationship between the level of erlotinib exposure and the degree of elevation of alanine aminotransferase or bilirubin (data not shown).

A relationship was evident between increasing predose levels of α -1 acid glycoprotein and increased erlotinib exposure (data not shown). However, due to the small numbers of patients studied, this finding was not conclusive. The fraction of unbound erlotinib seemed to remain constant regardless of the level of erlotinib exposure. Coadministration of FOLFOX did not affect the level of protein binding of erlotinib (data not shown).

Antitumor activity. Of 32 patients enrolled, 24 (75%) were evaluable for response (Table 4). No patients in cohort 1 achieved a complete response or partial response, but in cohorts 2 and 3, two (28.6%) and six (46%) patients, respectively, responded to treatment. One patient with colorectal cancer in cohort 3 had a complete response. A further nine (37.5%) patients had stable disease. The time to progression ranged from 43 to 119 days in cohort 1, 39 to 262 days in cohort 2, and 37 to 222 days in cohort 3.

Of the evaluable patients, data on HER1/EGFR status were available for 14 patients. One patient with a complete response had a HER1/EGFR score of 3+ and one with a partial response had a score of 2+, whereas four patients with partial responses had a score of 0. Two patients with stable disease had scores of 2+ and 3+. One patient with progressive disease scored 2+, whereas four patients with stable disease and one with progressive disease had a score of 0. There seemed to be no clear correlation between HER1/EGFR expression and clinical outcome. However, these results are inconclusive because of the small number of patients assessed.

Discussion

For many years, the standard chemotherapy regimen for patients with colorectal cancer was i.v. 5-FU/leucovorin (31). Although the combination achieved improved tumor response

Table 2. Summary of treatment-related adverse events in $\geq 15\%$ of patients

Adverse event	Cohort 1 (n = 6)		Cohort 2 (n = 9)		Cohort 3 (n = 17)	
	Grade		Grade		Grade	
	All (%)	3/4 (%)	All (%)	3/4 (%)	All (%)	3/4 (%)
Gastrointestinal disorders						
Diarrhea	3 (50)	0	9 (100)	3 (33)	14 (82)	2 (12)
Nausea	2 (33)	0	5 (56)	0	10 (59)	0
Stomatitis	0	0	5 (56)	0	9 (53)	0
Vomiting	2 (33)	0	2 (22)	0	6 (35)	0
Dyspepsia	1 (17)	0	3 (33)	0	3 (18)	0
Constipation	0	0	2 (22)	0	1 (6)	0
Hematochezia	1 (17)	0	0	0	0	0
Skin						
Rash	3 (50)	0	8 (89)	0	12 (71)	0
Alopecia	1 (17)	0	3 (33)	0	3 (18)	0
Acne	2 (33)	0	0	0	0	0
General disorders						
Fatigue	4 (67)	0	0	0	5 (29)	0
Asthenia	1 (17)	0	2 (22)	1 (11)	5 (29)	2 (12)
Lethargy	0	0	3 (33)	0	4 (24)	1 (6)
Mucosal inflammation	0	0	2 (22)	0	3 (18)	1 (6)
Nervous system disorders						
Parasthesia	1 (17)	0	6 (67)	0	7 (41)	0
Neuropathy	0	0	0	0	3 (18)	1 (6)
Dysethesia	0	0	2 (22)	0	2 (12)	0
Dysgesia	0	0	1 (11)	0	3 (18)	0
Respiratory, etc.						
Epitaxis	1 (17)	0	0	0	5 (29)	0
Hematologic						
Neutropenia	0	0	3 (33)	1 (11)	5 (29)	1 (6)
Thrombocytopenia	0	0	1 (11)	0	5 (29)	1 (6)
Anemia	1 (17)	0	0	0	1 (6)	0
Leukopenia	1 (17)	0	0	0	0	0
Lymphopenia	1 (17)	1	0	0	0	0
Metabolism and nutrition disorders						
Anorexia	0	0	0	0	8 (47)	4 (24)
Vascular disorders						
Petechiae	1 (17)	0	0	0	0	0
Renal and urinary						
Hematuria	2 (33)	0	0	0	1 (6)	0
Ear and labyrinth						
Vertigo	1 (11)	0	0	0	0	0

rates compared with 5-FU alone, this did not translate into significant improvements in survival for patients with mCRC (32, 33). More recently, the introduction of newer chemotherapeutic agents has begun to transform prospects for patients with mCRC. The combination of oxaliplatin with infusional

5-FU/leucovorin (FOLFOX) is now established as a standard regimen for the treatment of patients with mCRC.

Targeted therapies are also already established in the treatment of mCRC. Cetuximab produced significantly improved response rates when combined with irinotecan compared with cetuximab

Table 3. Mean (SE) pharmacokinetics variables of erlotinib given alone (cycle 1, day 14) or in combination with FOLFOX4 (cycle 2, day 1)

Variable (unit)	Oral erlotinib					
	Cohort 1 (n = 6)		Cohort 2 (n = 9)		Cohort 3 (n = 13)	
	Single agent	Combination	Single agent	Combination	Single agent	Combination
C_{max} (ng/mL)	1,519.50 \pm 257.06	1,460 \pm 159.54	1,380.11 \pm 184.99	1,296.33 \pm 145.86	2,831.38 \pm 488.91	2,834.69 \pm 502.07
T_{max} (h)	3.65 \pm 0.60	3.99 \pm 0.88	4.35 \pm 2.48	5.67 \pm 2.37	6.6 \pm 2.23	3.33 \pm 0.60
$AUC_{(0-24\text{ hr})}$ (ng·hr/mL)	22,809.48 \pm 4,256.52	22,741.60 \pm 4,004.25	18,853.62 \pm 2,924.06	19,945.63 \pm 2,646.45	40,982.29 \pm 8,162.19	45,278.58 \pm 8,837.97
$AUC_{(0-last)}$ (ng·hr/mL)	23,240.12 \pm 4,115.23	23,109.80 \pm 3,894.27	18,709.72 \pm 2,888.24	20,113.59 \pm 2,677.70	41,195.87 \pm 8,260.98	46,375.45 \pm 9,205.67
T_{last} (h)	24.65 \pm 0.43	24.41 \pm 0.38	23.70 \pm 0.30	24.23 \pm 0.13	24.18 \pm 0.18	24.47 \pm 0.16

Abbreviation: AUC, area under the curve.

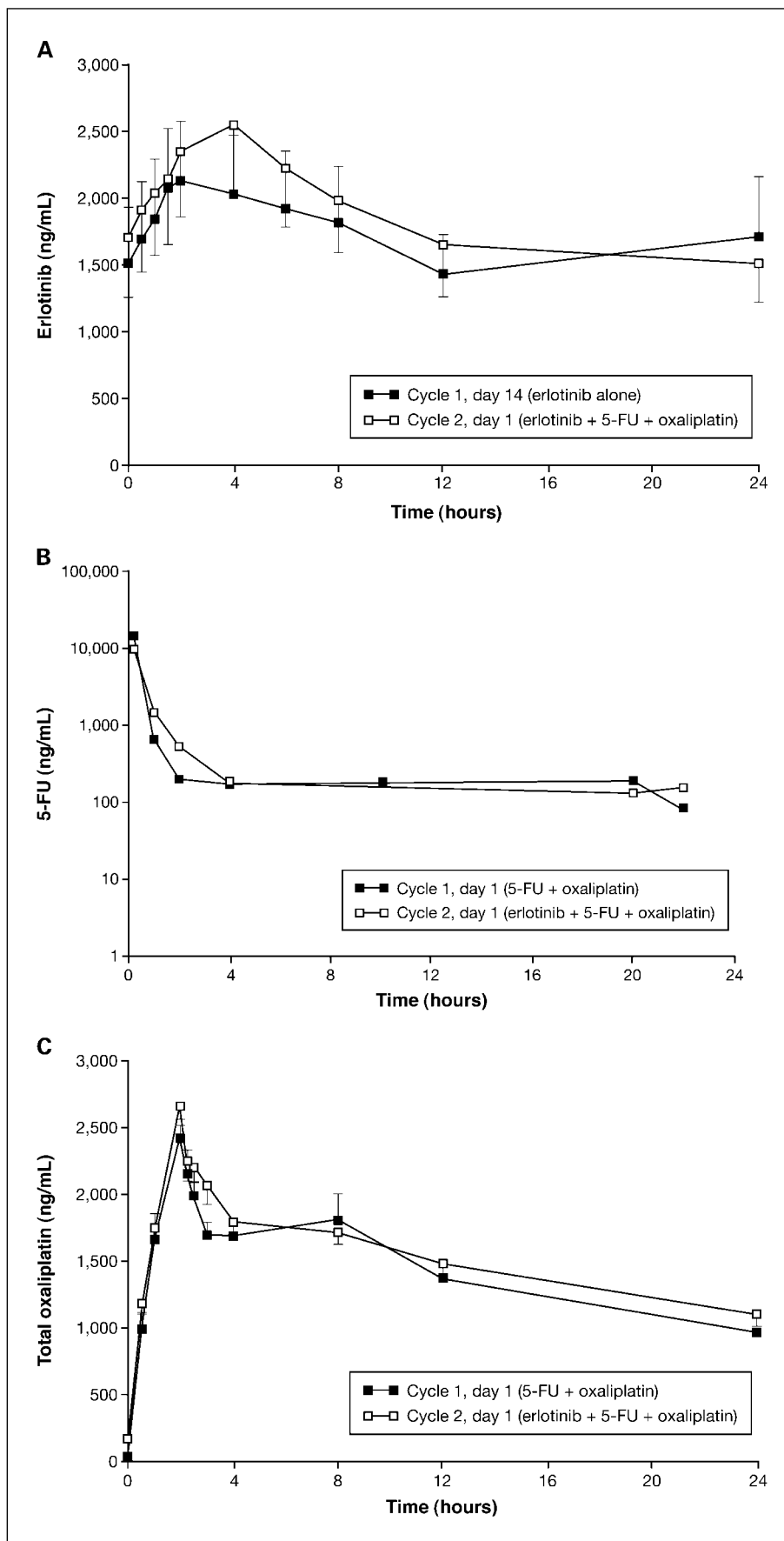


Fig. 2. Mean plasma concentration versus time for (A) erlotinib alone and in combination with FOLFOX4; (B) 5-FU (log mean); and (C) oxaliplatin in cohort 3. Data presented are only from subjects with data in both cycles ($n = 13$; B and C).

Table 4. Antitumor activity of erlotinib in combination with FOLFOX4

	No. patients (colorectal cancer patients)			
	Cohort 1	Cohort 2	Cohort 3	Total
No. evaluable patients	4 (0)	7 (6)	13 (10)	24 (16)
Complete response	0 (0)	0 (0)	1 (1)	1 (1)
Partial response	0 (0)	2 (2)	5 (4)	7 (6)
Stable disease	1 (0)	3 (3)	5 (3)	9 (6)
Progressive disease	3 (0)	2 (1)	2 (2)	7 (3)
Not evaluable	2	2	4	8
Total	6	9	17	32

alone (22.9% versus 10.8%, respectively; $P = 0.007$); progression-free survival was also significantly prolonged (4.1 versus 1.5 months; $P < 0.001$; ref. 7). In February 2004, cetuximab was approved in the United States for the treatment of patients with mCRC, either in combination with irinotecan (for patients refractory to irinotecan-based chemotherapy) or alone (for patients unsuitable for irinotecan). Furthermore, bevacizumab, in combination with chemotherapy, has shown impressive results. In a phase III trial in patients with previously untreated mCRC, the addition of bevacizumab to a chemotherapy regimen comprising irinotecan and 5-FU/leucovorin resulted in a statistically and clinically significant improvement in median survival compared with chemotherapy alone (20.3 versus 15.6 months; $P < 0.001$; ref. 34). Similar improvements have been observed when bevacizumab is added to 5-FU/leucovorin and FOLFOX4 (28, 35). These data have led to the approval of bevacizumab in combination with 5-FU-based chemotherapy for the first-line treatment of mCRC in many countries worldwide.

Combining erlotinib with FOLFOX also represents a feasible treatment option for patients with colorectal cancer and possibly other advanced solid tumors. Two phase II trials of erlotinib monotherapy in patients with mCRC have produced data, suggesting activity in this setting: in the first, 39% of patients experienced stable disease (16), whereas in the second, which enrolled patients who were treated second and third line, responses were seen in 8% of patients (17). The results of the current study show that when erlotinib was combined with the FOLFOX4 regimen used in cohorts 2 and 3, it was generally well tolerated, with no unexpected adverse events. The combination also showed preliminary evidence of antitumor activity.

The primary objective of this study was to determine the MTD for the combination. Dose escalation successfully reached cohort 3. A total of four patients (two in cohort 2 and two in cohort 3) experienced DLTs during the first two cycles of treatment: three grade 3 diarrhea and one staphylococcal sepsis. As this was within defined limits, cohort 3 was expanded to include a total of 12 patients with colorectal cancer and confirmed as the MTD cohort (as per the protocol definition): 150 mg/d erlotinib, 85 mg/m² oxaliplatin on day 1, 200 mg/m² leucovorin on days 1 and 2, and 400 mg/m² bolus 5-FU followed by 600 mg/m² continuous infusion 5-FU on days 1 and 2 of a 14-day cycle (FOLFOX4). It should be noted that the doses used in cohort 3 do not represent the MTD for this combination, as no further dose escalation was planned.

Whether higher doses of each drug in the combination would be tolerated is unknown.

Overall, the combination was generally well tolerated and there were no unpredicted adverse events (given the known tolerability profiles of the individual drugs). Adverse events were mainly mild to moderate in severity, and only one patient withdrew from the study because of an adverse event. The most common treatment-related adverse events were diarrhea, nausea, stomatitis, and rash. The occurrence of rash and diarrhea is not unexpected, as both are associated with HER1/EGFR inhibition (30), and have been observed in previous studies with erlotinib (12, 13, 18, 30, 36) as well as other HER1/EGFR inhibitors, such as gefitinib (37, 38). However, there was no apparent relationship between the level of erlotinib exposure and the severity and frequency of rash and diarrhea.

Rash has also been observed with 5-FU treatment, and both diarrhea and nausea have been seen with 5-FU and oxaliplatin (26, 32, 33). Thus, the overlap in toxicity profiles may have contributed to the severity and incidence of these adverse events. For this reason, lower than normally recommended starting doses for erlotinib, 5-FU and oxaliplatin were used. However, most side effects were mild to moderate in intensity in all three cohorts and doses were escalated to the known MTD for erlotinib (150 mg/d) and the standard recommended doses for oxaliplatin (85 mg/m²) and 5-FU (400 mg/m² bolus followed by 600 mg/m² continuous infusion 5-FU) when administered according to the FOLFOX4 regimen without causing an unacceptable incidence of DLTs. Thus, each of the drugs could be administered at a clinically active dose, maximizing the antitumor effect of the combination. The ability to use erlotinib at the MTD is particularly important as this allows maximal exposure of the tumor to the active agent (high exposure is thought to be necessary for adequate control of intracellular signaling; refs. 10, 39).

Neurologic toxicities (dysesthesia, paresthesia, and peripheral sensory neuropathy) were experienced by several patients. These events were primarily mild to moderate in severity. Neurotoxicity is commonly observed with oxaliplatin treatment (26). Hematologic toxicities are also seen with oxaliplatin and can be more pronounced in combination with 5-FU/leucovorin (26). In this study, mostly mild-to-moderate neutropenia and thrombocytopenia were observed, increasing in incidence at higher doses. The incidence and severity of these neurologic and hematologic events were as expected.

The pharmacokinetics analyses suggest that erlotinib exposure is unaffected by concomitant administration with FOLFOX4. Likewise, coadministration of erlotinib did not seem to affect 5-FU or oxaliplatin exposure. These results are encouraging because they indicate that the exposure to each drug (and therefore efficacy and safety) is unlikely to be altered when they are given in combination. The fact that the known clinically effective dose for each drug could be administered is further evidence that this combination can be administered at doses that achieve optimal exposure.

Other studies have investigated the potential of adding erlotinib to standard chemotherapy. In NSCLC, erlotinib has proven efficacy as monotherapy (18) and in combination with chemotherapy showed a survival benefit in a subgroup of never-smoking patients, but not overall (19–21). Erlotinib has

a proven survival advantage in pancreatic cancer when combined with gemcitabine (22). This result is important for a difficult to treat disease and further highlights the clinical benefits that can be achieved by combining a targeted agent with standard chemotherapy.

In the present study, the erlotinib plus FOLFOX4 combination showed encouraging preliminary evidence of antitumor activity in patients with mCRC. Of the 16 patients with mCRC evaluable for tumor response, 7 (44%) patients responded to treatment. In first-line advanced colorectal cancer, the addition of oxaliplatin to infusional 5-FU/leucovorin was showed previously to improve response rates from 22.3% to 50.7% ($P = 0.0001$) and progression-free survival from 6.2 to 9.0 months ($P = 0.0003$; ref. 26). In patients treated with FOLFOX4 second or third line, response rates are ~20% (28).

An area of ongoing investigation concerns the clinical outcomes that may be achieved by targeting more than one biological process, such as inhibition of both HER1/EGFR and vascular endothelial growth factor. In a phase I/II trial in patients with NSCLC, the combination of erlotinib and

bevacizumab was found to be well tolerated and showed encouraging antitumor activity (40). An ongoing phase III trial (DREAM) in mCRC will assess the efficacy and safety of combinations of first-line bevacizumab and erlotinib with oxaliplatin-containing chemotherapy. Although bevacizumab is already becoming an established first-line treatment option in mCRC, the DREAM trial and other ongoing studies may also confirm a future role for erlotinib in this setting.

In conclusion, the present study provides evidence that a targeted therapy, erlotinib, and a standard cytotoxic chemotherapy regimen, FOLFOX4, can be successfully combined. The combination was generally well tolerated and showed preliminary evidence of antitumor activity. Erlotinib in combination with FOLFOX4 has profound potential in the treatment of colorectal cancer, in particular, and warrants further clinical investigation.

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