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ORIGINAL ARTICLE

Phase I study of gefitinib, irinotecan, 5-fluorouracil and leucovorin in patients with metastatic colorectal cancer

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Abstract
Purpose To determine the maximum tolerated doses (MTD), toxicities, efficacy, and pharmacokinetics (PK) of gefitinib combined with irinotecan, 5-fluorouracil (5-FU) and leucovorin (IFL) in patients with previously untreated advanced colorectal cancer.
Experimental Design Starting doses were gefitinib 250 mg/day orally without interruption, irinotecan 100 mg/m^2 as a 90 min intravenous (i.v.) infusion, 5-FU 400 mg/m^2 bolus i.v. and leucovorin 20 mg/m^2 i.v. on days 1 and 8 of a 21-day cycle. Dose escalations involved increasing gefitinib to 500 mg then increasing irinotecan to 125 mg/m^2 and 5-FU to 500 mg/m^2.
Results Twenty-four patients received therapy. The starting doses proved to be the MTD, as attempts to increase the dose of either gefitinib or the chemotherapeutic agents resulted in dose-limiting toxicities. Gastrointestinal effects and bone marrow suppression were the principal toxicities; however, only 1/17 (6%) patients treated with the MTD had severe (grades 3–4) diarrhea and severe neutropenia occurred in only two (12%) patients. Partial responses occurred in 10/17 patients receiving the MTD and another five had stable disease. Median progression-free and overall survivals were 12.2 and 26.6 months, respectively. In ten patients treated with the MTD, the steady-state PK of gefitinib was not affected by IFL nor did gefitinib appear to influence the PK of either irinotecan or 5-FU.
Conclusions Gefitinib can be safely combined with an intermittent weekly schedule of IFL. Evidence of promising activity should encourage further clinical evaluation of epidermal growth factor receptor tyrosine kinase inhibitors, such as gefitinib, combined with multiagent chemotherapy for metastatic colorectal cancer.

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Introduction

Colorectal cancer is the fourth most common malignancy and second most frequent cause of cancer-related death in the United States, with 148,610 new cases and 55,170 deaths anticipated in 2006 [1]. The frequency of metastatic disease at the time of diagnosis is 19% [2] and metastases develop in nearly 30% of patients initially diagnosed with localized disease [3]. The addition of either irinotecan or oxaliplatin to treatment with a fluoropyrimidine has significantly

improved the efficacy of first-line cytotoxic chemotherapy for metastatic colorectal cancer [4]. However, median survival for these patients still remains under 2 years and the 5-year survival rate is less than 5%. Clearly, new treatment strategies need to be explored.

The introduction of molecularly targeted anticancer drugs into clinical practice has generated considerable interest in determining whether their use in combination with traditional cytotoxic chemotherapeutic agents is therapeutically advantageous. The epidermal growth factor receptor (EGFR) has emerged as an important target for therapeutic interventions in a variety of human cancers [5]. Alterations in the function of EGFR are associated with oncogenic transformation and abnormal regulation leads to cell growth, invasion, angiogenesis, and metastasis. In colorectal cancer, between 25 and 77% of tumors overexpress EGFR [6–8], which has been associated with a poorer prognosis [6, 8].

Monoclonal antibodies against EGFR have proven efficacious as monotherapy and in combination with irinotecan in patients previously treated for metastatic disease [9–12]. In contrast, synthetic, low molecular weight inhibitors of the EGFR intracellular domain, such as erlotinib and gefitinib, do not appear to have measurable activity against metastatic colorectal cancer as single agents [13–15], but may enhance the activity of cytotoxic chemotherapy [16, 17]. Gefitinib is an orally bioavailable anilinoquinazoline derivative that inhibits the EGFR tyrosine kinase. Further, preclinical evidence suggests a synergistic interaction between gefitinib and irinotecan [18–21]. We therefore conducted a phase I study combining gefitinib with bolus 5-fluorouracil (5-FU), irinotecan, and leucovorin (IFL) in patients with chemotherapy-naïve metastatic colorectal cancer to test the tolerability of the regimen, pharmacokinetics (PK) of each agent, and efficacy of this combination regimen.

Material and Methods

Eligibility

The protocol and informed consent document were approved by the Dana-Farber/Harvard Cancer Center Scientific Review Committee and Institutional Review Board and the Cancer Therapy Evaluation Program (CTEP) of the National Cancer Institute. All patients were required to sign written informed consent prior to participation in the study. Patients with histologically confirmed advanced colorectal adenocarcinoma without previous treatment were enrolled in this study. Eligibility criteria included: age at least 18 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; life expectancy greater than 12 weeks; measurable disease based on Response Evaluation Criteria In Solid Tumors (RECIST) [22]; and adequate hematological (white blood cell count $\geq$3,000 per deciliter, absolute neutrophil count $\geq$1,500 per deciliter, platelets $\geq$100,000 per deciliter), liver (total bilirubin in normal range; transaminases $\leq$five times upper limit of normal) and renal (creatinine within normal limits) function.

Patients were ineligible if they had prior chemotherapy for advanced colorectal cancer, prior radiotherapy to greater than 15% of bone marrow (standard radiation for rectal cancer excluded a patient), prior therapy with irinotecan, gefitinib or other anti-EGFR agents, uncontrolled other medical condition, predisposing colonic or small bowel disorder in which baseline pattern of greater than 3 loose stools per day (without a colostomy or ileostomy), or prior malignancy (except adequately treated basal or squamous cell skin cancer) unless disease-free for at least 5 years. Prior adjuvant chemotherapy for stage II or III disease was allowed, as long as 12 months had elapsed from the last treatment.

Drug administration and dose escalation

Gefitinib (Astra Zeneca, Wilmington, DE, USA) was supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute (Bethesda, MD, USA) as a yellow tablet containing 250 mg of the drug. Irinotecan, 5-FU, and leucovorin were obtained from commercial sources as the standard dosage form for injection. Gefitinib was given orally once every day without interruption. After receiving gefitinib alone for the initial 14 days, treatment was initiated with IFL given on two consecutive weeks followed by 1-week without dosing of the cytotoxic agents. A cycle was defined as 2 weeks of IFL followed by 1-week break from cytotoxic therapies (3-week cycles), although the first cycle also included a 2-week run-in period of gefitinib plus the 21-day cycle of IFL (5 weeks). Irinotecan was given as a 90-min continuous intravenous infusion followed by leucovorin as a 15-min intravenous infusion and bolus intravenous administration of 5-FU. Starting doses were gefitinib 250 mg, irinotecan 100 mg/m^2, 5-FU 400 mg/m^2 and leucovorin at 20 mg/m^2 (dose level 1). Dose escalations involved increasing the gefitinib dose to 500 mg, initially with the starting doses of the other drugs (dose level 2), then increasing the doses of irinotecan and 5-FU to 125 and 500 mg/m^2 (dose level 3), respectively.

Dose-limiting toxicities (DLT) were defined as toxicities experienced during the 5 weeks of the first cycle of treatment. DLTs included grade 4 neutropenia for greater than 3 days, any grade 4 neutropenia with a fever ≥100.5°F, grade 4 thrombocytopenia, grade 3 diarrhea requiring hospitalization or lasting more than 24 h despite aggressive anti-diarrheal therapy, any grade 4 diarrhea despite aggressive anti-diarrheal therapy, grade 4 vomiting despite optimal anti-emetic therapy, failure to administer full planned doses of irinotecan and 5-FU without dose delay or reduction during the first cycle or any other grade 3 or higher non-hematological toxicity (except nausea or alopecia).

Patients were enrolled into each dose level initially in cohorts of 3. Intrapatient dose escalation was not permitted. Escalation to the next dose level was permitted if all three patients treated at a given dose level were observed during cycle 1 without a DLT. In contrast, if two of the initial three patients experienced a DLT, then the previous dose was considered the maximum tolerated dose (MTD). However, if a dose-limiting toxicity was observed in one of the initial three patients, then three additional patients were treated at that dose level. The next dose level was administered if none of the three additional patients experienced a dose-limiting toxicity; otherwise, the previous dose was considered the MTD. Ten additional patients were treated at the MTD to further define the toxicity profile, better evaluate the tolerability of the regimen, and provide an adequate number of patients to achieve the objectives of the PK studies.

Toxicity classifications

All toxicities were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 2.0 [23]. Toxicities were assessed weekly for the first 8 weeks and then at least prior to administration of each week of IFL. Dose modifications during the first cycle were allowed only for DLTs whereas dose reductions were permitted for less severe toxicities that occurred during subsequent cycles of therapy. Because gefitinib is available as 250 mg tablets, a single dose reduction from 500 to 250 mg daily was provided in dose levels 2 and 3, whereas withdrawal from study was required if the minimal 250 mg daily dose was not tolerated. Gefitinib could be held for up to 14 consecutive days for skin toxicity, diarrhea, nausea or vomiting. Administration of the next cycle of IFL required the patient to have an absolute neutrophil count of 1,500/mm^3, diarrhea and other toxicities not greater than grade 1. The dose of irinotecan was reduced in increments of 25 mg/m^2 to a minimum dose of 50 mg/m^2. The 5-FU dose was reduced in increments of 100 mg/m^2 to a minimum dose of 200 mg/m^2. The leucovorin dose was not adjusted due to toxicity or 5-FU dose reduction. Patients that did not tolerate the minimum defined doses of irinotecan and/or 5-FU doses were removed from study.

Evaluation of response

Patients were required to have computed tomography of all measurable disease within 14 days before the first cycle of therapy. Over the course of the trial, therapeutic response was evaluated by computer tomography after the first 8 weeks of therapy (following completion of cycles 1 and 2) and then every 12 weeks thereafter. Patient response was classified according to RECIST criteria [22]. Responses were confirmed at least 4 weeks after documenting an initial response. Progression-free survival was defined as time from study entry to date of progression or death while on trial; patients who came off trial for other reasons were censored at the time of study withdrawal.

Pharmacokinetic studies

The PK of gefitinib, irinotecan, and 5-FU were characterized in a group of ten patients treated with the MTD of the combination regimen. Plasma samples were obtained immediately before dosing with gefitinib, when given alone during weeks 1 and 2, on days 1, 8, 15, and on two additional days between days 8 and 15. Plasma samples were also obtained before dosing with gefitinib on the day immediately after giving the first four infusions of irinotecan and 5-FU (days 16, 23, 37, and 44). Blood specimens (4 mL) were drawn from an arm vein into a tube with freeze-dried lithium heparin, mixed by inversion, centrifuged at 1,000×g for 10 min. The plasma was stored at −70°C until assayed. A validated analytical method based upon isocratic reversed-phase high performance liquid chromatography with electrospray ionization mass spectrometric detection was used to measure the concentration of gefitinib in plasma, as previously described [24] The lower limit of quantitation of the assay was 0.30 ng/mL. Precision of the assay as indicated by coefficients of variation for repeated determination of calibration standards with concentrations ranging from 0.50 to 1,000 ng/mL was ≤6.0% within-day and ≤5.2% between-day, respectively. Accuracy of the assay ranged from 91.0 to 97.7% across the entire concentration range of the calibration curve both within- and between-days.

Samples were also obtained to define the plasma concentration-time profiles of irinotecan and 5-FU for

the doses given on day 15. A sample was collected shortly before dosing and at the following times after beginning the irinotecan infusion: 0.5, 1.0, 1.5 (just before the end of the infusion), 2.0, 2.5, 3.3, 3.8, 5.5, 7.5, 24, and 30 h. The sampling schedule for 5-FU was as follows: 5, 10, 30, 45, 60, 90, and 120 min after bolus intravenous administration. At each time point, blood (8 mL) was drawn from a peripheral vein in the arm not used for infusing the drugs into a collection tube with freeze-dried sodium heparin, mixed by inversion, and promptly centrifuged for 10 min at 1,200×*g* and 4°C. The plasma was removed from the blood cells and stored at ≤−70°C. The total concentration (i.e., lactone plus carboxylate forms) of irinotecan and SN-38 was determined, as previously reported, by isocratic reversed-phase high performance liquid chromatography with fluorescence detection [25]. The concentration of SN-38 glucuronide was determined indirectly by measuring the total concentration of SN-38 liberated by hydrolysis with β-glucuronidase [26]. The lowest concentration of irinotecan included in the calibration curves, 10.0 ng/mL, was assayed with an accuracy of 111.2% and a precision of 4.2%. At all other concentrations, the between-day accuracy ranged from 95.3 to 102.0% and the precision ranged from 2.1 to 3.4%. The lowest concentration of SN-38 in the calibration curves, 2.50 ng/mL, was assayed with an accuracy of 105.2% and a precision of 7.0%. At all other concentrations, the between day accuracy ranged from 94.8 to 102.2% and the precision ranged from 1.9 to 5.4%. A validated analytical method based upon isocratic reversed-phase high performance liquid chromatography with UV detection, as previously reported, was used to measure the concentration of 5-FU in plasma [27]. The between-day accuracy was 107.4% at the 0.025 μg/mL lower limit of quantitation and 97.5–102.3% at all other concentrations. Corresponding values of the precision were 7.1% at the lower limit of quantitation and 1.7–13.7% at other concentrations.

The steady-state minimum concentration of gefitinib in plasma (C^{ss}_{min}) was calculated for each patient as the geometric mean of the determinations made before dosing on days 8–15 (without chemotherapy) and days 16–44 (with chemotherapy). Observations were excluded if the sample was collected after the administration of gefitinib or determined to be an outlier by Dixon's test. The overall mean C^{ss}_{min} for the group was calculated as the geometric mean of the individual patient values. The paired two-tailed *t*-test was used to compare the mean C^{ss}_{min} for gefitinib given alone and together with the chemotherapeutic agents using logarithmically transformed data. A value of $P < 0.05$ was considered to be significantly different.

Actual sample times were calculated from the beginning of the irinotecan and 5-FU infusions to the sample collection time. Plasma concentration-time curves of irinotecan, SN-38, and SN-38 glucuronide for individual patients were analyzed by standard non-compartmental methods using WinNonlin Professional 5.0 software (Pharsight Corp., Cary, NC, USA) [28]. Area under the plasma concentration-time curve (AUC) was estimated using the logarithmic-linear trapezoidal algorithm to the last data point, with extrapolation to time infinity using the estimated value of the slope of the terminal logarithmic-linear data. Plasma concentration-time curves for 5-FU were fit to model-independent equations for zero-order drug input and first-order elimination, with one or two exponential terms, by non-linear regression using WinNonlin Professional [27]. Final values of the iterated parameters in the best-fit equation for each plasma profile were used to calculate all PK variables according to standard equations [29]. PK parameters are reported as the geometric mean ± standard deviation of the estimated values for individual patients [30, 31]. Standard deviations for the geometric mean values were estimated by the jackknife method [32].

Immunohistochemistry of epidermal growth factor receptor

Paraffin-embedded tissue blocks were obtained at the time of resection of the primary tumor. Paraffin sections of tumor tissue were deparaffinized, incubated with 3% H_2O_2 (20 min) to block endogenous peroxidase, and then incubated with pepsin at 37°C (10 min). Protein block (Vector Laboratories, Burlingame, CA, USA) for 20 min was followed by application of primary anti-EGFR antibody (Zymed Laboratories, South San Francisco, CA, USA) (dilution 1:100; overnight at 4°C). Then, secondary anti-mouse antibody (Vector Laboratories) was applied (20 min), avidin biotin complex was added and sections were visualized by diaminobenzidine (5 min) and methyl-green counterstain. EGFR expression was recorded as negative (0), weakly positive (1+), positive (2+), or strongly positive (3+). Results were interpreted by a pathologist (S.O.) blinded from patients' identity and clinical outcomes data.

Results

Patient characteristics

Characteristics of the 25 patients enrolled into this phase I study of gefitinib in combination with IFL from September 2001 to June 2002 are summarized in

Table 1. One patient enrolled into dose level 2 was not evaluable because of a prolonged malignant small bowel obstruction that presented within hours after taking the first 500 mg dose of gefitinib. The median age of the patients was 57, three-quarters were males, and all but one were non-Hispanic Caucasian. Approximately half of all patients had a baseline ECOG performance status of 0 and 87% had received prior adjuvant therapy.

Determination of the maximum tolerated dose

At dose level 1 (gefitinib 250 mg/day, irinotecan 100 mg/m^2, bolus 5-FU 400 mg/m^2, and leucovorin 20 mg/m^2), one of the initial three patients experienced a DLT (grade 4 neutropenia). An additional three patients were treated at the same dose level and no further DLTs were detected. However, at dose level 2 (gefitinib increased to 500 mg/day with the same doses of IFL), two of four patients experienced DLTs. Both patients had grade 3 diarrhea and one patient also had grade 3 emesis. Four patients were treated at this dose level because 1 of the initial three experienced a small bowel obstruction after a single dose of gefitinib prior to IFL treatment and thus was replaced by an additional patient for the same cohort.

Due to the two DLTs at dose level 2, further treatment with 500 mg/day of gefitinib was discontinued and the protocol was amended to evaluate gefitinib 250 mg/day with an escalation of irinotecan and 5-FU to 125 and 500 mg/m^2, respectively (dose level 2a). However, two of the three patients treated with these doses of IFL experienced a DLT, both being grade 4 neutropenia. Consequently, dose level 1 was established as the MTD and an additional 11 patients were enrolled for treatment, which is one more than planned, because PK samples were not obtained from one of the patients in the expanded cohort at the MTD.

Table 1 Patient characteristics

Number of patients	24
Age (years)	
Median (range)	57 (36–74)
Gender	
Female	6 (25%)
Male	18 (75%)
ECOG Performance Status at study entry	
0	11 (46%)
1	13 (54%)
Prior adjuvant therapy	21 (87%)
Total bilirubin (ng/dL)	
Median (range)	0.4 (0.2–1.0)
CEA	
Median (range)	92 (4–5922)
Alkaline phosphatase	
Median (range)	102 (8–494)
Epidermal growth factor receptor immunohistochemistry[a]	
Negative	0%
Weakly positive (1+)	4%
Positive (2+)	21%
Strongly positive (3+)	33%
Not tested	42%

ECOG Eastern Cooperative Oncology Group, *CEA* carcinembryonic antigen

[a] EGFR testing was only performed for 14 patients based on tumor block availability

Toxicity

A total of 17 patients were treated with the MTD (dose level 1). The median number of cycles administered to these patients was ten (range 2–21 cycles). Treatment-related toxicities for all 24 patients, presented separately for the cohort evaluated at the MTD and the six patients treated at the two higher doses, are described in Table 2. The predominant adverse events were gastrointestinal toxicity and bone marrow suppression. Although most patients experienced diarrhea, the frequency of grade 3 diarrhea was appreciably lower among those treated at the MTD (6%) than the seven patients receiving the two higher dose levels (dose levels 2 or 2a) of the combination regimen (43%). Similarly, grade 3 or higher neutropenia occurred in 43% of patients receiving dose levels 2 or 2a as compared to 12% for those treated at dose level 1. Six patients experienced either a lower extremity deep venous thrombosis requiring anticoagulation ($n = 2$) or a pulmonary embolism ($n = 4$). Of these six patients, one had grade 2 vomiting and grade 3 diarrhea, one had grade 2 vomiting and one had grade 2 diarrhea with grade 1 nausea; the remaining three did not have significant gastrointestinal side effects. Each of these patients responded well to anticoagulation and none had to be removed from the study due to a thrombotic event.

Seven of the 17 patients (42%) treated with the MTD required dose reductions. Three of the patients (18%) had one dose reduction of irinotecan to 75 mg/m^2 and 5-FU to 300 mg/m^2. Four patients (24%) had irinotecan and 5-FU dose reduced twice, to 50 and 200 mg/m^2, respectively. One of these patients remained on study for over 1 year whereas the other three were removed from the study within 4 months due to disease progression or withdrawal of consent.

Efficacy and duration of treatment

Among the 17 patients treated at the MTD (dose level 1), 10 patients (59%) achieved a confirmed partial response (Table 3), with 1 additional patient having a

Table 2 Toxicity

Toxicity	Dose level 1[a] ($n = 17$)		Dose levels 2[b] and 2a[c] ($n = 7$)	
	Any grade	Grade 3/4	Any grade	Grade 3/4
Nausea	12 (71%)	0	4 (57%)	1 (14%)
Emesis	4 (24%)	0	2 (29%)	1 (14%)
Diarrhea	16 (94%)	1 (6%)	7 (100%)	3 (43%)
Abdominal cramps	7 (41%)	0	4 (57%)	0
Dehydration	1 (6%)	1 (6%)	2 (29%)	0
Neutropenia	11 (65%)	2 (12%)	3 (43%)	3 (43%)
Thrombocytopenia	2 (12%)	0	0	0
Fatigue	15 (88%)	0	6 (86%)	0
Mucositis	8 (47%)	0	1 (14%)	0
Anorexia	3 (18%)	0	4 (57%)	0
Skin rash	15 (88%)	0	6 (86%)	0
Thrombosis/pulmonary embolism	6 (35%)	5 (29%)	1 (14%)	1 (14%)

Based on worse grade toxicity in all cycles

[a] Dose level 1: Gefitinib 250 mg/day, irinotecan 100 mg/m^2 , bolus 5-FU 400 mg/m^2 , and leucovorin 20 mg/m^2

[b] Dose level 2: Gefitinib 500 mg/day, irinotecan 100 mg/m^2 , bolus 5-FU 400 mg/m^2 , and leucovorin 20 mg/m^2

[c] Dose level 2a: Gefitinib 250 mg/day, irinotecan 125 mg/m^2 , bolus 5-FU 500 mg/m^2 , and leucovorin 20 mg/m^2

Table 3 Efficacy of gefitinib, irinotecan, 5-FU and leucovorin

	All patients	Dose level 1 (MTD)
Number of patients	24	17
Response rate	46%	59%
Stable disease at least 12 weeks	38%	29%
Progression-free survival	12.2 months (95% CI, 6.9–14.7 months)	12.2 months (95% CI, 5.8–14.7 months)
Overall survival	23.0 months (95% CI, 16.0–30.7 months)	26.6 months (95% CI, 16.0–38.3 months)

partial response that was not confirmed (patient withdrew consent prior to confirmatory scan). The median duration of response was 8.5 months (95% confidence interval [CI], 5.7–13.4 months). Five patients (29%) had stable disease for at least 12 weeks. The median progression-free survival for these 17 patients was 12.2 months (95% CI, 5.8–14.7) and median overall survival was 26.6 months (95% CI, 16.0–38.3). As demonstrated in Table 3, the activity of this regimen was similar when data from the seven patients treated at the two higher dose levels was combined with that for patients evaluated at the MTD.

The degree of rash did not correlate with response rate. Tumor specimens were available for EGFR testing in 14 patients, of which 13 demonstrated moderate-to-strong positivity. Thus, we were unable to test the relationship between degree of EGFR staining and treatment efficacy.

Among all 24 evaluable patients, 11 (46%) came off study due to progression of disease. An additional three patients (13%) withdrew consent with evidence of disease progression that did not meet RECIST criteria requiring removal from the study. Seven patients (29%) stopped therapy due to toxicity from therapy. One patient who experienced a partial response underwent curative-intent surgery for liver metastases. Finally, two patients withdrew consent to pursue therapy at a center closer to their home. Among patients treated at the MTD (dose level 1), 53% of patients withdrew from study due to disease progression and 18% stopped due to treatment-related toxicity.

Pharmacokinetics

Pharmacokinetic data for 5-FU, irinotecan and gefitinib were obtained from a group of ten patients treated with the combination regimen. Mean values of selected PK parameters for each of the drugs are summarized in Table 4. The mean total body clearance (CL) of 5-FU 400 mg/m^2, 48.4 ± 15.7 L/h/m^2, was consistent with previously reported data from two clinical studies of the drug given as a short intravenous infusion demonstrating a dose-dependent decrease in the mean CL of 5-FU from 79.8 ± 13.9 to 34.5 ± 13.8 L/h/m^2 in adult cancer patients treated with doses ranging from 370 to 500 mg/m^2 [27, 33]. Similarly, mean PK variables for irinotecan,

Table 4 Mean pharmacokinetic parameters for ten patients treated at the maximum tolerated dose

Compound	Parameter	Mean ± SD
5-FU	C_{max} (nM)	291 ± 126
	$t_{1/2,z}$ (min)	10.0 ± 3.0
	CL (L/h/m^2)	48.4 ± 15.7
	V_z (L/m^2)	11.7 ± 4.6
Irinotecan	C_{max} (nM)	1666 ± 348
	$t_{1/2,z}$ (h)	6.3 ± 0.8
	CL (L/h/m^2)	14.1 ± 3.1
	V_z (L/m^2)	128.6 ± 29.2
SN-38	C_{max} (nM)	51 ± 24
	$t_{1/2,z}$ (h)	11.3 ± 11.8
	AUC (nM h)	620 ± 475
SN-38 glucuronides	C_{max} (nM)	112 ± 59
	$t_{1/2,z}$ (h)	11.9 ± 7.3
	AUC (nM h)	2071 ± 1339
Gefitinib	C^{ss}_{min} (ng/mL)	
	Prechemotherapy	258.2 ± 181.1
	Post-chemotherapy	260.3 ± 143.9*

C_{max} peak concentration in plasma, $t_{1/2,z}$ apparent biological half-life, CL total body clearance, V_z apparent total body volume of distribution, AUC area under the plasma concentration-time curve from zero to infinity, C^{ss}_{min} minimum concentration in plasma at steady-state for repeated dosing

*$P = 0.92$, for comparison of means using paired two-tailed t-test

its active metabolite SN-38, and the glucuronide conjugate of SN-38 were in excellent agreement with published data for irinotecan given alone or in combination with intravenous bolus 5-FU [25, 34, 35]. There was no difference ($P = 0.92$) between the mean C^{ss}_{min} of gefitinib resulting from oral administration of a 250 mg dose once every day either alone (258 ± 181 ng/mL) or together with irinotecan and 5-FU (260 ± 144 ng/mL).

Discussion

In this phase I study of patients with chemotherapy-naïve metastatic colorectal cancer, we determined that oral gefitinib at 250 mg daily could be safely combined with a weekly schedule of irinotecan, bolus 5-FU, and leucovorin (IFL). Diarrhea and neutropenia represented the principal DLT. The regimen at the MTD was considerably active, with a response rate of 59%, median progression-free survival of 12 months and median overall survival of greater than 2 years.

In 2000, the United States Food and Drug Administration approved combining irinotecan with 5-FU and leucovorin leading to a rapid change in paradigm of treating metastatic colorectal cancer. The original IFL (or "Saltz") regimen was four weekly treatments of intravenous irinotecan at 125 mg/m^2, bolus intravenous 5-FU at 500 mg/m^2, and bolus intravenous leucovorin at 20 mg/m^2, followed by a 2-week break period. This regimen led to a higher than anticipated number of treatment-related deaths when IFL was first widely utilized [36]. Variation of the original regimen include dose reductions of irinotecan and 5-FU to 100 and 400 mg/m^2, respectively, or switching to two weekly treatments followed by a 1-week rest [37, 38]. Consequently, in designing the current phase I study with gefitinib, the first dose level used slightly attenuated doses of both irinotecan and 5-FU as well as the modified 21-day cycle schedule. Of note, a recent National Cancer Institute combined cooperative group study found that such attenuated doses of irinotecan and 5-FU in the IFL regimen did not compromise treatment efficacy [39]. Nonetheless, IFL alone as first-line therapy has consistently demonstrated a 35–40% response rate, 7 months median progression-free survival and 15 months median overall survival [39, 40]. This current study suggests a promising improvement in efficacy with the addition of an oral EGFR inhibitor. In this current phase I study, a cohort of 17 patients ultimately received the MTD, thereby increasing the stability of the endpoints, including a 12-month progression-free survival.

Preclinical studies suggest considerable synergy when gefitinib is combined with irinotecan. Gefitinib has been shown to sensitize colon cancer cell lines to SN-38, the active metabolite of irinotecan, by increasing SN-38-mediated induction of protein-linked DNA single-strand breaks [18]. Synergy between irinotecan and gefitinib may be mediated by gefitinib inhibiting breast cancer resistance protein (ABCG2), a transporter that confers resistance to SN-38 [20, 21]. Thus, adding gefitinib to irinotecan-based therapy may have a dual role of improving the efficacy of irinotecan beyond inhibition of EGFR.

The major objective of the PK studies undertaken during this clinical trial was to ascertain whether the administration of irinotecan and 5-FU had any effect on the steady-state PK of gefitinib. It was found that the mean C^{ss}_{min} of gefitinib in a group of ten patients determined before and after initiating treatment with irinotecan and 5-FU were nearly identical and also in good agreement with previously reported data for similar doses of the drug given once every day [41, 42]. These findings suggest that the PK of gefitinib is not affected by the concurrent administration of irinotecan and 5-FU.

In a recently reported phase II clinical trial, cancer patients experienced unexpectedly severe toxicity when gefitinib 250 mg orally once a day was combined with the FOLFIRI regimen of irinotecan, infusional 5-FU and leucovorin [43]. The investigators proposed

that this may be the result of a PK interaction between gefitinib and irinotecan based upon the nature of the toxicity profile and preliminary reports indicating that gefitinib did not influence the tolerability or PK of 5-FU. The potential for a metabolic interaction between irinotecan and gefitinib has been evaluated in several preclinical studies. Gefitinib is extensively metabolized by human liver microsomes with cytochrome P-450 isoform 3A4 (CYP3A4) being the predominant enzyme involved in its metabolism [44, 45]. Nevertheless, formation of the major metabolite present in the plasma of humans treated with gefitinib, *O*-desmethyl-gefitinib, is catalyzed exclusively by CYP2D6. Although present in plasma at concentrations similar to gefitinib, the *O*-desmethyl compound is only a minor product of the in vitro microsomal metabolism of gefitinib [45]. CYP3A4 mediated hepatic metabolism appears to be a significant route of elimination for irinotecan in humans because its total body clearance is highly correlated with the clearance of midazolam, a CYP3A phenotyping probe drug [46]. It has been demonstrated that gefitinib decreases the total metabolic clearance of irinotecan in human liver microsomes when present at concentrations of at least 5 μM [47]. In comparison, daily oral administration of gefitinib 250 mg results in mean steady-state plasma concentrations that are only in the 0.51–0.74 μM range [42]. These in vitro studies suggest that gefitinib is unlikely to influence the PK of other drugs, such as irinotecan, for which oxidative hepatic metabolism represents an important elimination pathway because it does not significantly inhibit the activity of any CYP isoenzymes at systemically achievable concentrations [44, 47]. Furthermore, the total body clearance of intravenous irinotecan was shown to be unaffected by co-administering a single oral dose of gefitinib in mice [21].

Our clinical trial was not designed to specifically evaluate whether gefitinib influences the PK of irinotecan, as the patients did not receive the cytotoxic chemotherapeutic agents alone. However, mean values of the total body clearance of irinotecan and the area under the plasma concentration-time curves of SN-38 and SN-38 glucuronide were in close agreement with data from previously published studies of irinotecan given alone [34, 35]. Thus, consistent with evidence from preclinical studies, administering daily gefitinib does not appear to influence the PK of either irinotecan, the conversion of irinotecan to its active metabolite SN-38, or the subsequent glucuronidation of SN-38. Similarly, gefitinib, at the same dose and schedule used in our clinical trial, had no effect of the clinical PK of paclitaxel or docetaxel, both of which are metabolized by CYP3A4 [48–50].

The higher than expected rate of thrombosis in this trial is unclear. The IFL regimen was associated with a dehydration and thromboembolic syndrome when first widely utilized [36]. Three of the patients had significant gastrointestinal toxicities that may have predisposed them to dehydration and increased thrombosis. To our knowledge, gefitinib has not been reported to be associated with thrombosis, though it is possible the interaction of the four agents may lead to an increased risk. Gastrointestinal toxicity was the major side effect experienced by patients, particularly at dose levels 2 and 2a, which we presume was overlapping toxicities of gefitinib, 5-flurouracil and irinotecan.

The landscape of treatment for colorectal cancer has changed at a rapid pace during the past 5 years. Increasingly, first-line therapy for metastatic disease includes infusional 5-FU with either irinotecan or oxaliplatin in conjunction with bevacizumab, a monoclonal antibody to vascular endothelial growth factor [4]. In contrast, the use of intravenous bolus 5-FU with irinotecan is less commonly used, and, at the same time, the FDA has placed restrictions on the use of gefitinib due to a negative trial in non-small cell lung cancer [51, 52]. Nonetheless, this study provides important insights into the potential for combining an EGFR inhibitor with cytotoxic chemotherapy against metastatic colorectal cancer. Additional studies that add EGFR inhibitors to first-line chemotherapy for metastatic colorectal cancer should be encouraged.

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References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al (2006) Cancer statistics, 2006. CA Cancer J Clin 56(2):106–130
2. Ries L, Eisner M, Kosary C, Hankey B, Miller B, Clegg L, et al (2003) SEER Cancer statistics review, 1975–2000. Institute NC, editor. Bethesda, MD, 2003. p. http://seer.cancer.gov/csr/1975_2000/
3. Midgley R, Kerr D (2001) Conventional cytotoxic and novel therapeutic concepts in colorectal cancer. Expert Opin Investig Drugs 10(6):1011–1019
4. Meyerhardt JA, Mayer RJ (2005) Systemic therapy for colorectal cancer. N Engl J Med 352(5):476–487
5. Mendelson J (2000) Blockade of receptors for growth factors: an anticancer therapy—the fourth annual Joseph H Burchenal American association of cancer research clinical research award lecture. Clin Cancer Res 6(3):747–753
6. Mayer A, Takimoto M, Fritz E, Schellander G, Kofler K, Ludwig H (1993) The prognostic significance of proliferating cell nuclear antigen, epidermal growth factor receptor, and mdr gene expression in colorectal cancer. Cancer 71(8):2454–2460

7. Salomon DS, Brandt R, Ciardiello F, Normanno N (1995) Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 19(3):183–232
8. Hemming AW, Davis NL, Kluftinger A, Robinson B, Quenville NF, Liseman B, et al (1992) Prognostic markers of colorectal cancer: an evaluation of DNA content, epidermal growth factor receptor, and Ki-67. J Surg Oncol 51(3):147–152
9. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351(4):337–345
10. Saltz L, Rubin M, Hochster H, Tchekmeydian NS, Waksal H, Needle M, et al (2001) Cetuximab (IMC-C225) plus irinotecan (CPT-11) is active in CPT-11-refractory colorectal cancer (CRC) that expresses epidermal growth factor receptor (EGFR). Proc Am Soc Clin Oncol 20:3a
11. Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J, Mayer RJ (2004) Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 22(7):1201–1208
12. Malik I, Hecht JR, Patnaik A, Venook A, Berlin J, Croghan G, et al (2005) Safety and efficacy of panitumumab monotherapy in patients with metastatic colorectal cancer. Proc Am Soc Clin Oncol 23(16S):3520
13. Dorligschaw O, Kegel T, Jordan K, Harba A, Grothey A, Schmoll H-J (2003) ZD1839 (Iressa)-based treatment as last-line therapy in patients with advanced colorectal cancer. Proc Am Soc Clin Oncol 23:1494
14. Hidalgo M, Siu LL, Nemunaitis J, Rizzo J, Hammond LA, Takimoto C, et al (2001) Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. J Clin Oncol 19(13):3267–3279
15. Hightower M, Klem J, Lee D (2003) Epidermal growth factor receptor in not correlated with prognosis in patients with metastatic colorectal cancer treated with erlotinib (OSI-774, Tarceva). Clin Colorectal Cancer 3(1):10–11
16. Kuo T, Cho CD, Halsey J, Wakelee HA, Advani RH, Ford JM, et al (2005) Phase II study of gefitinib, fluorouracil, leucovorin, and oxaliplatin therapy in previously treated patients with metastatic colorectal cancer. J Clin Oncol 23(24):5613–5619
17. Meyerhardt JA, Zhu AX, Enzinger PC, Ryan DP, Clark JW, Kulke MH, et al (2006) Phase II study of capecitabine, oxaliplatin, and erlotinib in previously treated patients with metastastic colorectal cancer. J Clin Oncol 24(12):1892–1897
18. Braun AH, Stark K, Dirsch O, Hilger RA, Seeber S, Vanhoefer U (2005) The epidermal growth factor receptor tyrosine kinase inhibitor gefitinib sensitizes colon cancer cells to irinotecan. Anticancer Drugs 16(10):1099–1108
19. Koizumi F, Kanzawa F, Ueda Y, Koh Y, Tsukiyama S, Taguchi F, et al (2004) Synergistic interaction between the EGFR tyrosine kinase inhibitor gefitinib ("Iressa") and the DNA topoisomerase I inhibitor CPT-11 (irinotecan) in human colorectal cancer cells. Int J Cancer 108(3):464–472
20. Nakamura Y, Oka M, Soda H, Shiozawa K, Yoshikawa M, Itoh A, et al (2005) Gefitinib ("Iressa", ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, reverses breast cancer resistance protein/ABCG2-mediated drug resistance. Cancer Res 65(4):1541–1546
21. Stewart CF, Leggas M, Schuetz JD, Panetta JC, Cheshire PJ, Peterson J, et al (2004) Gefitinib enhances the antitumor activity and oral bioavailability of irinotecan in mice. Cancer Res 64(20):7491–7499
22. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al (2000) New guidelines to evaluate the response to treatment in solid tumors. European organization for research and treatment of cancer, national cancer institute of the United States, national cancer institute of Canada. J Natl Cancer Inst 92(3):205–216
23. Institute NC (2006) Common terminology criteria for adverse events (CTCAE) version 2.0. Cited 14 July 2006
24. Bai F, Iacono L, Johnston B, et al (2004) Determination of gefitinib in plasma by liquid chromatography with a C12 column and electrospray tandem mass spectrometry detection. J Liq Chromatogr 27:2743–2758
25. Gilbert MR, Supko JG, Batchelor T, Lesser G, Fisher JD, Piantadosi S, et al (2003) Phase I clinical and pharmacokinetic study of irinotecan in adults with recurrent malignant glioma. Clin Cancer Res 9(8):2940–2949
26. Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE, Ratain MJ (1994) Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. Cancer Res 54(14):3723–3725
27. Zhu AX, Puchalski TA, Stanton VP Jr, Ryan DP, Clark JW, Nesbitt S, et al (2004) Dihydropyrimidine dehydrogenase and thymidylate synthase polymorphisms and their association with 5-fluorouracil/leucovorin chemotherapy in colorectal cancer. Clin Colorectal Cancer 3(4):225–234
28. Gabrielsson JL, Weiner DL (1999) Methodology for pharmacokinetic/pharmacodynamic data analysis. Pharm Sci Technol Today 2(6):244–252
29. Gibaldi M, Perrier D (1982) Pharmacokinetics, 2nd edn. Marcel Dekker, NY
30. Mizuta E, Tsubotani A (1985) Preparation of mean drug concentration—time curves in plasma. A study on the frequency distribution of pharmacokinetic parameters. Chem Pharm Bull (Tokyo) 33(4):1620–1632
31. Lacey LF, Keene ON, Pritchard JF, Bye A (1997) Common noncompartmental pharmacokinetic variables: are they normally or log-normally distributed? J Biopharm Stat 7(1):171–178
32. Miller R (1974) The jacknife—a review. Biometrika 61:1–15
33. Grem JL, McAtee N, Murphy RF, Hamilton JM, Balis F, Steinberg S, et al (1994) Phase I and pharmacokinetic study of recombinant human granulocyte-macrophage colony-stimulating factor given in combination with fluorouracil plus calcium leucovorin in metastatic gastrointestinal adenocarcinoma. J Clin Oncol 12(3):560–568
34. Saltz LB, Kanowitz J, Kemeny NE, Schaaf L, Spriggs D, Staton BA, et al (1996) Phase I clinical and pharmacokinetic study of irinotecan, fluorouracil, and leucovorin in patients with advanced solid tumors. J Clin Oncol 14(11):2959–2967
35. Gupta E, Mick R, Ramirez J, Wang X, Lestingi TM, Vokes EE, et al (1997) Pharmacokinetic and pharmacodynamic evaluation of the topoisomerase inhibitor irinotecan in cancer patients. J Clin Oncol 15(4):1502–1510
36. Rothenberg ML, Meropol NJ, Poplin EA, Van Cutsem E, Wadler S (2001) Mortality associated with irinotecan plus bolus fluorouracil/leucovorin: summary findings of an independent panel. J Clin Oncol 19(18):3801–3807
37. Goldberg RM (1998) Is repeated treatment with a 5-fluorouracil-based regimen useful in colorectal cancer? Semin Oncol 25(5 Suppl 11):21–28
38. Hwang JJ, Eisenberg SG, Marshall JL (2003) Improving the toxicity of irinotecan/5-FU/leucovorin: a 21-day schedule. Oncology (Huntingt) 17(9 Suppl 8):37–43
39. Goldberg RM, Sargent DJ, Morton RF, Fuchs C, Ramanathan RK, Williamson SK, et al (2004) N9741: FOLFOX (oxaliplatin(Oxal)/5-fluorouracil (5-FU)/leucovorin (LV) or

reduced dose R-IFL (CPT-11 + 5-FU/LV) in advanced colorectal cancer (CRC): final efficacy data from an intergroup study. J Clin Oncol ASCO Annu Meet Proc 22(14S):3621
40. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, et al (2000) Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan study group. N Engl J Med 343(13):905–914
41. Herbst RS, Maddox AM, Rothenberg ML, Small EJ, Rubin EH, Baselga J, et al (2002) Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumors: results of a phase I trial. J Clin Oncol 20(18):3815–3825
42. Ranson M, Hammond LA, Ferry D, Kris M, Tullo A, Murray PI, et al (2002) ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. J Clin Oncol 20(9):2240–2250
43. Veronese ML, Sun W, Giantonio B, Berlin J, Shults J, Davis L, et al (2005) A phase II trial of gefitinib with 5-fluorouracil, leucovorin, and irinotecan in patients with colorectal cancer. Br J Cancer 92(10):1846–1849
44. Swaisland HC, Ranson M, Smith RP, Leadbetter J, Laight A, McKillop D, et al (2005) Pharmacokinetic drug interactions of gefitinib with rifampicin, itraconazole and metoprolol. Clin Pharmacokinet 44(10):1067–1081
45. McKillop D, McCormick AD, Millar A, Miles GS, Phillips PJ, Hutchison M (2005) Cytochrome P450-dependent metabolism of gefitinib. Xenobiotica 35(1):39–50
46. Mathijssen RH, de Jong FA, van Schaik RH, Lepper ER, Friberg LE, Rietveld T, et al (2004) Prediction of irinotecan pharmacokinetics by use of cytochrome P450 3A4 phenotyping probes. J Natl Cancer Inst 96(21):1585–1592
47. Fujita K, Ando Y, Narabayashi M, Miya T, Nagashima F, Yamamoto W, et al (2005) Gefitinib (Iressa) inhibits the CYP3A4-mediated formation of 7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin but activates that of 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin from irinotecan. Drug Metab Dispos 33(12):1785–1790
48. Miller VA, Johnson DH, Krug LM, Pizzo B, Tyson L, Perez W, et al (2003) Pilot trial of the epidermal growth factor receptor tyrosine kinase inhibitor gefitinib plus carboplatin and paclitaxel in patients with stage IIIB or IV non-small-cell lung cancer. J Clin Oncol 21(11):2094–2100
49. Manegold C, Gatzemeier U, Buchholz E, Smith RP, Fandi A (2005) A pilot trial of gefitinib in combination with docetaxel in patients with locally advanced or metastatic non-small-cell lung cancer. Clin Lung Cancer 6(6):343–349
50. Rowinsky EK (1997) The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. Annu Rev Med 48:353–374
51. Twombly R (2005) FDA Oncology Committee debates Iressa's status following negative trial results. J Natl Cancer Inst 97(7):473
52. Twombly R (2005) Failing survival advantage in crucial trial, future of Iressa is in jeopardy. J Natl Cancer Inst 97(4):249–250