

## Phase I Pharmacokinetic/Pharmacodynamic Study of EKB-569, an Irreversible Inhibitor of the Epidermal Growth Factor Receptor Tyrosine Kinase, in Combination with Irinotecan, 5-Fluorouracil, and Leucovorin (FOLFIRI) in First-Line Treatment of Patients with Metastatic Colorectal Cancer

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**Abstract Purpose:** To determine the recommended dose (RD) of EKB-569, an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, in combination with FOLFIRI chemotherapy in patients with metastatic colorectal cancer (mCRC).

**Methods:** Patients with previously untreated mCRC received FOLFIRI and EKB-569 at doses of 10, 25, 50, and 75 mg/day (EKB started on day 3). Three sequential skin biopsies were obtained in selected patients to assess the pharmacodynamic effects on EGFR signaling of FOLFIRI alone and with EKB-569. Complete pharmacokinetic sampling and tumor biopsies, when feasible, were conducted.

**Results:** Forty-seven patients were enrolled. Dose-limiting toxicities (grade 3 diarrhea or asthenia) were observed in 1/7 patients at 50 mg EKB-569 and in 2/3 at 75 mg. Two additional dose levels (35 mg EKB-569/day and 50 mg EKB-569/day plus modified FOLFIRI) were evaluated. The RD was 25 mg EKB-569/full dose FOLFIRI. Grades 3 to 4 toxicities in >10% of patients were diarrhea (30%), neutropenia (21%), and asthenia (17%). Twenty-one patients had an objective response [48%; 95% confidence interval (95% CI), 32-65%]. The median time to tumor progression was 7.7 months. At the RD, EKB-569 resulted in complete inhibition of phosphorylated EGFR (pEGFR) and downstream receptor signaling in skin and tumor samples. FOLFIRI alone did not affect pEGFR, but inhibited epidermal proliferation and activated mitogen-activated protein kinase (MAPK) and induced p27 expression in the skin.

**Conclusion:** The RD of EKB-569 is 25 mg/day when combined with FOLFIRI and results in complete EGFR inhibition. Chemotherapy alone interferes with pharmacodynamic markers, an observation to be taken into account in future studies of targeted agents with chemotherapy.

Colorectal cancer is the second most frequent cause of cancer-related death worldwide, and patients with metastatic colorectal cancer (mCRC) have a median survival of 8.5 months without chemotherapy (1). Current standard chemotherapy regimens such as FOLFIRI [irinotecan/5-fluorouracil (5-FU)/leucovorin (LV)] or FOLFOX (oxaliplatin/5-FU/LV) induce response rates of about 50%, prolong progression-free survival to about 8 months, and increase the median overall survival to 20 months (2-5). Despite these advances, the long-term prognosis of patients with mCRC remains poor, with <5%

surviving 5 years. Therefore, new treatment strategies have to be explored to improve the prognosis of this disease.

The epidermal growth factor receptor (EGFR) has emerged as an important therapeutic target in a variety of cancers including mCRC. Two different approaches have been clinically developed to target the EGFR: monoclonal antibodies (i.e., cetuximab and panitumumab) directed to the ectodomain of the receptor that have shown meaningful clinical activity in patients with mCRC refractory to standard chemotherapy (6); and small-molecule tyrosine kinase inhibitors (TKI).

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EKB-569 is an p.o. administered irreversible EGFR TKI that inhibits the growth of EGFR-overexpressing tumor cell lines (7). A recently published phase I study with EKB-569 monotherapy established the maximum tolerated dose (MTD) at 75 mg/day, the dose-limiting toxicity (DLT) being diarrhea. In this study, two heavily pretreated patients with mCRC achieved prolonged tumor stabilization (8).

Because of the known synergistic clinical effect of anti-EGFR agents in combination with irinotecan (9), we aimed to perform a pharmacokinetic/pharmacodynamic phase I trial of EKB-569 in combination with FOLFIRI in patients with mCRC. The objectives of this study were to assess the optimal recommended dose (RD) of this combination based on the safety profile and the molecular pharmacodynamic effects in sequential skin biopsies of FOLFIRI alone and EKB-569 in combination with FOLFIRI.

## Patients and Methods

**Inclusion criteria.** Patients with histologically or cytologically confirmed mCRC were enrolled. Prior chemotherapy for metastatic disease, adjuvant chemotherapy within the last 6 months of study entry, and prior treatment with EGFR inhibitors were not allowed. At study entry, Eastern Cooperative Oncology Group performance status (ECOG PS) of  $\leq 2$ , bilirubin  $\leq 1.5 \times$  upper limit of normal (ULN), aspartate aminotransferase (AST)/alanine aminotransferase (ALT)  $\leq 3 \times$  ULN (or  $\leq 5 \times$  ULN in case of liver metastases), alkaline phosphatase  $\leq 3 \times$  ULN, creatinine  $\leq 1.25 \times$  ULN ( $< 1.5 \times$  ULN and calculated creatinine clearance  $\geq 60$  mL/min), hemoglobin  $\geq 8.5$  mg/dL, absolute neutrophil count  $\geq 1500/\text{mm}^3$ , and platelet count  $\geq 100,000/\text{mm}^3$  were required. Pregnant or nursing women or patients without adequate contraception, patients with chronic inflammatory bowel disease, symptomatic cerebral metastases, or other severe concomitant diseases were excluded from this trial. All patients had signed a written informed consent. The

study was reviewed by the local Ethics Committees and done according to the Helsinki Declaration.

**Treatment and response evaluation.** Patients were treated in 28-day cycles. EKB-569 (Wyeth Pharmaceuticals) was administered p.o. at doses of 10, 25, 50, and 75 mg once daily with food, starting on day 3 of the first cycle. On days 1 and 15, patients received FOLFIRI [180 mg/m<sup>2</sup> irinotecan (1.5 h, i.v.), 400 mg/m<sup>2</sup> LV (2 h, i.v.), 400 mg/m<sup>2</sup> 5-FU bolus i.v., and 2,400 mg/m<sup>2</sup> 5-FU infusion]. At each dose level, enrollment of three to six patients was planned. The dose was escalated if none of the three patients experienced a DLT during the first cycle. If one of three patients had a DLT, additional three patients were enrolled at this level, and dose was escalated if no additional patient experienced a DLT. Otherwise, the escalation was stopped, and the last dose was considered the MTD. Once the MTD was reached and to better characterize the RD, 15 more patients were to be treated at this dose level.

All patients who received at least one dose of FOLFIRI or EKB-569 were evaluated for toxicity using the National Cancer Institute CTC v2.0. DLT was defined as febrile neutropenia, grade 4 neutropenia or thrombocytopenia lasting  $\geq 7$  days, grade 3 diarrhea of  $\geq 2$  days duration despite appropriate medical therapy (i.e., loperamide) or of any duration when associated with fever or dehydration, and any other nonhematologic EKB-569/FOLFIRI-related grade 3/4 toxicity (except grade 3 nausea, vomiting, or constipation, unless resistant to medical therapy). Because of the toxicities observed in cohorts of 50 and 75 mg EKB-569/day, the protocol was amended to expand the dose escalation by two additional cohorts: 35 mg EKB-569/day plus FOLFIRI and 50 mg EKB-569/day plus modified FOLFIRI (300 mg/m<sup>2</sup> 5-FU bolus, 2000 mg/m<sup>2</sup> 5-FU infusion). Treatment was planned for six cycles, with an option to continue the combined treatment or EKB-569 in case of clinical benefit.

**Pharmacokinetics.** Venous blood samples were collected for pharmacokinetic analysis to determine the concentration of EKB-569 and its *N*-desmethyl metabolite in the 25-mg EKB-569/FOLFIRI, 35-mg EKB-569/FOLFIRI, and 50-mg EKB-569/modified FOLFIRI cohorts on day 15 of cycle 1 at 0, 1.5, 2.5, 4, 6, 8, and 24 h. Concentrations were measured simultaneously in EDTA-treated plasma using a validated liquid chromatography/tandem mass spectrometry procedure.

**Table 1.** Patients baseline characteristics and treatment exposure

Dose level	1	2 and MTD	3	4	Intermediate	Modified FOLFIRI	Total
Treatment							
EKB-569 dose (mg)	10	25	50	75	35	50	
Chemotherapy	FOLFIRI	FOLFIRI	FOLFIRI	FOLFIRI	FOLFIRI	Modified FOLFIRI*	
Patients evaluable/enrolled	4/4	22/22 <sup>†</sup>	6/7	3/3	7/7	4/4	46/47
Age (y), median (range)	54.5 (49-60)	66.5 (41-77)	57 (19-66)	57 (53-65)	65 (56-69)	70.5 (62-72)	65 (19-77)
Gender (male/female)	3/1	15/7	4/3	3/0	5/2	3/1	33/14
ECOG status (n)							
0	2	12	4	2	7	3	30 (64%)
1	2	10	2	1	0	1	16 (34%)
2	0	0	1	0	0	0	1 (2%)
Prior chemotherapy <sup>‡</sup>	2	4	0	0	1	1	8 (17%)
Prior radiotherapy	0	3	0	0	1	0	4 (9%)
Treatment exposure							
Median number of EKB-569 treatment cycles	10.5	5.0	4.0	10.0	5.0	1.5	5.0
Median duration of EKB-569 treatment (wk)	43.2	19.0	15.9	47.6	20.6	6.6	20.2
Patients with dose reduction for EKB-569	0	0	1	3	3	1	8 (17%)
Patients with dose reduction for FOLFIRI	2	13 (62%)	4	3	4	2	28 (61%)

Abbreviation: DL, dose level.

\*Modified FOLFIRI: 300 mg/m<sup>2</sup> 5-FU bolus instead of 400 mg/m<sup>2</sup>, 2,000 mg/m<sup>2</sup> 5-FU infusion instead of 2,400 mg/m<sup>2</sup>.

<sup>†</sup>DL2: 5 patients; MTD cohort: 17 patients.

<sup>‡</sup>5-FU containing chemotherapy as adjuvant treatment or radiosensitizer. No patient received previously irinotecan, oxaliplatin, or therapy for metastatic disease.

**Table 2.** Toxicity

	EKB-569 dose level						Total (n = 47), n (%)
	10 mg (n = 4)	25 mg (n = 22), n (%)	35 mg (n = 7)	50 mg (mod)* (n = 4)	50 mg (n = 7)	75 mg (n = 3)	
Number of DLTs (first 28 d)	0	0	1	2	1	2	6
All grade toxicity †							
All adverse events, n (%)	4 (100)	22 (100)	7 (100)	4 (100)	7 (100)	3 (100)	47 (100)
Diarrhea	4	19 (86)	7	4	7	3	44 (94)
Asthenia	3	20 (91)	4	3	6	2	38 (81)
Nausea	4	17 (77)	4	2	5	1	33 (70)
Neutropenia	2	11 (50)	4	2	3	2	24 (51)
Vomiting	2	9 (41)	2	2	4	1	20 (43)
Anorexia	2	10 (45)	3	1	3	1	20 (43)
Alopecia	4	9 (41)	2	1	1	2	19 (40)
Stomatitis/mucositis	4	7 (32)	2	1	4	1	19 (40)
Rash	1	6 (27)	2	3	4	2	18 (38)
Abdominal pain	1	7 (32)	2	2	1	2	15 (32)
Anemia	0	6 (27)	1	1	1	1	10 (21)
Grade ≥3 toxicity ‡							
All grade ≥3 adverse events, n (%)	3 (75)	20 (91)	6 (86)	3 (75)	4 (57)	3 (100)	39 (83)
Diarrhea	0	4 (18)	2	2	3	3	14 (30)
Neutropenia	1	5 (23)	2	0	2	0	10 (21)
Asthenia	0	5 (23)	1	1	1	0	8 (17)
Hypertension	0	2 (9)	1	0	1	0	4 (9)
Deep vein thrombosis	0	2 (9)	1	0	1	0	4 (9)
Pulmonary embolism	0	2 (9)	1	0	0	0	3 (6)
Hyperglycemia	0	2 (9)	0	0	1	0	3 (6)
Nausea	0	2 (9)	0	0	1	0	3 (6)
Infection	0	1 (5)	0	0	0	1	2 (4)
Anemia	0	2 (9)	0	0	0	0	2 (4)

\*Modified FOLFIRI: 300 mg/m<sup>2</sup> 5-FU bolus instead of 400 mg/m<sup>2</sup>; 2,000 mg/m<sup>2</sup> 5-FU infusion instead of 2,400 mg/m<sup>2</sup>.

† Regardless of causality, occurring in ≥20% of patients.

‡ Occurring in >2.5% of patients.

**Pharmacodynamic evaluation.** The pharmacodynamic evaluation was done at one of the sites (Vall d'Hebron University Hospital) in patients who separately consented. Skin (punch needle) biopsies were to be obtained at screening, after the first administration of FOLFIRI and before the first dosing of EKB-569 on day 3, and at the end of the second cycle (between day 22 of cycle 2 and the start of cycle 3). Tumor (CT scan-guided) biopsies were obtained at screening and at the end of the second cycle. All biopsies were analyzed as previously described (10).

**End points.** The primary aim of the study was to evaluate the safety, tolerability, and the MTD of EKB-569 in combination with FOLFIRI. Secondary end points were to evaluate the pharmacokinetic profile of EKB-569, to obtain preliminary antitumor efficacy data (response rate

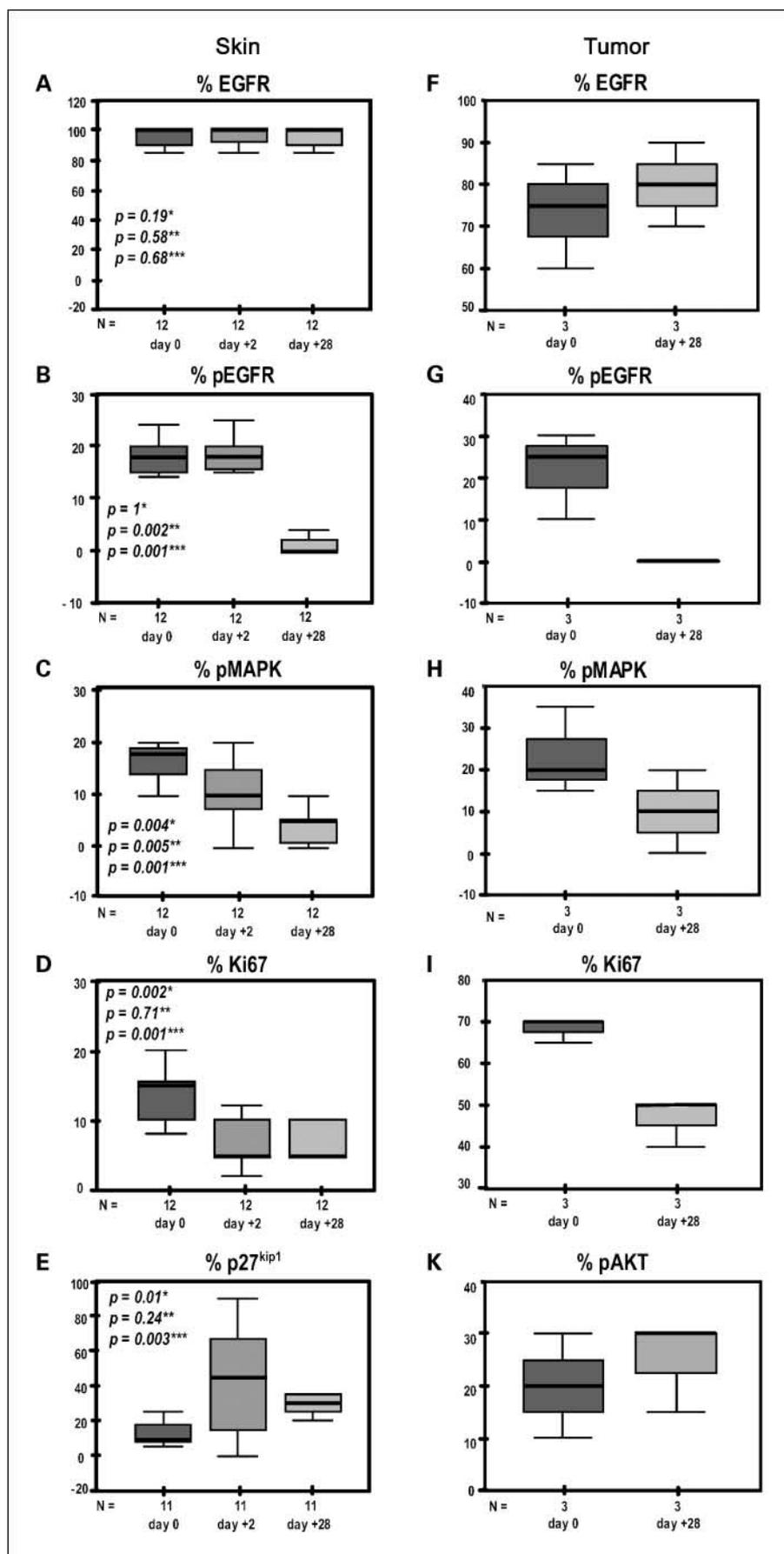
and time to tumor progression), and to describe the pharmacodynamic effects in sequential skin and tumor biopsies. Response was evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST; ref. 11). All patients who received ≥2 cycles of EKB-569/FOLFIRI therapy or who discontinued treatment due to disease progression were considered to be evaluable for efficacy.

## Results

**Patients' characteristics.** Forty-seven patients were enrolled in this phase I trial between April 2002 and October 2003. The

**Table 3.** Pharmacokinetic evaluation (mean ± SD) of EKB-569 and the *N*-desmethyl metabolite on day 15

		<i>C</i> <sub>max</sub> , ng/mL	AUC, h ng/mL	<i>t</i> <sub>1/2</sub>
25 mg EKB-569 FOLFIRI	EKB-569	16.6 ± 9.8 n = 16	235.9 ± 138.5 n = 16	22.4 ± 9.4 n = 11
	<i>N</i> -desmethyl Metabolite	2.5 ± 1.6 n = 12	27.6 ± 25.2 n = 12	12.9 ± 4.8 n = 7
35 mg EKB-569 FOLFIRI	EKB-569	26.7 ± 12.1 n = 7	398.0 ± 164.3 n = 7	14.4 ± 2.7 n = 7
	<i>N</i> -desmethyl Metabolite	2.9 ± 1.8 n = 7	42.9 ± 26.7 n = 7	13.5 ± 3.3 n = 7
50 mg EKB-569 mod. FOLFIRI	EKB-569	54.6 ± 26.8 n = 3	774.3 ± 439.1 n = 3	15.6 ± 3.0 n = 3
	<i>N</i> -desmethyl Metabolite	5.5 ± 2.1 n = 3	81.8 ± 28.5 n = 3	13.8 ± 0.3 n = 3



**Fig. 1.** Pharmacodynamic analysis of skin biopsies (A-E) and tumor biopsies (F-K) on days 0, +2, and +28 (A-E). Pharmacodynamic evaluation of skin biopsies was done on days 0, +2, and +28, evaluation of tumor biopsies on days 0 and +28. FOLFIRI started at day 1, and EKB-569 started at day 3. Boxes, 90% of the values. Bold lines, mean of the values. External lines, complete range when beyond 90% of the values. Wilcoxon signed-rank tests: \*, comparison between days +2 and 0 samples; \*\*, comparison between days +28 and +2 samples; \*\*\*, comparison between days +28 and 0 samples.

population had a median age of 65 years (range, 19-77 years) and a median ECOG PS of 0 (Table 1). All patients received FOLFIRI and were evaluated for toxicity. One patient in the 50-mg cohort refused treatment after the first dose of FOLFIRI and was replaced for the decision on dose escalation.

**Dose escalation.** The disposition of patients according to the dose escalation is shown in Table 2. During the initial escalation phase, none of the four patients treated with EKB-569 10 mg/day and none of the five patients treated at 25 mg/day had a DLT. At 50 mg EKB-569/day, one out of seven patients treated (one not evaluable for toxicity) had DLT consisting in grade 3 asthenia and diarrhea.

Dose escalation continued to 75 mg EKB-569/day. Two out of three patients treated had DLT with grade 3 diarrhea. Thus, enrollment was stopped at this dose level. Because all patients in the 50-mg EKB-569 cohort had diarrhea (controlled with loperamide) within the first 28 days of treatment, 50 mg EKB-569 were regarded as not feasible for further recruitment. A decision was made to include two additional cohorts: 35 mg EKB-569/day plus FOLFIRI and 50 mg EKB-569/day plus modified FOLFIRI (see Materials and Methods). However, the tolerability of these doses was inferior to the 25-mg EKB-569/day plus FOLFIRI dose because grade 3 diarrhea was observed in two out of seven patients (29%) in the 35-mg EKB-569/FOLFIRI cohort and in two out of four patients (50%) in the 50-mg EKB-569/modified FOLFIRI cohort. Hence, the MTD was chosen to be 25 mg EKB-569/day plus FOLFIRI, and this cohort was further expanded.

**Toxicity.** The most common toxicities were diarrhea, asthenia, nausea, neutropenia, vomiting, anorexia, alopecia, rash, abdominal pain, stomatitis, and anemia (Table 2). Thirty-nine (83%) patients had at least one grade 3 or 4 event. The most

frequent grade 3/4 toxicities were diarrhea (30%), neutropenia (21%), and asthenia (17%). No deaths occurred within 30 days of the last dose of EKB-569.

**Treatment duration.** Twenty-one out of the 47 patients completed the first six cycles. Twenty-six patients discontinued the treatment due to disease progression, 11 because of adverse events, and 10 for other reasons. The median number of EKB-569 treatment cycles was 5.0; the median duration of EKB-569 treatment was 20.2 weeks (Table 1). Dose was reduced in 17% of patients for EKB-569 and in 61% for FOLFIRI (Table 1).

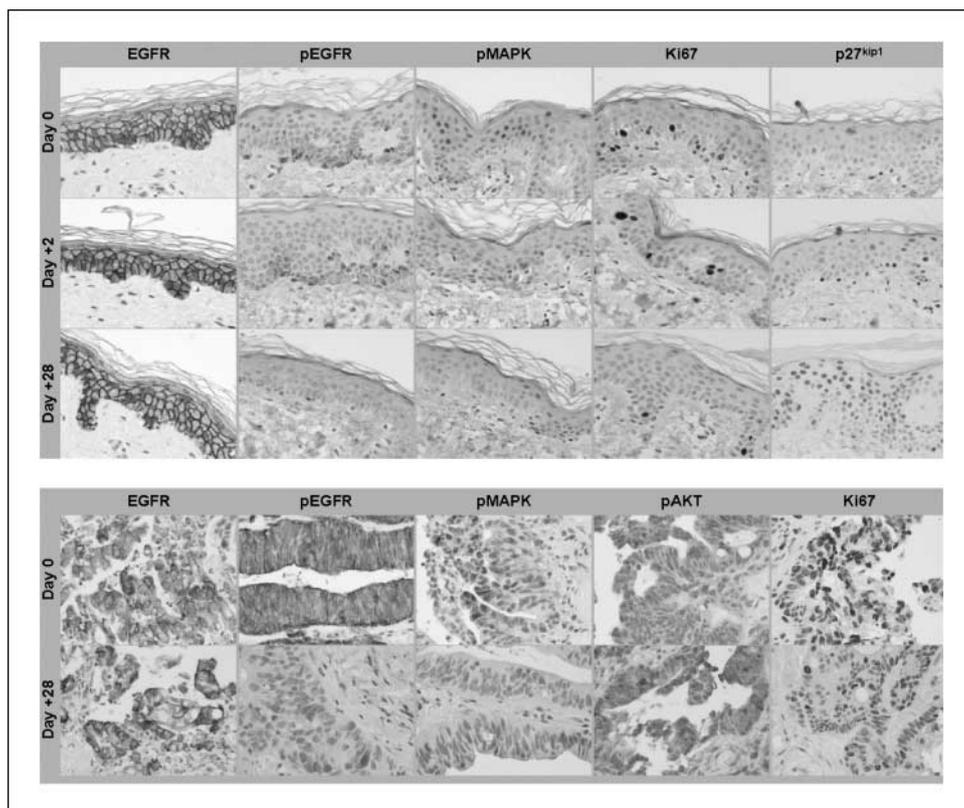
**Pharmacokinetics.** After p.o. administration of 25 mg EKB-569 in combination with FOLFIRI on day 15, mean EKB-569  $C_{max}$  was 16.6 ng/mL and mean  $AUC_{0-24}$  236 ng h/mL (Table 3). When EKB-569 dose was increased from 25 to 35 and 50 mg, the EKB-569 exposure ( $C_{max}$  and AUC) increased in a dose-related manner (Table 3). The observed pharmacokinetics of 25 mg EKB-569 after concomitant administration with FOLFIRI are comparable with those determined for single-agent EKB-569 (8), suggesting that there is no interference of FOLFIRI on the pharmacokinetics of EKB-569.

**Pharmacodynamic evaluation.** We conducted a pharmacodynamic evaluation of the EGFR pathway signaling inhibition with sequential skin biopsies in 12 patients and paired tumor biopsies in three patients, all treated with EKB-569 at doses  $\geq 25$  mg (Fig. 1).

To analyze whether chemotherapy alone induced changes, patients were started first with chemotherapy alone, and EKB-569 was not introduced until the third day. Skin biopsies were conducted pre-therapy, on chemotherapy alone (before day 3), and on chemotherapy plus EKB-569 (end of second cycle).

Total EGFR expression was not altered by FOLFIRI alone and FOLFIRI plus EKB-569 in both skin and tumor (Fig. 1A

**Fig. 2.** Immunohistochemistry studies in skin and tumor samples of one patient treated with EKB-569 at 25 mg and FOLFIRI. *A*, skin study: evaluation of total EGFR, pEGFR, pMAPK, Ki67, and p27<sup>Kip1</sup> at baseline (day 0), after FOLFIRI (day +2), and after FOLFIRI and EKB-569 (day +28). *B*, tumor study: evaluation of total EGFR, pEGFR, pMAPK, pAKT, and Ki67 at baseline (day 0) and after FOLFIRI and EKB-569 (day +28).



**Table 4.** Overview of studies with EGFR-tyrosine kinase inhibitors in colorectal cancer

Author	TKI/dose	Chemotherapy and doses (mg/m <sup>2</sup> )	Phase	First line/pre-treated
Combination with oxaliplatin/fluoropyrimidines				
Kuo (25)	Gefitinib, 500 mg	FOLFOX4	II	Pretreated
Fisher (23) (group A)*	Gefitinib, 500 mg	FOLFOX4	II	First line
Zampino (24)	Gefitinib, 250 mg	mFOLFOX6	II	First line
Hartmann (26)	Gefitinib, MTD: 250 mg	FUFOX, MTD: full dose; Ox: 50, FU 2000, FA 500	I	Pretreated
Hanauske (42)	Erlotinib	FOLFOX4	I	Pretreated
Spigel (28)	Erlotinib, 150 mg	FOLFOX4/bevacizumab (5 mg/kg)	II	First line
Meyerhardt (35)	Erlotinib, 150 mg	mFOLFOX6/bevacizumab (5 mg/kg)	II	First line
Meyerhardt (33)	Erlotinib, 150 mg	CapOx, Ox: 130, Cap: 2000 or 1500	II	Pretreated
Delord (27)	Erlotinib, 100 mg	CapOx, Ox: 130 mg/m <sup>2</sup> ; Cap: 1650 (recommended) or 2000	Ib	First line or pretreated
Tejpar (15)	EKB-569, 25 mg	FOLFOX4	I/II	First line
Combination with irinotecan/fluoropyrimidines				
Veronese (34)	Gefitinib (250 mg)	Irinotecan/LV5FU2; final dose Iri 120, FU (bol) 200, FU (inf) 500	II	First line
Messersmith (29)	Erlotinib, 100 mg	Irinotecan/LV5FU2, not feasible: Iri 150, FU (bol) 320, FU (inf) 480	I	First line
Hofheinz (30)	Gefitinib 250 mg	Irinotecan/5-FU/FA (AIO); MTD: Iri 60, FU 1600, FA 500	I	Pretreated
Redlinger (31)	Gefitinib 250 mg	Irinotecan/LV5FU2; final dose: Iri 120, FU (bol) 320, FU (inf) 600	II	First line
Arnold (32)	Gefitinib (250 mg)	Capecitabine/irinotecan; MTD: Iri 200, Cap 1600	I	Pretreatment Ox and 5-FU/FA
Monotherapy				
Mackenzie (19)	Gefitinib, 750 mg	n.a.	II	Pretreated or first line
Rothenberg (18)	Gefitinib, 250 or 500 mg	n.a.	Random phase II	Pretreated
Keilholz (20)	Erlotinib, 150 mg	n.a.	II	Pretreated

Abbreviations: RR, response rate; EFS, event-free survival; PSF, progression-free survival; TTP, time to progression; OS, overall survival. Cap, capecitabine; Iri, irinotecan; Ox, Oxaliplatin; FA, folinic acid; bol, bolus; inf, infusion; n.r., not reported; n.a., not applicable.  
\*Presented at American Society of Clinical Oncology 2004 in combination with preliminary results of ref. (25); toxicity reflects the pooled data from both study parts.  
† Confirmed response rate.

and F). FOLFIRI did not modify the total amount of activated (phosphorylated) EGFR (pEGFR), whereas EKB-569 markedly inhibited it in the skin ( $P \leq 0.002$ ; Fig. 1B), as well as in the tumors (Fig. 1G). This nearly complete inhibition of pEGFR by EKB-569 was seen at all dose levels analyzed ( $\geq 25$  mg/day). On the other hand, chemotherapy alone resulted in pharmacodynamic changes that are frequently seen as a result of EGFR inhibition (12). Hence, activated mitogen-activated protein kinase (pMAPK) expression in the skin was reduced after FOLFIRI treatment alone ( $P = 0.004$ ), and a further decrease was seen after treatment with EKB-569 ( $P = 0.005$ ; Fig. 1C). Ki67, as a marker for cell proliferation, decreased significantly in the basal epidermal cells of the skin after treatment with FOLFIRI ( $P = 0.002$ ) without further diminution with the introduction of EKB-569 (Fig. 1D). Similarly, the expression of the cyclin-dependent kinase inhibitor p27<sup>kip1</sup> was enhanced significantly by FOLFIRI ( $P = 0.01$ ), but no further increase was observed after treatment with EKB-569 (Fig. 1E). In the analyzed tumor samples (Fig. 1F-K), the pharmacodynamic findings observed by the combined administration of FOLFIRI and EKB-569 were similar to those

observed in the skin, with the exception of activated Akt (pAkt) status, not analyzed in the skin samples, that did not change with the treatment (Fig. 2).

**Efficacy.** Forty-four patients were considered evaluable for efficacy. Five patients (11%) had complete response, and 16 (36%) had partial response, resulting in an objective response rate of 48% [95% confidence interval (95% CI) 32-63%]. To evaluate a possible relation between EGFR expression in the primary tumor specimens and response, the EGFR status was determined in 26 evaluable patients. Five out of 13 patients with EGFR expression levels of 0 or 1+, and 9 out of 13 with EGFR  $\geq 2+$  achieved an objective response, this difference not being statistically significant. Overall, the median time to tumor progression was 7.7 months (95% CI 5.8-10.2 months).

## Discussion

The aim of this phase I study was to identify the RD for further development of EKB-569 combined with irinotecan-based chemotherapy. The rationale for combining FOLFIRI

**Table 4.** Overview of studies with EGFR-tyrosine kinase inhibitors in colorectal cancer (Cont'd)

<i>n</i>	RR (%)	Diarrhea gr. 3/4 (%)	Neutropenia gr. 3/4 (%)	Remarks
27	33	48	48	63% dose reduction for Ox., 48% dose reduction for 5-FU. EFS 5.4 mo, OS 12 mo
36	(53) <sup>†</sup>	54*	52*	TTP: 9.5 mo; OS not reached. One treatment-related death
56	71	16	18	
18	24			DLT: diarrhea; dose escalation to full-dose FUFOX was feasible with 250 mg gefitinib, but not with 500 mg gefitinib
15	23			
30	57	31	22	Early terminated because of toxicity (four possible treatment-related deaths) PFS: 10.9 mo, OS 12.4 mo
34	34	43	26	77% off study because of toxicity
32	25	38	3	Dose of Cap reduced to 1,500 mg/m <sup>2</sup> after 13 patients due to diarrhea gr. 3/4; 88% of patients with dose reduction PFS 5.4 mo; OS 14.7 mo
23	22			DLTs: 3/11 patients diarrhea gr. 2/11, rash gr. 3
29	36	8	32	DLTs: 1 diarrhea gr. 3, one febrile neutropenia
13	8	54	62	Dose reduction of chemotherapy by two dose levels and early termination because of toxicity
6	17	66	50	Recruitment stopped at first dose level because of toxicity; no interaction in pharmacokinetics
13	8	16		Early terminated because of toxicity (DLTs diarrhea, nausea/vomiting) and low activity
13	8		45	Dose reduction of chemotherapy by two dose levels because of neutropenia gr. 3/4 in 5/11 patients
13	n.a.	30	15	Recruitment stopped at second dose level (Cap 1600, Iri 225) due to diarrhea gr. 3 (3/3 patients), neutropenia gr. 3 (2/3 patients), and epistaxis gr. 3 (1/3 patients)
28	0	14	0	
110	1	5	n.r.	
51	4	18	n.r.	

with EKB-569 in patients with mCRC comes from preclinical studies that have shown a synergistic effect and a reversion of resistance to irinotecan with both the anti-EGFR antibody cetuximab and the TKI gefitinib in *in vitro* and *in vivo* models (9, 13). These effects have been shown in the clinical setting with the combination of cetuximab and irinotecan in patients with mCRC refractory to irinotecan-based chemotherapy (6).

In this study, we have shown that it is feasible to administer full-dose FOLFIRI plus EKB-569 at a pharmacodynamic active dose. However, the MTD and RD of EKB-569 in this study (25 mg/day) in combination with FOLFIRI is markedly lower than that for EKB-569 as a single agent (75 mg/day; ref. 8) due to overlapping toxicities of the two treatments. Regarding the low rate of skin toxicity, it may be discussed whether an adequate dose of the EGFR-TKI was achieved, especially regarding the correlation of skin toxicity in anti-EGFR treatment with efficacy (14). However, additional cohorts could not establish a higher dose of EKB-569 than 25 mg. In another phase I study of EKB-569 in combination with FOLFOX-4 (with an additional dose level of 35 mg EKB-569/day), the MTD of EKB-569 has also been found to be 25 mg/day (15). The combination of EKB-569 25 mg/day and FOLFIRI is well tolerated and results in a near-complete inhibition of EGFR activation in the skin and in tumor samples.

An important contribution of our study was to analyze the effects on pharmacodynamic markers induced by chemother-

apy alone before the introduction of EKB-569. Although FOLFIRI by itself did not result in the inhibition of EGFR phosphorylation, it did interfere substantially with epidermal proliferation and with activation of MAPK as well as with p27 levels. The implication of our findings is that chemotherapy alone may interfere, probably through a variety of nonspecific mechanisms, with the activation status of some of the pharmacodynamic markers that are frequently analyzed in clinical trials with targeted agents directed at signal transduction pathways. Therefore, caution should be introduced with the interpretation of pharmacodynamic studies of targeted agents when given in combination with chemotherapy.

It is worthwhile noting that activated Akt (pAkt) was not inhibited in the tumors after administration of FOLFIRI and EKB-569. One might speculate on the reasons underlying the lack of inhibition of pAkt in the tumor specimens after treatment. First, the activation of phosphoinositide-3-kinase (PI3K)/Akt is also mediated via other growth factor receptors. Second, the loss of activity of the tumor suppressor gene PTEN or presence of PI3K mutations results in enhanced PI3K/Akt activity (16). Third, the limited number of paired tumor samples makes possible the fact of a lack of inhibition by chance. Finally, the timing of the last biopsy may be, perhaps, too late to show meaningful changes in the inhibition of pAkt. It can be speculated that the limited inhibition of proliferation associated with the lack of inhibition of Akt activation in tumor

samples may, in part, explain the absence of important additional efficacy effects of EKB-569 over FOLFIRI alone.

The objective response rate of 48% observed with this combination of FOLFIRI plus EKB-569 is in the range of the known response rates achieved with the FOLFIRI regimen alone for patients with similar characteristics (2, 17). These data suggest that the addition of EKB-569 to FOLFIRI did not translate into clinical efficacy, although a firm conclusion cannot be made without a randomized study.

The future role of anti-EGFR TKIs in combination with chemotherapy in mCRC is unknown at this time. In contrast to non-small cell lung carcinoma (NSCLC), the efficacy of EGFR TKIs as single agents in mCRC is limited. In mCRC, gefitinib at doses of 250 to 750 mg and erlotinib at 150 mg resulted in objective response rates of <1% (18, 19) and 4% (20), respectively. This low single agent activity in mCRC does not preclude activity of these classes of compounds when given in combination with other agents. For example, response rates to oxaliplatin (21) and bevacizumab (22) are  $\leq$ 3% when used as a single agent in mCRC, but they clearly provide an important clinical benefit when combined with other agents.

The clinical efficacy results of this study are in concordance with most other studies investigating EGFR TKIs plus chemotherapy in mCRC (Table 4). Although limited phase II studies of the combination of gefitinib/FOLFOX have reported an encouraging antitumoral activity with response rates of 74% to 77% in first-line therapy (23, 24) and 33% in pretreated patients (25), other trials have not been so successful. Some

studies have failed to achieve full doses of chemotherapy in combination with RDs of anti-EGFR TKIs (26–34), whereas others have shown an unfavorable safety profile (26–34) and/or, more importantly, have not suggested higher efficacy results than those obtained with chemotherapy alone (15, 27, 29–34). In addition, it may not be possible to extrapolate from the results of EGFR TKIs plus chemotherapy in other disease types because results from phase III studies have been mixed: the addition of EGFR TKIs to standard chemotherapy did not improve outcome in metastatic NSCLC (36–38), whereas it resulted in significant benefit in pancreatic cancer (39). Taking all these considerations together, it is not possible at this time to predict the role of EGFR TKIs in combination with chemotherapy in patients with mCRC. The answer to this question would require the conduct of a well-designed phase III study. However, the already proven clinical benefit of chemotherapy and anti-EGFR monoclonal antibodies in a similar clinical setting (40, 41) has decreased the enthusiasm for such a study.

In conclusion, the combination of EKB-569 with FOLFIRI is feasible and has an acceptable toxicity profile at a dose level of EKB-569 that results in profound EGFR signaling inhibition. The observed clinical activity was consistent with other prior studies with anti-EGFR TKIs and chemotherapy in mCRC. Our findings that FOLFIRI interferes with epidermal proliferation and with pharmacodynamic markers used as end points in studies with targeted agents should be considered when interpreting pharmacodynamic studies of these agents given in combination with chemotherapy.

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# Clinical Cancer Research

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