A Pharmacodynamic Study of the Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor ZD1839 in Metastatic Colorectal Cancer Patients


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ABSTRACT

Purpose: Epidermal growth factor receptor (EGFR) appears to play an important role in the pathogenesis of colorectal cancer. We have performed a Phase I/II study of the EGFR tyrosine kinase inhibitor ZD1839 in metastatic colorectal cancer patients in which serial biopsies were taken pre- and posttreatment to assess biological activity.

Experimental Design: Paired biopsies were obtained from colorectal cancer patients before and after treatment. Proliferation and apoptosis were assessed using Ki67 immunohistochemistry and terminal deoxynucleotidyl transferase-mediated nick end labeling assays, respectively. Immunohistochemistry for EGFR, activated EGFR, phosphorylated Akt, phosphorylated ERK, p27Kip1, and β-catenin was also performed.

Results: Posttreatment samples showed a statistically significant reduction in the cancer cell proliferation index (mean proliferation index pretreatment 31%; posttreatment 12%; P = 0.047). The mean cancer cell apoptosis index also increased from 6 to 12% in posttreatment samples, although this difference did not achieve statistical significance. All pretreatment samples showed strong staining for activated EGFR, phosphorylated Akt, and phosphorylated ERK in cancer cells. Serine phosphorylation was observed in some patients after treatment. p27Kip1 was absent in the cancer cells of most pretreatment biopsies; two patients showed a marked increase in staining for nuclear p27Kip1 after treatment with ZD1839. These two patients also showed large increases in apoptotic index.

Conclusions: ZD1839 inhibits EGFR signaling and proliferation in the cancer cells of patients with metastatic colorectal cancer. ZD1839 may also induce cancer cell apoptosis in a subset of colorectal cancer patients via up-regulation of p27Kip1.

INTRODUCTION

The EGFR tyrosine kinase (1) is a membrane glycoprotein with intrinsic protein tyrosine kinase activity. Activation of the receptor by binding of any of its six known ligands leads to receptor dimerization and transautophosphorylation on tyrosine residues. Phosphorylated tyrosine residues on the receptor then serve as binding sites to recruit and activate downstream signaling pathways that control cellular proliferation and apoptosis in both normal cells and cancer cells.

ZD1839 (Iressa) is a selective inhibitor of the EGFR tyrosine kinase (1). Structurally, it belongs to the quinazoline class of TKIs (2). Its binding to EGFR is competitive with respect to ATP, and it inhibits EGFR in vitro with an IC50 of 27–33 nM. ZD1839 is very selective, having an ~70-fold lower affinity for HER2, a second member of the EGFR family that has a highly homologous tyrosine kinase domain (3). In animals, ZD1839 has ~50% bioavailability, and the drug is distributed through most tissues (1). ZD1839 has shown antitumor activity in many different mouse xenograft models, both on its own and in combination with established chemotherapy agents. Phase I studies have shown that the drug is well-tolerated, with diarrhea and rash as the most frequent side effects (4–7). Phase I studies also showed that ZD1839 is active; partial responses were seen in non-small cell lung cancer, and disease stabilization was seen in a number of other tumor types, including colorectal cancer (5–7). Phase II and III trials are currently underway to additionally assess the activity of ZD1839 in non-small cell lung cancer (1). Phase I and II studies of ZD1839 in other tumor types, including breast, head and neck, renal cell, and prostate cancer, are also underway.

Colorectal cancer is the third most common cause of can-
cancer-related deaths in North America. There is a large body of evidence supporting a role for EGFR in the development of this disease. EGFR expression is common in colorectal cancer (8, 9), and it has been proposed that overexpression of EGFR may promote the formation of liver metastases in this disease (10, 11). Studies in mouse models of colorectal cancer have shown a role for EGFR in this disease: when APC<sup>min</sup> mice, which develop colorectal cancer at a high frequency, are crossed with mice carrying a mutant, kinase-deficient EGFR, tumors develop at a much lower frequency (12). ZD1839 and other EGFR-TKIs have been evaluated in mouse xenograft models of colorectal cancer and shown to be active (12, 13). Thus, immunohistochemical, genetic, and pharmacological data all support a role for EGFR in the development of colorectal cancer.

Here, we describe the results of a trial of ZD1839 in patients with metastatic colorectal cancer in which laboratory correlative studies were carried out to assess the activity of the drug at the cellular and molecular level in patients. Details of the clinical results will be described elsewhere (14); here, we focus on the results of the laboratory correlative studies in which pre- and posttreatment biopsies samples were examined immunohistochemically for proliferation, apoptosis, and markers of EGFR signaling.

**MATERIALS AND METHODS**

**Patient Biopsies.** Biopsies were obtained with informed consent from colorectal cancer patients. Pretreatment samples were obtained 1 or 2 days before initiation of treatment, and posttreatment samples were obtained after 28 days of oral dosing with 750 mg/day ZD1839. All biopsies discussed in this article were from liver metastases, except for 1 patient (patient no. 5), where a suprapubic node was biopsied. Core biopsies of liver metastases were generally obtained using an 18-g needle and ultrasound guidance. The same lesion was biopsied pre- and posttreatment. Samples were transferred to cryovials and immediately frozen in liquid N<sub>2</sub>. Samples were shipped on dry ice and stored at −80°C. All laboratory tests were performed blinded to patient clinical data; results were then reported to the National Cancer Institute of Canada Clinical Trials Group and assessed together with clinical data.

**Antibodies.** The following antibodies were used in this study: Neomarkers Ab-10 (clone 111.6) anti-EGFR mouse monoclonal IgG1 antibody (1:100 dilution); Chemicon MAB3052-antiactiivated EGFR mouse monoclonal IgG1 antibody (1:100 dilution); Cell Signaling Technology antiphospho-p44/42 mitogen-activated protein kinase (Thr202/Tyr204) E10 mouse monoclonal IgG1 antibody (1:50 dilution); Cell Signaling Technology antiphosphorylated Akt (Ser473) 4E2 mouse monoclonal IgG2a antibody (1:50 dilution); Dako anti-Ki67 clone Ki-S5 mouse IgG1 monoclonal antibody (1:500 dilution); Neomarkers anti-MMP-2 (72-kDa collagenase-IV) Ab-4 mouse IgG1 monoclonal antibody (1:100); Transduction Laboratories anti-β-catenin mouse IgG1 monoclonal antibody (1: 100); Neomarkers anti-p27<sup>Kip1</sup> Ab-1 mouse IgG1 monoclonal antibody, clone DCS-72.F6 (1:50); Neomarkers mouse IgG1 antibody Ab-1, clone NCG01 (negative control, used at same dilution as test antibody); and Neomarkers mouse IgG2a antibody Ab-1, clone NCG2A.01 (negative control, used at same dilution as test antibody).

**Immunohistochemistry.** Frozen samples were embedded in Cryomatrix embedding resin (Thermo Shandon, Pittsburgh, PA), and 5-μm sections were prepared using a cryostat. Sections were transferred to precoated slides and air-dried 5 min. Samples were fixed with 4% paraformaldehyde for 20 min, washed with PBS, and permeabilized by incubation in 0.2% Triton X-100 for 10 min. Endogenous peroxidase activity was quenched by incubation in 3% H<sub>2</sub>O<sub>2</sub> in PBS for 15 min. Immunohistochemical detection was done using the Envision + Polymer Detection System (Dako), according to the manufacturer’s instructions. This method was found to be similar in sensitivity to the avidin-biotin complex staining method but gave lower nonspecific staining in fibroblasts and hepatocytes. Control immunohistochemical staining with an isotype-matched irrelevant antibody was carried out with each assay. TUNEL assays were evaluated using the In Situ Cell Death Detection Kit (Roche Molecular Diagnostics, Laval, Quebec, Canada). Slides were evaluated by two investigators and staining was scored as −, +, ++, or +++ based on relative staining intensity. For quantitation of proliferation and apoptosis, ×40 microscope fields were randomly chosen; the number of Ki67-positive cancer cells (proliferation) or TUNEL-positive cancer cells (apoptosis) were then counted and divided by the total number of cancer cells/field.

**Assessment of Tumor Burden.** Tumor burden was assessed using computed tomography scans. Baseline measurements were made in the 28-day period before the initiation of treatment. All measurable lesions documented at this time were followed in subsequent assessments, which were done at 4-week intervals after the initiation of treatment. Any new lesions that appeared during treatment were also included in the measurement of tumor burden. Tumor burden was defined as the sum of the products of the two largest perpendiculars of all measured lesions in a patient.

**Statistical Analysis.** Statistical analysis was performed using StatView 5 software (SAS Institute, Cary, NC). Comparisons between pre- and posttreatment samples were done using the Wilcoxon signed rank test, using a two-sided 0.05 level of significance. Fig. 1 was generated using GraphPad Prism version 3.00 for Windows software (San Diego, CA).

**RESULTS**

**Summary of Sampling.** Twenty-seven patients with colorectal cancer were enrolled in the trial. Paired biopsies were obtained from 17 patients in total. Single samples were obtained from another 8 patients. Of the 17 paired samples obtained, 11 were evaluable; in the other pairs, there were few or no cancer cells in either the pre- or posttreatment samples. All but 2 of the patients for which paired biopsies were obtained had previously undergone chemotherapy; this was stopped a minimum of 28 days before initiation of ZD1839 treatment.

**Histopathology.** H&E staining was used to examine tumor morphology. In most specimens, the tumor tissue contained...
An important question was whether the laboratory data on proliferation and/or apoptosis would show a correlation with changes in tumor burden, determined radiologically. (All laboratory analyses described in this article were performed blinded to patient clinical data.) Fig. 1 shows a plot of the proliferation index determined after 28 days of treatment versus percentage changes in tumor burden for each patient; simple regression analysis gives an $R^2$ value of 0.597; a $t$ test shows that there is a significant linear relationship between the growth index and the percentage change in tumor burden ($P = 0.0088$). There was no significant linear relationship between posttreatment apoptosis index values and the percentage change in tumor burden ($R^2 = 0.285, P = 0.118$). This suggests that the proliferation index measurements reflect the clinical disease behavior in most patients.

**EGFR.** EGFR was detected by immunohistochemistry in 16 of 16 patients for which pretreatment biopsies were available (Table 1 and Fig. 2). Staining was always much stronger in the cancer cells than in adjacent stromal fibroblasts or hepatocytes when these were present. Staining intensity was scored as +, ++, or ++++, with +++ being the most intense. There was no correlation between expression levels and the changes in apoptosis or proliferation index seen with different patients. In 7 of 10 patients, EGFR levels were unchanged after treatment with ZD1839. The other three showed a decrease in EGFR levels after treatment: 2 of these were patients that also showed a large increase in apoptosis index after treatment.

We also assessed levels of active EGFR using an antibody that specifically recognizes the activated form of this receptor. This monoclonal antibody was originally isolated from a mouse immunized with tyrosine phosphorylated proteins purified from epidermal growth factor-stimulated cells but appears to recognize an activated conformation of the receptor rather than a specific phosphorylation site (15). The same antibody has been used previously to study pharmacological inhibition of EGFR in mouse xenograft models (16) and to study the effects of ZD1839 on EGFR in patient skin biopsies (17). Immunohistochemical staining for activated EGFR was detected reproducibly in 1 patient (Fig. 2). This signal was absent in controls where an isotype-matched irrelevant primary antibody was used. The signal was also absent in the posttreatment sample. This suggests that ZD1839 was directly inhibiting its target in this patient. Our initial evaluation of this antibody using cultured cells indicated that it only detects activated EGFR when it is present at high levels; it is likely that other patients studied here have activated EGFR but at levels that are below the detection limit.

**PKB/Akt Phosphorylation.** EGFR is able to activate the protein kinase PKB/Akt via a signaling pathway that involves the enzymes phosphoinositide 3-kinase and phosphoinositide-dependent kinase 1 (18). PKB/Akt is activated by phosphorylation at two sites, Thr$^{308}$ and Ser$^{473}$; phosphorylation at both these sites is dependent on phosphoinositide 3-kinase activation. We used immunohistochemistry with an antibody specific for Ser$^{473}$-phosphorylated PKB/Akt to assess the activation status of this enzyme in patients treated with ZD1839. Phosphorylated PKB/Akt was detected in 2 of 10 patients evaluated (Table 1 and Fig. 2). This was detected in the pretreatment samples from both patients but in both cases was absent after treatment. There was no clear correlation between the presence of phosphorylated PKB/Akt and changes in proliferation or apoptosis indices. As with the antibody to activated EGFR (above), our evaluation of this antibody using cultured cells suggests that it may only detect relatively high levels of phosphorylated PKB/Akt.

**ERK Phosphorylation.** The mitogen-activated protein kinases ERK1 and ERK2 are known to be activated downstream
In the 2 patients where p27Kip1 was detected before treatment, including data from patients with pretreatment biopsies only. This protein has a well characterized role in the degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 shows the results of immunohistochemical analysis of p27Kip1 degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 presents in the nucleus of cells and can be inactivated either by degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 shows the results of immunohistochemical analysis of p27Kip1 degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 presents in the nucleus of cells and can be inactivated either by degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 shows the results of immunohistochemical analysis of p27Kip1 degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 presents in the nucleus of cells and can be inactivated either by degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 shows the results of immunohistochemical analysis of p27Kip1 degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 presents in the nucleus of cells and can be inactivated either by degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 shows the results of immunohistochemical analysis of p27Kip1 degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 presents in the nucleus of cells and can be inactivated either by degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 shows the results of immunohistochemical analysis of p27Kip1 degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 presents in the nucleus of cells and can be inactivated either by degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 shows the results of immunohistochemical analysis of p27Kip1 degradation (20) or by cytoplasmic sequestration (21, 22). Table 1 presents in the nucleus of cells and can be inactivated either by degradation (20) or by cytoplasmic sequestration (21, 22).

![Table 1](https://example.com/table1.png)

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<th>% TUNEL positive</th>
<th>% change in tumor burden</th>
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Fig. 2 Immunohistochemistry for EGFR, activated EGFR, phosphorylated Akt, and phosphorylated ERK. Sections from samples taken before treatment are shown on the left and after treatment on the right. Patient number and antigen stained for are indicated on the right-hand side. a EGFR, activated EGFR; pAkt, phosphorylated Akt; pERK, phosphorylated ERK. Immunohistochemistry was performed as described in “Materials and Methods.” No staining was seen when immunohistochemistry was performed with isotype-matched negative control antibodies.
DISCUSSION

One goal of this study was to determine whether ZD1839 could effectively inhibit EGFR activity in colorectal cancer metastases. We assessed four different markers of EGFR activity, i.e., activated EGFR, phosphorylated Akt, phosphorylated ERK, and nuclear p27\textsuperscript{kip1}. Multiple studies in tissue culture and animal models support the use of these antigens as markers of EGFR activity. In addition, studies of skin biopsies taken from patients undergoing treatment with ZD1839 have shown that ZD1839 can inhibit ERK activation and increase p27\textsuperscript{kip1} levels in normal skin (17). This supports the use of these markers of EGFR activity in patients. We observed a decrease in staining for activated EGFR in 1 patient, a decrease in staining for phosphorylated Akt in 2 patients, a decrease in tumor cell phosphorylated ERK in 1 patient, and an increase in staining for nuclear p27\textsuperscript{kip1} in 2 patients. There were no instances where positive staining for a marker of EGFR activity was present in pre- and posttreatment samples or where markers changed in the opposite direction to that which is predicted for EGFR inhibition. The probability of this being a random occurrence is very small ($P = 0.001$). Therefore, although we only detected individual markers in small numbers of patients, when taken together, the data from the four different markers of EGFR activity provide reasonable evidence that ZD1839 is able to inhibit EGFR signaling in metastatic colorectal cancer.

An unexpected finding arising from this study was that active, phosphorylated ERK is absent in the metastatic colorectal cancer cells of most patients. This result suggests that ERK does not have a major role in the growth of most colon cancer liver metastases (although it is also possible there is a low level of ERK activation in these cells that we are not able to detect). Raf, the first enzyme in the kinase cascade that activates ERK, does not transform rat intestinal epithelial cells, although it is able to transform fibroblasts (25), suggesting a limited role for ERKs in intestinal epithelial cell transformation. Also there is evidence that sustained activation of EGFR can partially antagonize ERK activation in some cell types because of up-regulation of an enzyme that is able dephosphorylate ERK (26). In contrast to the situation in cancer cells, phosphorylated ERK was present in the stromal fibroblasts of colorectal cancer liver metastases in all patients. It will be of great interest to determine the role that activated ERK plays in this cell compartment. ZD1839 treatment reduced stromal fibroblast levels of activated ERK in 5 of 9 patients, raising the possibility that some of antitumor activity of ZD1839 might be attributable to inhibition of stromal fibroblast function. Stromal fibroblasts are known to produce some of the MMPs that are necessary for growth and invasion of tumors. Specifically, MMP-2 and MMP-9 are thought to be produced exclusively by stromal fibroblasts (27). In vitro studies have shown that activation of MMP-2 and MMP-9 expression in fibroblasts is dependent on ERKs (28). We asked whether MMP-2 expression would be altered in stromal fibroblasts that showed reduced activated ERK after ZD1839 treatment. Immunohistochemistry for MMP-2 showed that it was only expressed in stromal fibroblasts, in agreement with previous studies (data not shown). However, the expression was unchanged after ZD1839 treatment in the 2 patients that we evaluated (nos. 7 and 10). This suggests that other signaling pathways might be involved in the antitumor activity of ZD1839.
pathways control MMP-2 expression in stromal fibroblasts in vivo.

A second goal of this study was to determine the effects of ZD1839 on metastatic colorectal cancer cell proliferation and apoptosis in patients. We observed a statistically significant reduction in proliferation in colorectal cancer cells after treatment with ZD1839. This result closely parallels results seen in a preclinical study on the effects of ZD1839 on a human colon cancer xenograft grown in nude mice (29), where a significant reduction in proliferation (also assessed by Ki67 immunohistochemistry) was observed. We also observed an increase in mean apoptosis levels after treatment; however, the increase in mean apoptosis was entirely because of large changes in 2 patients and was not significant for the population as a whole. Again, this parallels preclinical studies, where low concentrations of ZD1839 were able to cause significant growth inhibition in a colon cancer cell line without increasing apoptosis (13). This was seen at a concentration in tissue culture of 50 μM, which is considerably higher than the peak plasma levels obtained in patients (5). EGFR-TKIs have been shown to be active in at least three mouse xenograft models of colon cancer (12). In these preclinical models, regression of large, established tumors was not observed; the antitumor activity was predominantly growth inhibitory. In this study of metastatic colorectal cancer patients, ZD1839 behaved as predicted by preclinical models of colon cancer in that we observed a decrease in growth rate without pronounced tumor regression.

Phase I studies show that only a subset of non-small cell lung cancer patients respond to ZD1839 (5). An important area of future study will be in the identification of markers that predict response to EGFR-TKIs. For Herceptin, which targets the erbB2 member of the EGFR family, it is known that patients overexpressing erbB2 because of gene amplification are most likely to respond (30). Preclinical studies have shown that levels of EGFR expression do not correlate with response to ZD1839 (31). There are probably several reasons for this: (a) although overexpression of EGFR clearly plays a role in its activation in some cancers, EGFR can also be activated without overexpression via autocrine expression of its ligands; or (b) Herceptin is an antibody that kills tumor cells, in part, by antibody-dependent cellular cytotoxicity (32), and this cytotoxic mechanism is probably dependent on expression levels of Her2. This cytotoxic mechanism is obviously not a factor with ZD1839. In this study, the behavior of p27<sup>Kip1</sup> was most interesting with respect to the problem of why patients may respond differently to ZD1839. We did not detect p27<sup>Kip1</sup> in 6 of 7 pretreatment biopsies from patients; this is consistent with previous work showing that p27<sup>Kip1</sup> is a negative prognostic marker in colorectal cancer and that its expression is decreased in metastatic tumors compared with primary tumors (20, 33). Loss of expression of p27<sup>Kip1</sup> in colorectal cancer has been shown to be attributable to increased degradation, rather than changes in transcription (20). In the 1 patient where p27<sup>Kip1</sup> was present, its staining was cytoplasmic rather than nuclear. A cytoplasmic p27<sup>Kip1</sup> staining pattern was also seen in the same patient posttreatment and in a posttreatment sample from a second patient for which there was no more pretreatment sample to assess. Cytoplasmic sequestration has been shown to be an alternate mechanism for inactivation of p27<sup>Kip1</sup> (21). This appears to be a major mechanism for inactivation of p27<sup>Kip1</sup> in thyroid carcinoma, where increased expression of cyclin D3 causes cytoplasmic sequestration of p27<sup>Kip1</sup> (22). Increased cytoplasmic expression of p27<sup>Kip1</sup> is also seen in a subset of primary colorectal cancer tumors (34). Overall, the available data suggest that p27<sup>Kip1</sup> is inactivated in most or all metastatic colorectal cancers but that there are at least two mechanisms by which this can be accomplished: either by proteasomal degradation of p27<sup>Kip1</sup> induced by growth factors or by cytoplasmic sequestration. There is strong evidence from in vitro studies that up-regulation of p27<sup>Kip1</sup> is essential for growth inhibition by EGFR-TKIs (19). A reasonable hypothesis is that patients with cytoplasmic staining for p27<sup>Kip1</sup> are probably not inactivating p27<sup>Kip1</sup> via the EGFR and should not respond to ZD1839. Our data support this hypothesis: the 2 patients that show cytoplasmic staining for p27<sup>Kip1</sup> are also the only 2 patients that do not show a decrease in proliferation index after ZD1839 treatment. We are carrying out additional studies with ZD1839 that involve larger numbers of patients and which may allow definitive testing of this hypothesis.

Although nuclear p27<sup>Kip1</sup> was not present in any pretreatment samples, very marked nuclear staining was present in the posttreatment samples from 2 patients. Aside from its properties in inducing growth arrest, p27<sup>Kip1</sup> is also able to induce apoptosis in some cancer cell lines. This role has been demonstrated both by exogenous delivery of p27<sup>Kip1</sup> using viral vectors (35, 36) and by inhibition of endogenous p27<sup>Kip1</sup> using antisense RNA (37, 38) or neutralizing antibody (37). The 2 patients that showed nuclear staining of their cancer cells after treatment with ZD1839 showed marked decreases in proliferation, although other patients also show this without detectable increases in p27<sup>Kip1</sup>. However, the 2 patients that showed nuclear p27<sup>Kip1</sup> staining after treatment were also the only 2 patients that showed large increases in apoptosis after treatment. These patients were also 2 of 3 patients that showed a decrease in tumor burden. This suggests that in a subset of metastatic colorectal cancer patients, ZD1839 can cause some tumor regression by p27<sup>Kip1</sup>-induced apoptosis. Many of the activities of p27<sup>Kip1</sup> are a consequence of its ability to inhibit cyclin-dependent kinase 2. Cyclin-dependent kinase 2 inhibition is able to induce apoptosis when it occurs in the presence of upstream cell cycle signals; this is thought to provide a mechanism by which cells with malfunctioning growth-regulatory mechanisms can be eliminated (39). β-Catenin is frequently up-regulated in colorectal cancer and can activate upstream cell cycle activation signals via transcriptional activation of cyclin D1 (40, 41). We assessed β-catenin levels in paired samples from 2 patients, including 1 of the patients that showed a marked increase in p27<sup>Kip1</sup> and apoptosis. In both cases, β-catenin levels were unaffected by ZD1839. This suggests a mechanism in which apoptosis would result as a consequence of ZD1839-induced up-regulation of p27<sup>Kip1</sup> in the face of continued upstream cell cycle signaling activated by β-catenin. Although our data are consistent with this mechanism, clearly much more research will be necessary to determine whether it is correct. In 5 of 9 patients, p27<sup>Kip1</sup> was not detected after treatment. One possibility is that tyrosine kinase receptors other than EGFR are promoting p27<sup>Kip1</sup> degradation in these patients.

ZD1839 was developed based on its ability to specifically inhibit EGFR, a target that extensive preclinical studies had
implicated as having a role in cancer. This distinguishes it from many standard chemotherapy agents that were developed based on their ability to preferentially kill cancer cells. It has been argued that new molecularly targeted agents may need to be evaluated by different criteria than those used for cytotoxic agents (42, 43). In this trial of ZD1839 in 27 metastatic colorectal cancer, there were no objective responses, although modest decreases in tumor burden were seen in some patients. However, the laboratory correlative studies do provide evidence that ZD1839 is able to inhibit EGFR activity in metastatic colorectal cancer. In addition, these studies suggest that ZD1839 decreases tumor cell proliferation. This suggests that ZD1839 would be most beneficial when used to treat colorectal cancer at an early stage, perhaps as adjuvant therapy after surgery. An important point is that preclinical studies showed that ZD1839, along with decreasing growth rate, also significantly sensitized colon cancer cells to apoptosis induced by chemotherapy agents (13). Our evidence that ZD1839 is biologically active in metastatic colorectal cancer justifies additional studies in which ZD1839 is tested in combination with other chemotherapy agents to treat both early and advanced colorectal cancer.

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