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


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INTRODUCTION

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 21%; 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 EGFR. Loss of immunohistochemical staining for activated EGFR, phosphorylated Akt, and phosphorylated ERK in cancer cells 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.

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REFERENCES

1. The abbreviations used are: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; Ab, antibody; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; ERK, extracellular-regulated kinase; MMP, matrix metalloproteinase; PKB, protein kinase B; APC, adenomatous polyposis coli.
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 APCmin 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 supraclavicular 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 transferee to cryovials and immediately frozen in liquid N2. 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-antiactivated 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 monoclonal antibody (1:50 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-p27Kip1 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% H2O2 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 perpendiculants 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

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4 H. Hirte _et al._, manuscript in preparation.
epithelial neoplastic cells with an irregular glandular pattern within fibrotic stroma. In some other samples, scattered sheets or individual tumor cells in blood or ascites were observed. Obvious inflammatory cell infiltration was present in only a few samples.

**Proliferation.** Proliferation was assessed by immunohistochemistry for Ki67 (Table 1). A minimum of 70 cancer cells/sample was scored as positive or negative for Ki67; most estimates of proliferation index were from counting 100 cancer cells in both pre- and posttreatment samples. Ten paired samples were analyzed for proliferation (one paired sample contained too few cancer cells to obtain an accurate estimate of proliferation). Proliferation indices ranged from 8 to 57% in pretreatment samples and 5 to 53% in posttreatment samples. Eight of 10 patients showed decreased proliferation after treatment. The mean proliferation index pretreatment was 31%, as compared with 21% posttreatment. This difference was statistically significant when analyzed by a Wilcoxon signed rank test (P = 0.047). These data suggest that ZD1839 treatment slows the rate of cell division in metastatic colorectal cancer.

**Apoptosis.** Apoptosis was assessed using TUNEL assays, using the same numbers of cells as for the proliferation index (Table 1). Apoptosis indices ranged from 2 to 14% in pretreatment samples and from 2 to 39% in posttreatment samples. Six of 10 patients showed increases in apoptosis index after treatment; 2 showed no change, and 2 showed a decrease. The mean apoptosis index was 6% in pretreatment samples and 12% in posttreatment samples. This difference was not statistically significant when analyzed as above (P = 0.26). Two patients (nos. 8 and 9) showed large increases in apoptosis along with large decreases in proliferation; these may represent a subpopulation of patients that are more responsive to ZD1839 treatment.

**Relationship between Proliferation, Apoptosis, and Changes in Measurable Disease.** 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 R² 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 (R² = 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...
of EGFR in cultured cells, via activation of ras. ERK1 and ERK2 are activated by phosphorylation on specific tyrosine and threonine residues. We evaluated ERK1/2 activation using an antibody specific for phosphorylated ERK. Staining for activated ERK was only detected in the tumor cells from 1 patient for which paired samples were obtained (Table 1 and Fig. 2) and 1 additional patient where only a pretreatment sample was obtained (12 pretreatment samples were analyzed in total). In the 1 positive patient where a posttreatment sample was available, staining was absent after treatment with ZD1839. This suggests that ZD1839 blocked ERK activation in this patient. Although activated ERK was detected infrequently in cancer cells, it was invariably present in tumor stromal fibroblasts (Fig. 3). In 5 of 9 patients, levels of activated ERK in stromal fibroblasts were decreased after ZD1839 treatment. In some patients, the number of stromal fibroblasts staining positive was decreased, whereas in others, activated ERK staining in stromal fibroblasts was completely absent in the posttreatment sample.

**p27^Kip1 Immunohistochemistry.** Levels of the cyclin-dependent protein kinase inhibitor p27^Kip1 were also assessed in biopsies from patients. This protein has a well characterized role in the inhibition of the G1-S transition during the cell cycle, and activation of EGFR has been shown to decrease levels of p27^Kip1 by promoting its degradation (19). Active p27^Kip1 is present 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 p27^Kip1 expression and subcellular localization in paired biopsies. p27^Kip1 was absent in 7 of 9 pretreatment samples assayed (including data from patients with pretreatment biopsies only). In the 2 patients where p27^Kip1 was detected before treatment, much of the staining was cytosolic rather than nuclear (Fig. 3). Two patients showed strong staining for p27^Kip1 in their posttreatment biopsies; this staining was predominantly cytosolic and was undetectable in their pretreatment samples (Fig. 3). These 2 patients were also the only 2 patients that showed a large increase in apoptosis index with treatment (Table 1). p27^Kip1 staining that was predominantly cytosolic was observed in 2 posttreatment biopsies; in 1 patient, it was also present in the

### Table 1

Analysis of paired biopsy samples. Pre- and posttreatment results are shown separated by a “/”. The total number of cells that were assessed for Ki67 and TUNEL staining is shown in the second column. Activated EGFR is abbreviated as aEGFR. The type of cell that stained positive for phosphorylated (p)ERK is indicated in the column immediately to the left of the phosphorylated (p)ERK column (str; stromal cells; tu; tumor cells; no str indicates that no stromal cells were present in the sample). The subcellular location of p27^Kip1 staining is indicated in the column immediately to the left of the pAKT column (n, nuclear; c, cytoplasmic). The * symbol indicates a test was not performed because of insufficient sample. Additional data from patients that were biopsied only before treatment is discussed in the text.

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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 numbers 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.](clincancerres.aacrjournals.org)
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^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^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^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 the role that activated ERK plays in this cell compartment.

β-Catenin Immunohistochemistry. β-Catenin is overexpressed in many colorectal cancers because of inactivation of the APC gene product, which normally promotes the degradation of β-catenin (23, 24). We assayed β-catenin expression in 7 pretreatment patient samples (3 from the paired samples and 4 from patients for which only a pretreatment sample was available; data not shown). All of these showed high levels of β-catenin expression. We also analyzed two paired samples (patient nos. 5 and 9) for β-catenin expression (data not shown): ZD1839 treatment did not affect expression in either case. This is consistent with animal studies showing that EGFR inhibition does not affect β-catenin levels in mice with mutations in the APC gene (12).

Fig. 3 Immunohistochemistry for phosphorylated ERK in stromal fibroblasts and p27^Kip1 in tumor cells. The top panel shows an example of positive staining for phosphorylated ERK in stromal fibroblasts, which was detected in all patients where stromal fibroblasts were present. A larger field is shown to illustrate the distribution of phosphorylated ERK staining. In the posttreatment sample, the number of fibroblasts staining positive was reduced. The middle two sets of panels show sections from patient nos. 8 and 9, stained for p27^Kip1. Staining for p27^Kip1 is predominantly cytoplasmic in this patient and is present both before and after treatment.

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
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 p27Kip1 was most interesting with respect to the problem of why patients may respond differently to ZD1839. Although nuclear p27Kip1 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, p27Kip1 is also able to induce apoptosis in some cancer cell lines. This role has been demonstrated both by exogenous delivery of p27Kip1 using viral vectors (35, 36) and by inhibition of endogenous p27Kip1 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 showed this without detectable increases in p27Kip1. However, the 2 patients that showed nuclear p27Kip1 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 p27Kip1-induced apoptosis. Many of the activities of p27Kip1 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 p27Kip1 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 p27Kip1 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, p27Kip1 was not detected after treatment. One possibility is that tyrosine kinase receptors other than EGFR are promoting p27Kip1 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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