Brain Tumors in Mice Are Susceptible to Blockade of Epidermal Growth Factor Receptor (EGFR) with the Oral, Specific, EGFR-Tyrosine Kinase Inhibitor ZD1839 (Iressa)\textsuperscript{1}

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ABSTRACT

Iressa (ZD1839) is a p.o.-active, selective, epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) that blocks signal transduction pathways implicated in cancer cell proliferation, survival, and host-dependent processes promoting cancer growth. EGFR is up-regulated in primary malignant tumors of the central nervous system (CNS) and in many systemic tumors that metastasize to the CNS. The purpose of our study was to evaluate the efficacy and toxicity of p.o.-administered ZD1839 for the treatment of established intracerebral (i.c.) tumors expressing EGFR or the tumorigenic mutated variant EGFRvIII, which is constitutively phosphorylated. Oral administration of ZD1839 at 50 or 100 mg/kg/day for 3 weeks in athymic mice with established i.c. A431 human epidermoid carcinoma expressing EGFR increased median survival by 88% (P = 0.009) and 105% (P < 0.001), respectively. Additionally, there was no evidence of systemic or CNS toxicity. However, ZD1839 failed to inhibit either s.c. or i.c. in vivo tumor growth when tumorigenicity was conferred by EGFRvIII. Western blotting revealed that treatment with ZD1839 virtually ablated phosphorylation of EGFR Tyr-1173 in A431 tumors. However, treatment of NR6M tumors with ZD1839 only partially decreased phosphorylation of EGFRvIII Tyr-1173 while up-regulating overall expression, suggesting that EGFRvIII may not be susceptible to the same molecular mechanisms of tyrosine kinase inhibition as EGFR. In conclusion, ZD1839 is active in a brain tumor model expressing EGFR, but not EGFRvIII, as EGFR mutations may lead to relative therapeutic resistance. On the basis of these observations, we believe that clinical trials of ZD1839 against brain tumors expressing EGFR are warranted, but that special consideration should be given to tumors that coexpress EGFRvIII.

INTRODUCTION

The EGFR\textsuperscript{4} gene is frequently overexpressed in malignant primary tumors of the brain (1) and in tumors that commonly metastasize to the CNS, such as breast and non-small cell lung carcinomas (2). EGFR-TKIs have been developed to block overactive autocrine pathways that provide growth factor-induced proliferation within tumor cells. ZD1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy) quinazoline (molecular weight, 446.9) is a p.o.-administered EGFR-TKI of the quinazoline class with demonstrated \textit{in vitro} efficacy against lung, colorectal, prostate, ovarian, and breast cell lines and \textit{in vivo} growth delay in a range of s.c. human tumor xenografts that express EGFR (data on file at AstraZeneca, Cheshire, United Kingdom).

Tumors that express wild-type EGFR also express a mutated version of this receptor (3), EGFRvIII, characterized by a consistent in-frame deletion of 801 bp from the extracellular domain that splits a codon and produces a novel glycine amino acid at the fusion junction (1). The EGFRvIII alone confers enhanced tumorigenicity (4) in transfected cell lines and is frequently coexpressed with EGFR. Many systemic tumors overexpress wild-type EGFR and EGFRvIII and find refuge within the CNS, a site typically refractory to traditional chemotherapeutics. Because the majority of malignant primary brain tumors also overexpress wild-type EGFR and EGFRvIII, we sought to determine the \textit{in vivo} efficacy of ZD1839 against i.c. tumors expressing these receptors and its effect on receptor phosphorylation.

Recent studies have suggested a direct correlation between EGFR phosphorylation and cell cycle initiation (5, 6). In addition, Su Huang et al. (7) and Downward et al. (8) have shown that Tyr-1173 is the favored autophosphorylation site in ligand-activated wild-type EGFR and appears to be the major site of

\textsuperscript{1}The abbreviations used are: EGFR, epidermal growth factor receptor; CNS, central nervous system; EGFR-TKI, EGFR-tyrosine kinase inhibitor; EGFRvIII, EGFR variant III; i.c., intracerebral; DPBS, Dulbecco’s PBS; TBS, Tris-buffered saline.
autophosphorylation in mutant EGFRvIII. It is believed that phosphorylation of Tyr-1173 is important to EGFR-mediated signal transduction (7), because mutational analysis of Tyr-1173 has demonstrated that this phosphorylated residue is required for the enhanced tumorigenesis characteristic of mutant EGFRvIII (7). The studies reported herein demonstrate that oral treatment with ZD1839 has marked efficacy against i.c. tumors expressing wild-type EGFR, resulting in increased survival and nearly complete inhibition of receptor phosphorylation with minimal systemic or CNS toxicity. However, ZD1839 fails to inhibit the growth of tumors dependent on EGFRvIII, possibly because of its inability to completely inhibit EGFRvIII phosphorylation.

Materials and Methods

Assessment of Toxicity. To evaluate systemic toxicity, athymic male BALB/c nude mice without tumors received ZD1839 on weekdays at doses of 50 mg/kg/day (n = 5) or 100 mg/kg/day (n = 5) or the carrier as a control (n = 5) over 28 days for a total of 20 doses. Toxicity was monitored by daily weights, neurological examinations, and postmortem H&E histological examination of the brain, skin, spleen, heart, bone marrow, bowel, lung, liver, and kidney. Mice were euthanized with CO2 on day 28 of treatment and immediately autopsied, with organs fixed with formalin and histologically examined.

Xenografts. The human epidermoid carcinoma A431 (9) that expresses high levels of EGFR, approximately 2.7–3.8 × 10⁶ wild-type EGFR receptors/cell (10), was obtained from Ira Pastan (Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD). NR6M is a murine Swiss 3T3-fibroblast cell line that lacks endogenous wild-type EGFR expression that was stably transfected with the full-length human EGFRvIII cDNA and expresses 7.3–7.9 × 10⁵ mutant EGFRvIII receptors/cell (10). Tumorigenicity and consistent in vivo growth are conferred solely by the expression of EGFRvIII in this cell line, as has been demonstrated previously (11). Both cell lines were grown in zinc option medium (Life Technologies, Inc., Gaithersburg, MD) containing 10% (vol/vol) fetal bovine serum and were verified to be free of Mycoplasma contamination (12).

Tumor Implantation. For s.c. tumors, A431 or NR6M cells at logarithmic growth in vitro were harvested and washed twice with PBS. Cellular viability was >95%, as determined by trypan blue exclusion. s.c. tumors were initiated by implantation of 1 × 10⁶ A431 or NR6M suspended in 100 μl of PBS into the right flank of athymic mice.

For i.c. tumors, cells were resuspended in 2.5% methylcellulose, and the lethal tumorigenic dose of 1 × 10⁶ A431 cells or 2.5 × 10⁵ NR6M cells was injected i.c. into athymic mice (Jackson Laboratory, Bar Harbor, ME) in a volume of 1 μl using a 250-μl Hamilton syringe and injector (Hamilton Co., Reno, NV) with an attached 25-gauge needle. The needle was positioned along the coronal suture, 2 mm to the right of bregma and 4 mm below the surface of the skull, by using a stereotactic frame (Kopf Instruments, Tujunga, CA).

Treatment. Iressa (ZD1839) was supplied in lyophilized format from AstraZeneca and suspended in a carrier of 0.5% Tween 80 (Sigma Chemical Co., St. Louis, MO) and sterile H₂O at a concentration of 12.5 mg/ml (50-mg/kg dose) or 25 mg/ml (100-mg/kg dose), divided into daily doses, and stored in polypropylene vials at −135°C. Before administration, the vials were thawed, sonicated for 10–30 s, and administered to athymic mice in a volume of 100 μl via oral gavage.

For s.c. tumors, mice were treated daily starting 10 days after tumor implantation, when the tumors achieved an average volume of 0.4 cm³, with ZD1839 at 100 mg/kg/day for a total of 13 days. For i.c. tumors, treatment began 3 days after tumor implantation, when sample tumors were histologically evident, and consisted of a total of 15 weekday doses over 21 days with ZD1839 at 50 or 100 mg/kg/day. In vivo efficacy was assessed by comparing the treated and the control groups for tumor volume of s.c. tumors and for median survival time of mice with i.c. tumors.

Western Blot Analysis. NR6M and A431 cells were untreated or treated with ZD1839 and were cultured for 7 days, as indicated in the figure legends. At the time of harvest, cells were washed once in 1× DPBS, centrifuged, and lysed in 100 μl of Triton X-100 lysis buffer [50 mM HEPEC (pH 7.6), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 10% glycerol, 20 mM β-glycerophosphate, 1 mM NaVO₄, 1 mM NaF, 1 mM benzamidine, 5 mM p-nitrophenylphosphate, 1 mM DTT, 1 mg/ml leupeptin, 1 mg/ml aprotinin, 10 mM pepstatin, and 1 mM phenylmethylsulfonyl fluoride]. Protein concentrations were determined using BCA Protein Assay Reagent (Pierce Chemical Co., Rockford, IL). Proteins (50 μg of protein/lane) were separated by SDS-PAGE (4–20% acrylamide) according to the Laemml method, along with low-range molecular weight markers (Bio-Rad Laboratories, Hercules, CA), and transferred to Hybond-P chemiluminescence (ECL) polyvinylidene difluoride (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) at 15 V for 1.2 h at room temperature, using a semidy transfer chamber (Bio-Rad). The polyvinylidene difluoride membranes were blocked for 1 h with 5% nonfat milk in 1× TBS/0.1% Tween 20 and incubated for 2 h with antirabbit IgG (Cell Signaling) or antinoise IgG (Cell Signaling) conjugated to horseradish peroxidase. The membranes were washed six times in 1× TBS/0.1% Tween 20 and then probed with phospho-Tyr-1068 EGFR (Cell Signaling, Beverly, MA), phospho-Tyr-1173 EGFR (Santa Cruz Biotechnology, Santa Cruz, CA), pan EGFR (Cell Signaling, Beverly, MA), or β-actin (Sigma Chemical Co.) antibodies diluted in 5% nonfat milk in 1× TBS/0.1% Tween 20. The membranes were washed three times in 1× TBS/0.1% Tween 20 and incubated for 2 h with anti-rabbit IgG (Cell Signaling) or antimouse IgG (Cell Signaling) conjugated to horseradish peroxidase. The membranes were washed six times in 1× TBS/0.1% Tween 20 and phospho-EGFR, EGFR, or β-actin was visualized using the Phototope-HRP Western Blot Detection System (Cell Signaling), according to the manufacturer’s instructions.

Flow Cytometry Cell Cycle Analysis. In vivo untreated and ZD1839-treated NR6M and A431P cells were harvested with 0.125% trypsin, washed twice in 1× DPBS with 0.1% glucose, counted, and fixed overnight with 70% (vol/vol) ethanol. Cells were then centrifuged at 1333 × g for 10 min; resuspended at a concentration of 1 × 10⁶ cells/ml in 1× DPBS with 0.1% glucose, 50 mg/ml propidium iodide (Sigma), and 100 units/ml of RNase A (Sigma); and analyzed by flow cytometry. Gating and subsequent fluorescence-activated cell sorter analysis was assessed relative to M1 representing subcellular fractions (apoptosis or necrosis), M2 representing cells with normal diploid constitution, M3 representing cells under-
RESULTS

ZD1839 Maximally Tolerated Dose. To evaluate the toxicity of ZD1839, non-tumor-bearing mice were treated every day for 3 weeks with ZD1839 at 100 or 50 mg/kg/day or the carrier control. Daily treatment of ZD1839 at 100 mg/kg/day over 3 weeks in athymic mice, however, resulted in a 30% mortality rate. Dose-limiting toxicity has been attributed to renal papillary necrosis (data on file at AstraZeneca). When ZD1839 was delivered only during the weekdays, we observed no mortality, no mean weight loss >10%, and no neurological deficit in either treatment group over an observation period of 4 weeks. On day 28, organs were formalin fixed. Detailed H&E histological examination of the spleen, skin, kidney, lung, heart, sternal bone marrow, and gastrointestinal tract organs by an observer blinded to the treatment groups failed to reveal any significant differences among athymic mice treated with either dose of ZD1839 or the carrier control (Table 1). H&E histological evaluation of the brain failed to demonstrate any gliosis, hemorrhage, necrosis, or edema after administration in any treatment group.

ZD1839 Is Efficacious against Intracranial Tumors Expressing EGFR. To determine whether oral administration of ZD1839 was efficacious against i.c. tumors, athymic mice with established A431 i.c. tumors were treated for 3 weeks at 50 mg/kg/day during weekdays. This resulted in a median survival of 34 days, which is an 88% (P = 0.009) increase compared with tumor-bearing mice receiving the carrier control, which had a median survival of 18 days (Fig. 1A). Additionally, 10% (1 of 10) of mice treated at the 50-mg/kg/day dose during weekdays survived >50 days without clinical or histological evidence of tumor. Oral administration of ZD1839 at 100 mg/kg/day during weekdays resulted in a median survival of 37 days, which is a 105% (P < 0.001) increase compared with tumor-bearing mice receiving the carrier control. The survival difference between mice dosed with 100 mg/kg/day and those dosed with 50 mg/kg/day was also statistically significant (P = 0.022), suggesting a dose response.

To confirm that ZD1839 was efficacious against this xenograft when implanted s.c., we evaluated the efficacy of oral ZD1839 in treating mice with s.c. A431 tumors. During the treatment interval, there was marked suppression of A431 growth; however, there was not total elimination of tumor (Fig. 1B). Upon withdrawal of the ZD1839, all mice eventually had regrowth of s.c. tumors.

Tumorigenicity Conferred by EGFRvIII Is Not Susceptible to Growth Inhibition by Treatment with ZD1839. To determine whether oral administration of ZD1839 is efficacious against EGFRvIII-mediated tumorigenicity, NR6M cells, which had been stably transfected with the EGFRvIII and are dependent on the expression of EGFRvIII for tumorigenicity in vivo, were injected into athymic mice. The mice were subsequently treated with ZD1839 3 days after i.c. challenge, when tumors were evident histologically. Oral administration of ZD1839 at 100 mg/kg/day resulted in a median survival of 10 days, which is not a statistically significant (P = 0.407) increase compared with mice receiving the carrier control with a median survival of 9 days (Fig. 2A). Treatment duration was limited by the demise of all tumor-bearing mice in all treatment groups.

To eliminate the possibility that failure of ZD1839 to treat EGFRvIII-dependent tumors was related to a failure to achieve therapeutic levels within the CNS or the short survival times of the mice with i.c. tumors, we evaluated the efficacy of oral ZD1839 in treating mice with s.c. NR6M tumors and again found that there was no growth suppression of NR6M, which is dependent on the EGFRvIII for growth (Ref. 10; Fig. 2B).

Phospho-Tyrosine EGFR Expression Is Ablated in s.c. A431 Tumors, but not NR6M Tumors, Treated with ZD1839. To further investigate the effects of ZD1839 treatment on growth, untreated and 100 mg/kg/day ZD1839-treated s.c. NR6M and A431 tumors were grown in athymic mice and were analyzed for phospho-Tyr-1173 expression by Western blot analysis. ZD1839 was administered daily for 15 days and produced reversible dose-dependent growth inhibition of A431 tumors but had no effect on NR6M tumor growth. Previous studies have shown that daily oral administration of ZD1839 to nude mice (50 mg/kg) over 14 days yields a peak measured blood concentration of 5.9 μM 2 h after dosing and has a terminal half-life of ~3.6 h (data on file at AstraZeneca). At the end of treatment, tumors that were not harvested resumed a growth rate comparable with that of controls (data not shown).

**Table 1** Systemic histopathological effects of ZD1839 in athymic mice

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<th>CNS</th>
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* a Erythroid hyperplasia.

* b NA, not applicable.
In both untreated NR6M and A431 tumors, phospho-Tyr-1173 was constitutively expressed (Fig. 3A). Although treatment of NR6M tumors with 100 mg/kg/day ZD1839 did decrease overall phospho-Tyr-1173 expression, this same expression was virtually abolished in ZD1839-treated A431 tumors (Fig. 3A).

To support these observations, total EGFR and \(\beta\)/H9252-actin expression were determined by Western blotting, as well. Notably, total EGFRvIII proteins were up-regulated after treatment with ZD1839, whereas \(\beta\)/H9252-actin expression was found to be unchanged (Fig. 3A). A graphical representation of these data shows that there is a 2.8-fold decrease in phospho-Tyr-1173 expression in ZD1839-treated NR6M tumors (Fig. 3B), when compared with the expression in untreated tumors and normalized to total EGFR and \(\beta\)-actin \((P = 0.024)\). The observations are striking in ZD1839-treated A431 tumors, in which there is a 50-fold decrease in phospho-Tyr-1173 expression (Fig. 3B), when compared with the same expression in untreated tumors and normalized to total EGFR and \(\beta\)-actin \((P = 0.002)\).

**Cell Death Is Significantly Increased Whereas DNA Synthesis Is Significantly Decreased in A431 Tumors, but not NR6M Tumors, Treated in Vivo with ZD1839.** To further investigate the effects of ZD1839 in vivo, we wanted to confirm whether cell death was induced by treatment with the drug as well as to determine whether it had antitumor effects aside from cell death for s.c. NR6M and A431 tumors. Untreated and 100 mg/kg/day ZD1839-treated s.c. NR6M and A431 tumors were grown in vivo in athymic mice and harvested, counted, stained with propidium iodide, and studied by flow cytometry. NR6M cells treated with ZD1839 failed to induce statistically significant cell death (16%) compared with untreated cells (12%; \(P = 0.215\)), as determined by the mean percentage of subcellular events indicating cell death and denoted as M1 (Fig. 3C). In addition, ZD1839 treatment of NR6M cells did not decrease DNA synthesis to statistical significance (32%) either, when compared with untreated cells (34%; \(P = 0.392\)), as determined by the mean percentage of cells in the S, M, and G2 phases of the cell cycle and denoted as M3/M4. However, A431 cells treated with ZD1839 did induce statistically significant cell death (12%) when compared with untreated cells (4%; \(P = 0.018\)), whereas there was a statistically significant decrease in the mean percentage of cells in the S, M, and G2 phases of the cell cycle (8%), compared with untreated cells (23%; \(P = 0.005\); Fig. 3C).

**DISCUSSION**

There are few p.o. administered chemotherapeutics currently available with demonstrated efficacy against i.c. tumors. The principal finding in our study is that ZD1839, a p.o.-active EGFR-TKI, is efficacious against i.c. tumors expressing the
wild-type EGFR. This is important not only for EGFR-expressing primary malignant tumors of the CNS but also metastatic tumors that also frequently overexpress EGFR and that commonly seek refuge within the CNS. Previous studies have shown that oral administration of ZD1839 to rats (5 mg/kg) yields a terminal half-life of approximately 3–13.8 h in male animals and 5–8.2 h in female animals (data on file at AstraZeneca). The absolute bioavailability after oral dosing at 5 mg/kg was 49.8%. Consequently, when dosage was increased to 12.5 mg/kg, the absolute bioavailability was 76.7 and 87.6% in male and female animals, respectively. On the basis of this information, we had reason to believe that in our experiments treating with nontoxic doses 10–20-fold higher than previously reported would yield absolute bioavailabilities approaching 100% and would be therapeutically relevant. Specifically, we found that oral administration of ZD1839 at 50 or 100 mg/kg/day to treat an established i.c. A431 xenograft in athymic mice increased median survival by 88 and 105%, respectively (Fig. 1A). These results are especially notable because there is only a low distribution of radiolabeled [14C]ZD1839 to the CNS, as assessed by quantitative whole-body autoradiography 2 h after single oral dosing during peak concentrations to non-tumor-bearing rats (data on file at AstraZeneca). This discrepancy may be a consequence of the altered blood-brain barrier within tumor-bearing animals (14, 15). Despite the autoradiography data, the in vivo findings in this report support the use of ZD1839 in the treatment of established primary and metastatic brain tumors expressing EGFR.

The second purpose of our study was to evaluate the efficacy of ZD1839 against tumors expressing the constitutively phosphorylated EGFR mutant, EGFRvIII, which is commonly coexpressed on a variety of tumors that overexpress EGFR. EGFRvIII has been reported to enhance tumorigenicity through constitutively active signal transduction pathways similar to those used by EGFR (16, 17), and, presumably, the intracellular domains of EGFR and EGFRvIII are structurally the same. Prigent et al. (16) have suggested that EGFRvIII signal transduction proceeds through the Ras-Raf-MAPK pathway, whereas Moscatello et al. (17) have proposed that the phosphatidylinositol-3'-inositol kinase pathway is, in part, responsible for EGFRvIII signal transduction. Currently, this question has not been resolved conclusively and may be a cell line-specific observation or, rather, EGFRvIII could possibly use both pathways differentially, depending on extracellular conditions. In light of these possibilities, we did not observe s.c. growth delay (Fig. 2B) or an increase in median survival in athymic mice with established i.c. NR6M-derived tumors (Fig. 2A), which are solely dependent on EGFRvIII for growth. In addition, although there is some decrease in EGFRvIII phosphorylation in NR6M tumors, the extent of phosphorylation that is present (∼36%) is sufficient to
promote EGFR-mediated signal transduction and cellular proliferation (Fig. 3, A and B). This notion is supported by previously published reports that suggest that only 10% of typical EGFR phosphorylation levels are necessary for EGFRvIII signaling and cell growth (7, 16).

In conjunction with the data obtained from Western blotting, the lack of in vivo efficacy of ZD1839 against NR6M tumors raises the possibility that EGFRvIII is not as sensitive to the same molecular mechanisms responsible for inhibition of EGFR tyrosine kinase activity by ZD1839. It is thus plausible that there may exist inherent differences between the stroma of NR6M and A431 cells, which allow NR6M cells to effectively exclude the drug or minimize its efficacy. Alternatively, because EGFRvIII is constitutively phosphorylated in vivo, ZD1839 may not be able to “shut off” this tyrosine kinase activity as effectively, once the receptor has been phosphorylated. Consequently, ZD1839 may not be able to effectively inhibit or decrease autophosphorylation, or decreased phosphorylation may lead to an increase in nascent EGFRvIII proteins (Fig. 3A).

Treatment-induced up-regulation of certain oncogenic markers and proteins as a mechanism of positive feedback inhibition by transformed cells has been reported previously and may have a role here as well (18, 19). Most notably, the MDR1 gene, which encodes the drug efflux pump P-glycoprotein, is expressed in the blood-brain barrier and plays a significant role in modulating multidrug resistance in a variety of human cancers. Brain metastases treated with conventional chemotherapeutics have been shown to increase the expression of P-glycoprotein, which is commonly associated with poor prognosis. A similar mechanism for NR6M tumors treated with ZD1839 may apply. Nevertheless, the data we present here are consistent with the notion that whatever differences do exist between EGFR and EGFRvIII signal transduction or A431 and NR6M cells represent the feedback up-regulation of EGFRvIII, which is central to the pathogenesis of NR6M tumors. Finally, there exists the possibility that there is differential susceptibility of EGFRvIII signal transduction to ZD1839. The tyrosine kinase signal pathway has been demonstrated to be down-regulated in some cell lines that express EGFRvIII (20, 21).

Another promising feature for the therapeutic use of ZD1839 is the drug’s overall effect on the cell cycle in EGFR-expressing cells. The data presented here are consistent with the notion that ZD1839 treatment decreased A431 tumor proliferation in vivo. In addition, propidium iodide staining analyses demonstrated that ZD1839 treatment significantly increased the number of subcellular events while decreasing the number of A431 cells undergoing DNA synthesis (Fig. 3C). These results were not upheld with treatment of NR6M tumors but clearly make a case for therapeutic use of ZD1839 in intracranial EGFR-positive cancers, especially in the absence of EGFRvIII mutations.

Our model system using NR6M cells was developed to isolate the activity of ZD1839 against EGFRvIII. From a therapeutic perspective, however, the significance of this differen-
tial effect is uncertain. Many human tumors usually express a combination of EGFR and EGFR-III receptor subtypes (1, 3, 22). Whether cells expressing wild-type EGFR and EGFR-III would be affected by ZD1839 is not known. Although ZD1839 inhibited EGFR-III kinase and growth of NR6M cells, NR6M xenografts were not affected by ZD1839 under the conditions used. Further studies will be required to establish whether similar observations apply to other EGFR-III-expressing tumors. It is conceivable that elimination of cells expressing wild-type EGFR could lead to recurrence with highly malignant cells expressing only EGFR-III. The minimal level of expression of the wild-type EGFR in tumors expressing both wild-type EGFR and EGFR-III that would be responsive to ZD1839 in vivo is currently unknown and would entail testing a panel of tumors with variable expression levels of EGFR and EGFR-III.

In conclusion, the p.o.-active ZD1839 could potentially treat an extensive range of systemic cancers that express EGFR, including CNS metastatic manifestations that confer a poor prognosis. Additionally, because there are relatively few p.o.-administered chemotherapeutics currently available for treatment of primary brain tumors, with their dismal prognosis and overexpression of EGFR (10), ZD1839 represents a promising new agent with a novel mechanism of action for the treatment of these tumors.

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