

ZD1839 (Iressa): An Orally Active Inhibitor of Epidermal Growth Factor Signaling with Potential for Cancer Therapy

Alan E. Wakeling,¹ Simon P. Guy, Jim R. Woodburn, Susan E. Ashton, Brenda J. Curry, Andrew J. Barker, and Keith H. Gibson

Department of Cancer and Infection Research, AstraZeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom

ABSTRACT

The epidermal growth factor receptor (EGFR) is a promising target for anticancer therapy because of its role in tumor growth, metastasis and angiogenesis, and tumor resistance to chemotherapy and radiotherapy. We have developed a low-molecular-weight EGFR tyrosine kinase inhibitor (EGFR-TKI), ZD1839 (Iressa²). ZD1839, a substituted anilinoquinazoline, is a potent EGFR-TKI ($IC_{50} = 0.033 \mu M$) that selectively inhibits EGF-stimulated tumor cell growth ($IC_{50} = 0.054 \mu M$) and that blocks EGF-stimulated EGFR autophosphorylation in tumor cells. In studies with mice bearing a range of human tumor-derived xenografts, ZD1839 given p.o. once a day inhibited tumor growth in a dose-dependent manner. The level of expression of EGFR did not determine xenograft tumor sensitivity to ZD1839. Long-term ZD1839 (>3 months) treatment of mice bearing A431 xenografts was well tolerated, and ZD1839 completely inhibited tumor growth and induced regression of established tumors. No drug-resistant tumors appeared during ZD1839 treatment, but some tumors regrew after drug withdrawal. These studies indicate the potential utility of ZD1839 in the treatment of many human tumors and indicate that continuous once-a-day p.o. dosing might be a suitable therapeutic regimen.

INTRODUCTION

In the 15 years since EGFR³ (erbB1) was recognized as the cellular homologue of the viral oncogene *v-erb*, two strands of research have come together to make a compelling case for EGFR as an attractive target for cancer chemotherapy (1, 2). Complete description of the biochemical pathways that link growth factor binding at the cell surface to cell division and recognition that many common solid tumors of epithelial origin express high levels of EGFR or the homologous type I growth factor receptor erbB2/HER2/neu, which predicts a poor prognosis for the patient, led to several different approaches to drug discovery targeted at this pathway. Recent studies demonstrating that an anti-HER2 monoclonal antibody produces durable objective responses in breast cancer patients with HER2-overexpressing tumors and is well tolerated provided clinical “proof of hypothesis” for this approach (3). An alternative approach to targeting these growth factor receptors involves inhibition of their TK activity (4). This laboratory, in common with many others, adopted this approach and was the first to describe the anilinoquinazoline class of TKIs (5), which has subsequently provided a rich source of drug candidate molecules (4), including ZD1839 (Iressa; Ref. 6).

At the outset of the drug discovery program, two significant issues

were recognized. First, because EGFR is one of four members of the type I family of RTKs, which share a high degree of sequence homology in their kinase domains, and because the type I receptors are themselves part of a much larger “superfamily” of RTKs, would it be possible to synthesize EGFR-selective inhibitors? Second, given that EGFR is commonly expressed in normal tissues as well as in tumors, would EGFR-targeted inhibitors cause unacceptable toxicity? The synthesis of tyrophostins that inhibit EGFR-TK activity without affecting insulin receptor TK activity provided an important precedent for a small-molecule synthetic approach (7). The issue of toxicity could be addressed only with the inhibitors at hand, but attenuation, rather than complete blockade, of the abnormally active signal in tumors, implied by the high levels of EGFR expression, could provide an opportunity to define an acceptable therapeutic ratio between efficacy and toxicity.

This study was undertaken to examine the efficacy of ZD1839 for the growth inhibition of a range of human tumor xenografts based on a once-a-day p.o. dosing regimen and to evaluate the pharmacology of this targeted therapy.

MATERIALS AND METHODS

ZD1839. ZD1839 (Fig. 1) was synthesized as described by Barker *et al.* (6).

RTK Inhibition. ZD1839 inhibition of EGFR TK activity was measured by a substrate inhibition assay with EGFR prepared from the human vulval squamous carcinoma cell line A431 (which overexpresses EGFR), as described previously (5). Additionally, an ELISA was used to determine the ability of ZD1839 to inhibit kinase activity of baculovirus-expressed EGFR, erbB2, KDR, and Flt-1 TK domains, as described previously (8), but with the ATP concentration fixed at the K_m for each enzyme. ZD1839 inhibition of raf, MEK-1, and ERK-2 (MAPK) was measured by previously described assays (9). IC_{50} data were interpolated by nonlinear regression (four-parameter logistic equation) using Microcal Origin software (version 3.78; Microcal Software Inc., Northampton, MA). Enzyme kinetics with respect to ATP and substrate were determined as described by Ward *et al.* (5). ZD1839 inhibition of EGFR autophosphorylation was measured in lysates of tumor cells [HT29 (colon), KB (oral squamous), Du145 (prostate), A549 (lung)] by electrophoresis and Western blotting with antiphosphotyrosine antibodies (UBI 4G10; Upstate Biotechnology Inc., Lake Placid, NY), as described by Fry *et al.* (10).

Cell Growth Assays. A standard MTT assay was used to measure cell growth (11). Briefly, KB cells were seeded into 96-well culture plates (1.25×10^3 cells/well) in DMEM containing 5% (v/v) charcoal-treated FCS (to deplete endogenous growth factors), 2.0 mM L-glutamine, and 1% (v/v) nonessential amino acids; after attachment, cells were incubated for 72 h at 37°C with ZD1839 in the absence (control) or presence of EGF (10 ng/ml). After incubation, 15 μ l of MTT (Sigma) solution (10 mg/ml) were added to each well, and the plates were then incubated for 1 h at 37°C. Medium was replaced with 100 μ l of acid alcohol (90% ethanol, 5% acetic acid, 5% deionized water) per well. Absorbance at 540 nm was measured using a Titertek Multiscan. Cell growth was calculated by subtracting the mean day 0 absorbance value from the mean absorbance value at day 3. Cell growth inhibition was confirmed, with KB cells grown under similar conditions in 12-well dishes, by trypsinization and cell counting (Coulter) after 3 or 5 days of growth in the absence or presence of EGF and ZD1839 (0.05–25 μ M). ZD1839 inhibition of EGF-, FGF- and VEGF-stimulated growth of HUVECs was measured as described (8). Briefly, HUVECs isolated from umbilical

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¹ To whom requests for reprints should be addressed, at Department of Cancer and Infection Research, AstraZeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom. Phone: 44 1625 515116; Fax: 44 1625 513624.

² Iressa is a trademark of the AstraZeneca group of companies.

³ The abbreviations used are: EGFR, epidermal growth factor receptor; TK, tyrosine kinase; TKI, tyrosine kinase inhibitor; RTK, receptor tyrosine kinase; MEK, mitogen-activated protein/extracellular signal-related kinase kinase; ERK, extracellular signal-related kinase; MAPK, mitogen-activated protein kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HUVEC, human umbilical vascular endothelial cell; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; TGF α , transforming growth factor α .

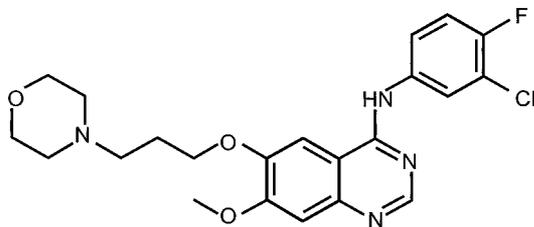


Fig. 1. Structure of ZD1839 (Iressa).

cords were seeded into 96-well plates (1×10^3 cells/well) and incubated for 4 days with ZD1839 in the absence or presence of EGF (10 ng/ml), FGF (0.3 ng/ml), or VEGF (3 ng/ml). Four h before harvesting, [^3H]thymidine (1 $\mu\text{Ci/ml}$) was added. After the medium was removed and the cells were washed with PBS/A, 20 μl of trypsin-EDTA (2.5% trypsin, 1.6 g/l EDTA) solution was added to each well. The cells were harvested by use of a 96-well plate harvester (TomTek) onto filtermats (Wallac Printed Filtermat A). Once dry, scintillation fluid was added (Wallac β -plate Scint) to the filtermats, and they were assayed in a β -plate scintillation counter (Wallac 1205 β -plate liquid scintillation counter) for incorporation of ^3H .

Tumor Xenograft Studies. Female nude mice (Alderley Park strain, derived from Swiss *nu/nu*; AstraZeneca) approximately 8–10 weeks of age and weighing >18 g were used. Mice were housed in air-filtered laminar flow cabinets and handled using aseptic procedures with a 12-h light cycle and food and water *ad libitum*. Procedures involving animals and their care were conducted in accordance with the institutional guidelines that comply with United Kingdom national policies [Animals (Scientific Procedures) Act 1986]. Fragments of tumor tissue (A431, Du145, CR10, HCT15, HT29, HX62, P246, MDA-MB-231, and MCF-7) or tumor cells (A549, LoVo, KB, MIA PaCa2, MKN45, and AR42J) were injected or implanted s.c. (left flank) under anesthesia. Tumors were allowed to establish growth, and at a designated time after implantation, treatment with ZD1839 commenced. ZD1839 at doses of 3–200 mg/kg was administered p.o. once a day as a ball-milled suspension in 0.5% (v/v) polysorbate 80. Mice were monitored daily for signs of toxicity and were weighed regularly. The maximum dose of ZD1839 administered was 200 mg/kg; this dose did not induce any significant adverse effect on body weight or produce any other signs of toxicity. Tumor growth was assessed at intervals by caliper measurement of tumor diameter in the longest dimension (L) and at right angles to that axis (W). Tumor volume was estimated by the formula, $\pi/6 \times L \times W \times W$, for prolate ellipsoids, and efficacy was determined as percentage inhibition compared with vehicle-treated controls. To assess the pharmacodynamic action of ZD1839 treatment, A431 tumors were excised 6 h after the last of four daily doses of 0, 12.5, 50, and 200 mg/kg ZD1839 or at 2, 4, 6, 24, 30, and 36 h after a single 50 mg/kg dose. Total RNA was extracted from the tumors to quantify *c-fos* mRNA by reverse transcription-PCR.

RESULTS

ZD1839 Enzyme Inhibition Profile. ZD1839 is a submicromolar inhibitor of EGFR TK activity (Table 1). Selectivity was demonstrated *versus* erbB2 and the VEGF TKs KDR and Flt-1, with at least a 100-fold difference in IC_{50} for EGFR compared with the other RTKs. Similarly, ZD1839 did not inhibit the activity of the serine-threonine kinases raf, MEK-1, and ERK-2 (MAPK). Enzyme kinetic studies to determine the characteristics of ZD1839 inhibition of EGFR TK (see Ref. 5), in which peptide substrate concentration was fixed at 2 mM (~ 6 -fold higher than the K_m) and the ATP concentration was varied, showed that ZD1839 is a competitive inhibitor with respect to ATP ($K_i = 2.1 \pm 0.2$ nM). Similarly, when the ATP concentration was fixed at 50 μM (~ 6 -fold higher than the K_m) and peptide concentration was varied, ZD1839 showed noncompetitive kinetics ($K_i = 15.0 \pm 1.0$ nM).

ZD1839 Cell Growth Inhibition Profile. ZD1839 is a potent and selective inhibitor of EGF-stimulated KB tumor cell growth *in vitro* (Table 2).

Selectivity was demonstrated by the >100 -fold difference in IC_{50} for cells grown in the presence ($\text{IC}_{50} = 0.054$ μM) or absence ($\text{IC}_{50} = 8.8$ μM) of EGF. Similarly, ZD1839 selectively inhibited EGF-stimulated growth of HUVECs compared with FGF- or VEGF-stimulated growth (Table 2).

ZD1839 Inhibits EGFR Autophosphorylation. Expression of phosphorylated EGFR varied among the selected tumor cell lines (Fig. 2A, Lane 1), but brief treatment with EGF (100 ng/ml for 5 min) markedly increased tyrosine phosphorylation of EGFR in all four cell lines (Fig. 2A, compare Lanes 1 and 2). Incubation with ZD1839 for 2 h before EGF stimulation produced a dose-dependent inhibition of EGFR autophosphorylation in all of the tumor cell lines (Fig. 2A, Lanes 3–8). ZD1839 completely blocked EGF-stimulated EGFR phosphorylation at 0.16 μM in Du145 (prostate) and A549 (lung) cells, whereas complete inhibition was achieved at 0.8 μM in KB (oral squamous) and HT29 (colon) tumor cells.

Sustained inhibition of autophosphorylation by ZD1839 was observed in “washout” studies in KB cells. After exposure for 2 h to 0.8 or 4 μM ZD1839 and replacement with culture medium without drug before EGF stimulation, complete blockade of autophosphorylation persisted for at least 24 h (Fig. 2B).

Tumor Xenograft Activity. In athymic nude mice bearing A431-derived xenografts, p.o. treatment once a day with ZD1839 (from day 7 after implantation for 3 weeks) inhibited tumor growth in a dose-dependent manner (Fig. 3). The ED_{50} was ~ 50 mg/kg, and the highest dose, 200 mg/kg ZD1839, prevented tumor growth. Similarly, ZD1839 inhibited the growth of A549 lung (Fig. 3) and Du145 prostate (Fig. 3) tumor xenografts in a dose-dependent manner.

Dose-dependent growth inhibition was also observed in colon (HCT15, HT29, LoVo), and squamous (KB) tumor xenografts. The maximum efficacy of ZD1839 (at 200 mg/kg p.o. once a day) differed markedly among different xenografts (Table 3).

Long-term treatment with ZD1839 (200 mg/kg p.o. once a day) was investigated in mice bearing A431 xenografts. Dosing for more than 3 months (up to 100 days) beginning 7 days after tumor implantation completely blocked tumor growth (Fig. 4). No tumors grew during treatment, but drug withdrawal allowed tumors to grow in five of the eight mice during 44 days of follow-up in this experiment. A more stringent test was provided by tumors that were 1 month old at the start of therapy. ZD1839 treatment (200 mg/kg p.o. once a day) for 90

Table 1. *In vitro* profile of ZD1839

Enzyme	ZD1839 IC_{50}^a (μM)
TKs	
EGFR (A431 membrane prep)	0.033 ^b (0.024–0.059; $n = 3$)
EGFR (baculovirus lysate)	0.027 ^b (0.009–0.054; $n = 5$)
erbB2	3.7–10 ($n = 5$)
KDR	3.7–10 ($n = 2$)
Flt-1	>100 ($n = 2$)
Serine-threonine kinases	
Raf	>10 ($n = 2$)
MEK-1	>10 ($n = 3$)
ERK-2 (MAPK)	>100 ($n = 2$)

^a n , number of experiments.

^b Values are the mean (range).

Table 2. Selectivity of ZD1839 in cell proliferation assays

Cell line (stimulus)	ZD1839 IC_{50}^a (μM)
KB (control)	8.8 ^b (8.64–9.03; $n = 5$)
KB (EGF)	0.054 ^b (0.024–0.084; $n = 5$)
HUVECs (EGF)	0.03–0.1
HUVECs (FGF)	1–3
HUVECs (VEGF)	1–3

^a n , number of experiments.

^b Values are the mean (range).

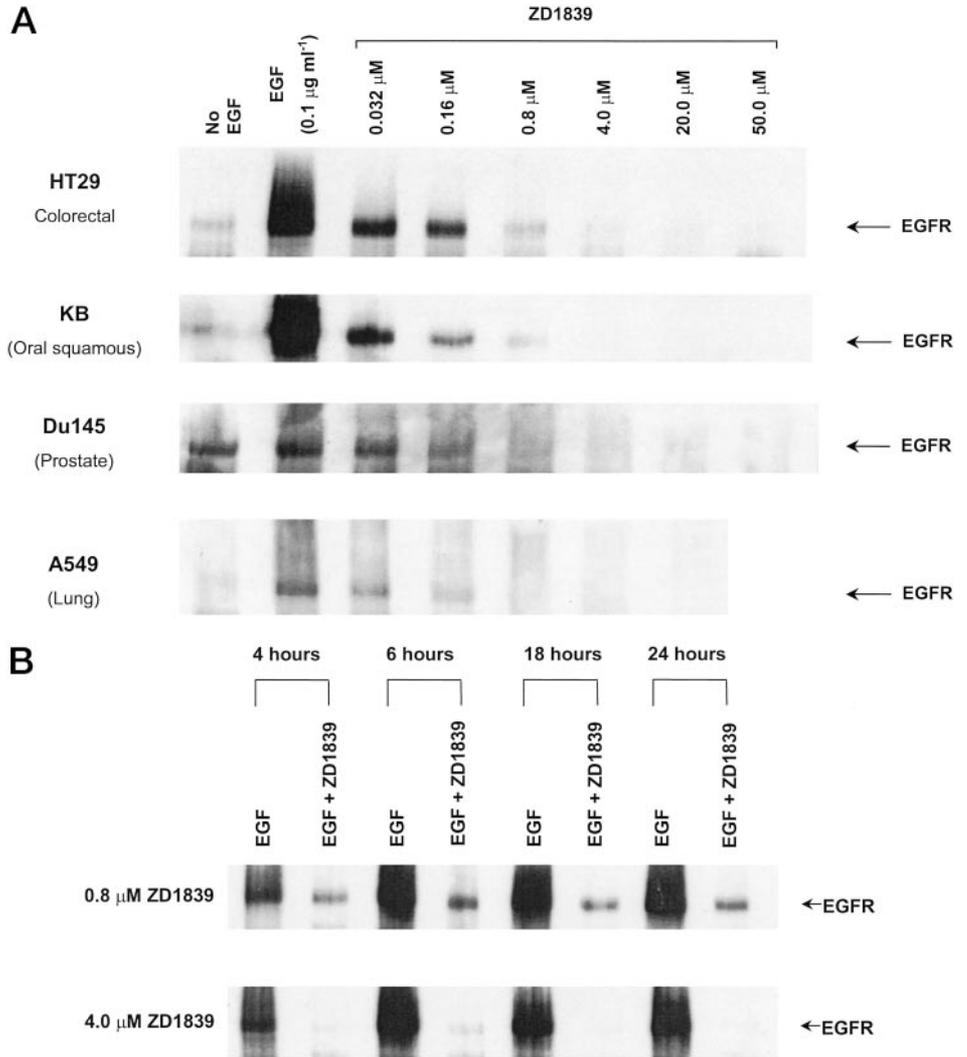


Fig. 2. Inhibition of autophosphorylation with ZD1839 in HT29 (colon), KB (oral squamous), Du145 (prostate), and A549 (lung) cell lines. Tumor cells were incubated in the absence or presence of ZD1839 (final concentration, 0.032–50 μM) for 2 h; EGF (0.1 μg/ml) was added 5 min before cell lysis. Samples were electrophoresed and electroblotted on to polyvinylidene difluoride membranes. For immunodetection of phosphotyrosine residues, membranes were incubated with primary antibody (UBI 4G10 antiphosphotyrosine antibody; 0.5 μg/ml) for 2 h before incubation with secondary antibody (Amersham antimouse horseradish peroxidase-conjugated immunoglobulin) for 1 h (A) and single conjugated antibody (Affiniti Recombinant RC20-horseradish peroxidase; washout studies; B). Detection was with electrochemiluminescence (Pharmacia) reagents according to the manufacturer's instructions. Membrane(s) were exposed to chemiluminescence-sensitive film (Kodak X-Omat).

days produced rapid tumor regression in these more advanced tumors in all mice and, on drug withdrawal, four of six tumors rapidly reestablished (Fig. 5).

Expression of c-fos mRNA in mice bearing A431 xenografts was

inhibited by ZD1839 in a dose-dependent manner after 4 days of treatment. Tumor expression of c-fos was reduced by 94% at the ED₅₀ dose of ZD1839 (50 mg/kg) and was completely blocked (>99%) at the 200 mg/kg dose (data not shown). In mice treated with a single 50

Fig. 3. Effect of ZD1839 on growth of human tumor xenografts. Either fragments of tumor tissue (A431 and Du145) or cells (A549) were suspended in growth medium and implanted s.c. (left flank) under anesthesia in nude mice. Tumors were allowed to establish growth (A431 for 7 days, A549 for 11 days, Du145 for 15 days) after implantation before treatment commenced. Vehicle (◆) or ZD1839 [3.125 mg/kg (■), 12.5 mg/kg (▲), 50 mg/kg (●), or 200 mg/kg (△)] was administered by oral gavage once daily. Data are expressed as mean (bars, SD) tumor volume.

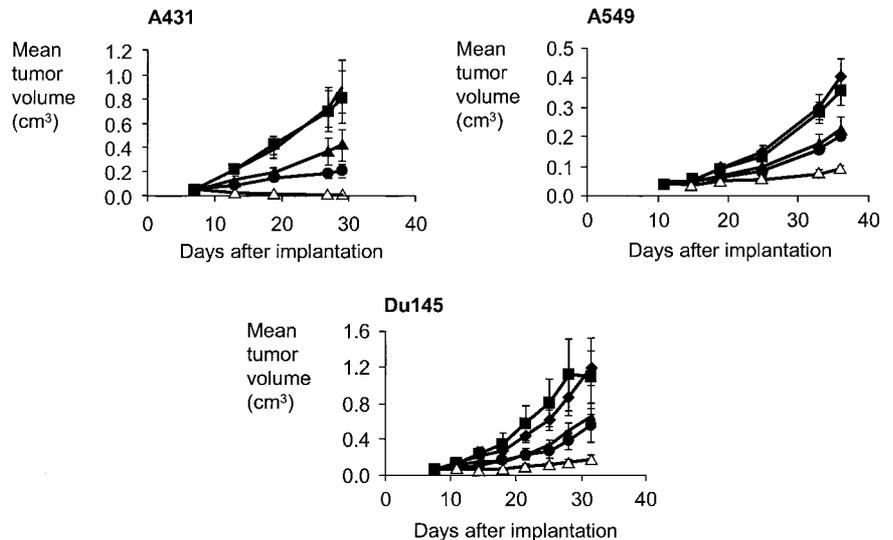


Table 3 Maximum effect of ZD1839 treatment on tumor xenograft growth

Xenograft (origin)	Inhibition of tumor volume relative to vehicle control ^a (%)
KB (oral squamous)	62 ($P < 0.05$)
HX62 (ovary)	56 ($P < 0.01$)
LoVo (colon)	80 ($P < 0.001$)
CR10 (colon)	96 ($P < 0.01$)
HCT15 (colon)	56 ($P < 0.05$)
HT29 (colon)	43 ($P < 0.001$)
MIA PaCa-2 (pancreas)	8
MDA-MB-231 (breast)	6
MKN45 (gastric)	1

^a Inhibition was measured after 14–28 days of treatment.

mg/kg p.o. dose of ZD1839, c-fos expression reached a nadir (5% versus control) at 6 h and did not fully recover until 36 h later (data not shown).

DISCUSSION

ZD1839 is a potent EGFR-TKI *in vitro*, and although it is a competitive inhibitor of ATP and therefore binds at the ATP site on EGFR, it demonstrates remarkable selectivity for EGFR compared with other RTKs that share sequence homology in the ATP binding domain. Other quinazoline RTK inhibitors similarly show a high degree of selectivity for their specific targets (8). Importantly, ZD1839 has no effect on the activity of the serine-threonine kinases raf, MEK-1, and MAPK, which transmit the EGF proliferative signal(s) downstream of EGFR.

The requirement for an *in vitro* test that would distinguish between specific EGFR-TKI-mediated effects on EGF-stimulated growth and serum-stimulated cell growth was recognized at the outset of the drug discovery program and led to the selection for this purpose of the human vulval squamous tumor-derived KB cell line. KB cells grow well in growth factor-depleted medium (*i.e.*, in 5% serum treated with charcoal to deplete growth factors), and addition of EGF increases KB cell growth rate in a reproducible manner. The selectivity of ZD1839 for inhibition of EGF-driven KB cell growth was demonstrated by the large difference in IC₅₀ in the presence or absence of EGF. Cytotoxicity was not observed at ZD1839 concentrations up to 25 μ M. The selectivity of ZD1839 for inhibition of EGF-stimulated cell growth was further exemplified in HUVECs. EGF-stimulated growth of HUVECs was potently inhibited by ZD1839, but bFGF- and VEGF-stimulated growth was relatively unaffected by ZD1839 at concentrations that block EGF effects.

Initiation of EGF-stimulated cell growth is triggered by *trans* autophosphorylation in ligand-binding-activated EGFR/erbB homo- or heterodimers. Western blots of several different human tumor-derived cell lines treated with ZD1839 before brief exposure to EGF showed that ZD1839 blocks EGF-stimulated EGFR autophosphorylation in a dose-dependent and complete manner. Drug washout studies showed that this inhibition is sustained for at least 24 h after a 2-h drug treatment. Other investigators also noted sustained inhibition of autophosphorylation *in vitro* by ZD1839 after drug washout and attributed this to drug sequestration in cells (12). More recent studies indicate that quinazoline EGFR-TKIs sequester EGFR into signaling-inactive receptor-ligand complexes (13).

Other investigators have studied the effects of ZD1839, alone and in combination with other drugs or radiation, on tumor cell proliferation. Ciardiello *et al.* (14) demonstrated that ZD1839 inhibits the proliferation of ovarian, breast, and colon cancer cells and provides a synergistic enhancement of the inhibitory action of cytotoxic drugs. The effect of ZD1839 was cytostatic, but higher doses increased apoptotic cell death, and in combination with cytotoxic drugs, ZD1839 markedly enhanced apoptotic cell death. Ciardiello *et al.* (15) also showed that ZD1839 inhibits tumor cell synthesis of tumor growth factor α and of the proangiogenic growth factors VEGF and bFGF. The IC₅₀ for tumor cell growth inhibition by ZD1839 was not strongly influenced by the level of expression of EGFR (14, 15). Preliminary data indicate that combination of ZD1839 with ionizing radiation also has additive or synergistic effects in non-small cell lung cancer cell lines *in vitro* (16). ZD1839 also effectively inhibits the growth of antiestrogen-resistant human breast cancer cells (17) and human tumor cells that overexpress HER2 (18). Additionally, ZD1839 inhibits ERK-1/2 (MAPK) activity, a downstream maker of EGFR signaling, in EGF-dependent human tumor cells at concentrations similar to those that inhibit cell proliferation (19).

Testing of drug candidate TKIs for *in vivo* activity was carried out with xenografts generated from KB cells, which have been used by others to demonstrate *in vivo* activity of TKIs (20). This model identified the structural features of quinazolines necessary for *in vivo* antitumor activity (21) and indicated the importance for *in vivo* activity of sustaining sufficient drug concentrations in the blood to inhibit EGFR-TK activity throughout each 24-h period after once-a-day dosing (6). ZD1839 was chosen as a candidate for development because it achieves high and sustained blood levels *in vivo* over a 24-h period (6).

The antitumor activity of ZD1839 was demonstrated in tests with

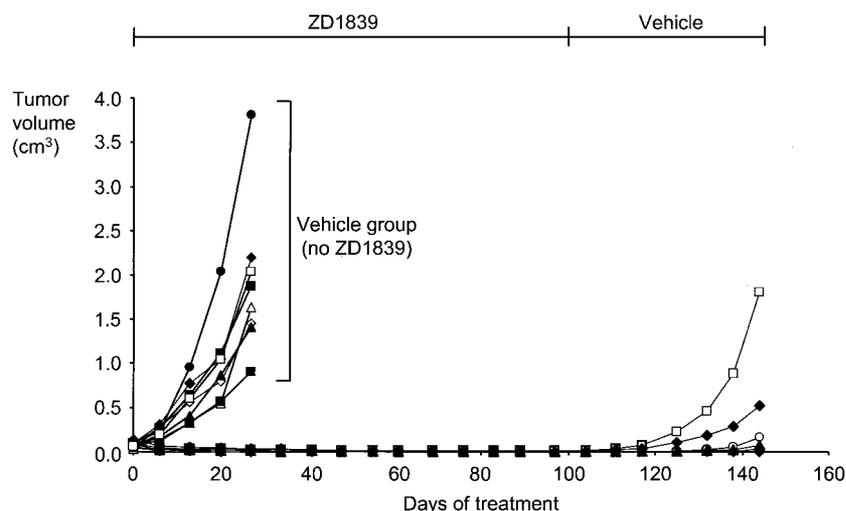


Fig. 4. Effect of prolonged dosing of ZD1839 (200 mg/kg) on growth of A431 (vulval squamous carcinoma) human tumor xenografts and effects of withdrawal of therapy. Dosing began 1 week after tumor implantation (day 0). The ZD1839 group was treated for 100 days, after which ZD1839 was withdrawn and vehicle alone was administered on days 101–144. The vehicle group was treated on days 0–27. Symbols indicate individual tumors.

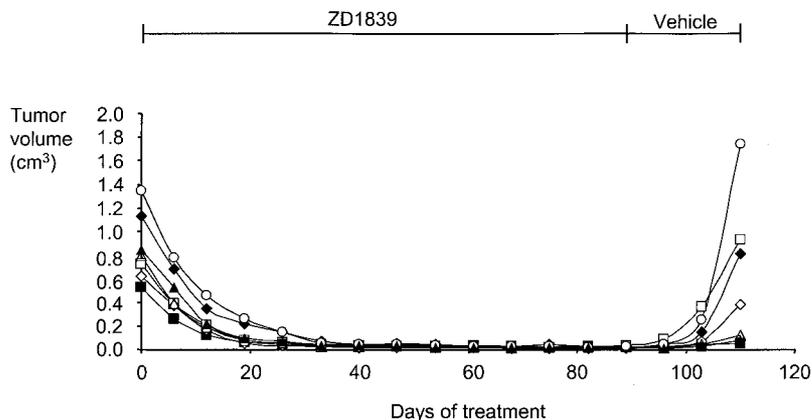


Fig. 5. Effect of prolonged dosing of ZD1839 (200 mg/kg) on growth of advanced (1 month old) A431 (vulval squamous carcinoma) human tumor xenografts and effects of withdrawal of therapy. The ZD1839 group was treated on days 0–89, after which ZD1839 was withdrawn and vehicle alone was used on days 90–110. Symbols indicate individual tumors.

tumor xenografts derived from a range of different human tissues. ZD1839 was particularly effective against A431 xenografts, a recognized model for the testing of biological effects on EGFR signaling (22). ZD1839 inhibited the growth of A431 xenografts in a dose-dependent manner, and complete inhibition was observed in animals receiving a daily p.o. doses of 200 mg/kg ZD1839. Long-term treatment (3–4 months) completely suppressed A431 tumor growth, and withdrawal of drug treatment allowed some tumors to resume growth in the 44-day follow-up period. When ZD1839 treatment was applied to large, well-established A431-derived tumors, rapid tumor regression was observed, which was sustained for the duration of drug treatment (3–4 months). The majority of these tumors resumed growth in the 21-day period after withdrawal of drug treatment, supporting the importance of continuous drug treatment to maintain antitumor activity. No evidence for the development of drug resistance emerged during these studies with A431 tumors because no tumor regrew during long-term ZD1839 treatment.

Dose-dependent tumor growth inhibition by ZD1839 was also demonstrated in mice bearing xenografts of human lung (A549), colon (LoVo, HT29, and HCT15) and prostate (Du145) tumors; additionally, antitumor activity was demonstrated in a breast (MCF-7) and a pancreatic (MIA PaCa-2) model, but some tumors failed to respond to drug treatment [P246 (broncho-epithelial), MKN45 (gastric), and AR42J (pancreatic)]. Review of literature reports on the level of EGFR expression in the cells from which these xenografts were derived reveals a very wide range of expression of EGFR; however, there was no clear correlation between the level of EGFR expression and xenograft sensitivity to ZD1839. Other investigators have also noted that the level of expression of EGFR in cells or tumors did not predict sensitivity to ZD1839 (14, 23). The level of EGFR expression may not indicate the degree to which any individual tumor or tumor cell line is dependent on the EGFR signaling pathway for growth. Additional biomarkers that would more specifically indicate this dependence have yet to be defined. Additionally, coexpression of other members of the erbB family that heterodimerize with EGFR but whose signaling would also be inhibited by ZD1839 through its action on the EGFR component of such heterodimers may also play an important role in determining drug sensitivity (18). Increased EGFR expression is only one mechanism by which enhancement of EGFR signaling drive can be achieved: increased levels of ligand, heterodimerization of EGFR, decreased intracellular phosphatase (which prolongs the activation of EGFR), and mutations in EGFR that lead to constitutive activation of the TK can all contribute to signaling drive. EGFRvIII is a mutated EGFR in which part of the ligand-binding domain of EGFR is missing and the TK is constitutively active. EGFR-TKIs are likely to target this mutant receptor because the kinase domains of EGFRvIII and EGFR are identical.

In addition to the studies reported by Barker *et al.* (6) discussed above, which indicated the importance to biological activity of sustained exposure to drug throughout the dosing interval (24 h in a once-a-day p.o. dosing regimen), additional biomarker studies were performed in tumor-bearing mice. *c-fos* transcription represents one end point of EGFR signaling in tumors because *c-fos* is transiently expressed only in proliferating cells transiting the early, G₁ phase of the cell cycle. Reverse transcription-PCR measurements of *c-fos* mRNA in extracts of A431 tumor xenografts from mice treated with ZD1839 showed that 4 days of drug treatment reduced *c-fos* in a dose-dependent and complete manner, paralleling drug effects on tumor size. When *c-fos* was measured in A431 tumors after a single p.o. dose of 50 mg/kg ZD1839, *c-fos* transcription reached a nadir (5% of control) 6 h after dosing, partially recovered at 24 h (20% of control), and was completely restored at 36 h. Although no complete pharmacokinetic/pharmacodynamic relationship for ZD1839 in the mouse has been defined, these studies indicate that once-a-day p.o. dosing effectively inhibits EGFR signaling throughout the dosing interval and that once-a-day p.o. administration might be a regimen suitable for therapeutic studies in humans.

The studies reported here have indicated the potential utility of ZD1839 as an antitumor agent, but because patients with locally advanced or metastatic cancer undergoing treatment with cytotoxic chemotherapy are most often the target population in which new anticancer agents are tested, combinations of ZD1839 with various cytotoxic drugs with different mechanisms of action have been investigated. These studies showed that combining ZD1839 with platinum (cisplatin, oxaliplatin, carboplatin), taxanes (paclitaxel, docetaxel), and topoisomerase inhibitors (doxorubicin, etoposide, topotecan) or the antimetabolite raltitrexed markedly potentiated cytotoxic drug activity *in vitro* and *in vivo* (14, 23). They also suggest that the addition of ZD1839 to the treatment regimen for patients undergoing cytotoxic chemotherapy might confer significant additional clinical benefits.

In conclusion, these studies demonstrate the potential of ZD1839 (Iressa) in the treatment of many tumors and indicate that continuous once-a-day p.o. dosing may be a suitable therapeutic regimen.

REFERENCES

- Woodburn, J. R. The epidermal growth factor receptor and its inhibition in cancer therapy. *Pharmacol. Ther.*, 82: 241–250, 1999.
- Ciardiello, F., and Tortora, G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin. Cancer Res.*, 7: 2958–2970, 2001.
- Cobleigh, M. A., Vogel, C. L., Tripathy, D., Robert, N. J., Scholl, S., Fehrenbacher, L., Wolter, J. M., Paton, V., Shak, S., Lieberman, G., and Slamon, D. J. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.*, 17: 2639–2648, 1999.

4. Boschelli, D. H. Small molecule inhibitors of receptor tyrosine kinases. *Drugs Future* 24: 515–537, 1999.
5. Ward, W. H. J., Cook, P. N., Slater, A. M., Davies, D. H., Holdgate, G. A., and Green, L. R. Epidermal growth factor receptor tyrosine kinase. Investigation of catalytic mechanism, structure-based searching and discovery of a potent inhibitor. *Biochem. Pharmacol.*, 48: 659–666, 1994.
6. Barker, A. J., Gibson, K. H., Grundy, W., Godfrey, A. A., Barlow, J. J., Healy, M. P., Woodburn, J. R., Ashton, S. E., Curry, B. J., Scarlett, L., Henthorn, L., and Richards, L. Studies leading to the identification of ZD1839 (IRESSA): an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor targeted to the treatment of cancer. *Bioorg. Med. Chem. Lett.*, 11: 1911–1914, 2001.
7. Yaish, P., Gazit, A., Gilon, C., and Levitzki, A. Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors. *Science (Wash. DC)*, 242: 933–935, 1988.
8. Hennequin, L. F., Thomas, A. P., Johnstone, C., Stokes, E. S., Plé, P. A., Lohmann, J. J., Ogilvie, D. J., Dukes, M., Wedge, S. R., Curwen, J. O., Kendrew, J., and Lambert-van der Brempt, C. Design and structure-activity relationship of a new class of potent VEGF receptor tyrosine kinase inhibitors. *J. Med. Chem.*, 42: 5369–5389, 1999.
9. Alessi, D. R., Cohen, P., Ashworth, A., Cowley, S., Leever, S. J., and Marshall, C. J. Assay and expression of mitogen-activated protein kinase, MAP kinase kinase and raf. *Methods Enzymol.*, 255: 279–290, 1995.
10. Fry, D. W., Nelson, J. M., Slintak, V., Keller, P. R., Rewcastle, G. W., Denny, W. A., Zhou, H., and Bridges, A. J. Biochemical and antiproliferative properties of 4-[ar(alkylamino)pyridopyrimidines, a new chemical class of potent and specific epidermal growth factor receptor tyrosine kinase inhibitor. *Biochem. Pharmacol.*, 54: 877–887, 1997.
11. Mossman, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65: 55–63, 1983.
12. Smaill, J. B., Palmer, B. D., Rewcastle, G. W., Denny, W. A., McNamara, D. J., Dobrusin, E. M., Bridges, A. J., Zhou, H., Showalter, H. D. H., Winters, R. T., Leopold, W. R., Fry, D. W., Nelson, J. M., Slintak, V., Elliot, W. L., Roberts, B. J., Vincent, P. W., and Patmore, S. J. Tyrosine kinase inhibitors. 15. 4-(Phenylamino)quinazoline and 4-(phenylamino)pyrido[d]pyrimidine acrylamides as irreversible inhibitors of the ATP binding site of the epidermal growth factor receptor. *J. Med. Chem.*, 42: 1803–1815, 1999.
13. Lichtner, R. B., Menrad, A., Sommer, A., Klar, U., and Schneider, M. R. Signaling-inactive epidermal growth factor receptor/ligand complexes in intact carcinoma cells by quinazoline tyrosine kinase inhibitors. *Cancer Res.*, 61: 5790–5795, 2001.
14. Ciardiello, F., Caputo, R., Bianco, R., Damiano, V., Pomato, G., De Placido, S., Bianco, A. R., and Tortora, G. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin. Cancer Res.*, 6: 2053–2063, 2000.
15. Ciardiello, F., Caputo, R., Bianco, R., Damiano, V., Fontanini, G., Cuccato, S., De Placido, S., Bianco, A. R., and Tortora, G. Inhibition of growth factor production and angiogenesis in human cancer cells by ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor. *Clin. Cancer Res.*, 7: 1459–1465, 2001.
16. Raben, D., Helfrich, B., Phistry, M., and Bunn, P. ZD1839 (Iressa), an EGFR-TKI, potentiates radiation/chemotherapy cytotoxicity in human non-small cell lung cancer (NSCLC) cell lines. 11th NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy, Amsterdam, 2000.
17. McClelland, R. A., Barrow, D., Madden, T. A., Dutkowsky, C. M., Pamment, J., Knowlden, J. M., Gee, J. M. W., and Nicholson, R. I. Enhanced epidermal growth factor receptor signalling in MCF7 breast cancer cells after long-term culture in the presence of the pure antiestrogen ICI 182,780 (Faslodex). *Endocrinology*, 142: 2776–2788, 2001.
18. Moasser, M. M., Basso, A., Averbuch, S. D., and Rosen, N. The tyrosine kinase inhibitor ZD1839 (“Iressa”) inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. *Cancer Res.*, 61: 7184–7188, 2001.
19. Albanell, J., Codony-Servat, J., Rojo, F., Del Campo, J. M., Sauleda, S., Anido, J., Raspall, G., Giral, J., Rosello, J., Nicholson, R. I., Mendelsohn, J., and Baselga, J. Activated extracellular signal-regulated kinases: association with epidermal growth factor receptor/transforming growth factor α expression in head and neck squamous carcinoma and inhibition by anti-epidermal growth factor receptor treatments. *Cancer Res.*, 61: 6500–6510, 2001.
20. Yoneda, T., Lyall, R. M., Alsina, M. M., Persons, P. E., Spada, A. P., Levitzki, A., Zilbestein, A., and Mundy, G. R. The antiproliferative effects of tyrosine kinase inhibitors tyrphostins on a human squamous cell carcinoma *in vitro* and in nude mice. *Cancer Res.*, 51: 4430–4435, 1991.
21. Wakeling, A. E., Barker, A. J., Davies, D. H., Brown, D. S., Green, L. R., Cartledge, S., and Woodburn, J. R. New targets for therapeutic attack. *Endocr. Relat. Cancer*, 4: 1–5, 1997.
22. Robinson, S. P., Dean, B. J., and Petty, B. A. Characterization of the A431 tumour xenograft as an *in vivo* model for testing epidermal growth factor-receptor antagonists. *Int. J. Oncol.*, 1: 293–298, 1992.
23. Sirotnak, F. M., Zakowski, M. F., Miller, V. A., Scher, H. I., and Kris, M. G. Efficacy of cytotoxic agents against human tumour xenografts is markedly enhanced by co-administration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. *Clin. Cancer Res.*, 6: 4885–4892, 2000.

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