

# The Tyrosine Kinase Inhibitor ZD1839 (“Iressa”) Inhibits HER2-driven Signaling and Suppresses the Growth of HER2-overexpressing Tumor Cells<sup>1</sup>

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## ABSTRACT

The epidermal growth factor receptor (EGFR) is commonly overexpressed in many human tumors and provides a new target for anticancer drug development. ZD1839 (“Iressa”), a quinazoline tyrosine kinase inhibitor selective for the EGFR, has shown good activity in preclinical studies and in the early phase of clinical trials. However, because it remains unclear which tumor types are the best targets for treatment with this agent, the molecular characteristics that correlate with tumor sensitivity to ZD1839 have been studied. In a panel of human breast cancer and other epithelial tumor cell lines, HER2-overexpressing tumors were particularly sensitive to ZD1839. Growth inhibition of these tumor cell lines was associated with the dephosphorylation of EGFR, HER2, and HER3, accompanied by the loss of association of HER3 with phosphatidylinositol 3-kinase, and down-regulation of Akt activity. These studies suggest that HER2-overexpressing tumors are particularly susceptible to the inhibition of HER family tyrosine kinase signaling and suggest novel strategies to treat these particularly aggressive tumors.

## INTRODUCTION

Deregulated expression of tyrosine kinases through mutation, amplification, or deregulation is a hallmark of malignancy. Overactivity of these signaling proteins can lead to abnormal growth control and cellular transformation. Thus, selective inhibitors of specific tyrosine kinases might prove to be effective anticancer agents. Studies of the HER family of tyrosine kinases have been at the forefront of these efforts.

Four members of the HER family of transmembrane RTKs<sup>3</sup> are known: EGFR (HER1, ErbB1), HER2 (Neu, ErbB2), HER3 (ErbB3), and HER4 (ErbB4). These proteins share a common structural organization and an overall amino acid homology of 40–50%. Growth factor signaling by the HER family of RTKs is marked by complexity at every step because of the multiplicity of ligands, receptors, effectors, and downstream pathways. More than 25 ligands are known, which bind these RTKs with various affinities, although a high-affinity ligand specific for HER2 has not yet been reported, and this receptor may not have a physiological ligand. Although the four members of the HER family share many structural and functional characteristics, differences in tissue expression and different phenotypes in gene-disrupted mice reveal that each member performs nonredundant functions, and it remains unclear what unique functions distinguish the individual family members (reviewed in Ref. 1). The HER family of RTKs controls a complex process of lateral signaling through ligand-induced homo- and heterodimeriza-

tions. These intra- and inter-receptor interactions define a hierarchical array of possible signaling partners, which greatly complicates efforts to understand their unique functions and pathways (2). Activated receptors interact with numerous cytoplasmic signaling proteins, and these interactions are dependent on specific dimerization partners generating additional signal diversification (1).

Deregulated activity of HER family RTKs is commonly seen in human tumors and is oncogenic in many experimental systems. EGFR and HER2 are transforming in cell culture models and tumorigenic in transgenic mice when activated by overexpression, autocrine stimulation, or mutation (3–5). Amplification of EGFR and HER2 occurs in a subset of human tumors including breast, lung, ovarian, and kidney cancers, and glioblastomas (6). Overexpression of HER2 in breast and ovarian cancers is associated with a particularly aggressive course and poor prognosis (7). Because these cancers share a well-defined common genetic abnormality, much attention has focused on the development of therapies specifically directed toward this subset of human cancers.

Therapeutic mAbs targeting the extracellular domains of EGFR and HER2 have been developed and have shown efficacy in the treatment of patients with cancer, and anti-HER2 antibodies are now in widespread use in the management of patients with breast cancer (8). Advances in pharmaceutical technologies have led to the development of small-molecule inhibitors of tyrosine kinases with a high degree of molecular specificity for HER family RTKs, and many of these agents are now ready for clinical testing. Agents that selectively inhibit these targets afford a useful tool for determining their oncogenic function in certain tumors.

The tyrosine kinase inhibitor ZD1839 (“Iressa”) is a synthetic anilinoquinazoline tyrosine kinase inhibitor selective for EGFR. It inhibits EGFR *in vitro* with an IC<sub>50</sub> of 27–33 nM<sup>4</sup> and is competitive with ATP and noncompetitive with peptide substrates. It has much lower activity against related RTKs, with an IC<sub>50</sub> of 2 μM and 20 μM for HER2 and KDR, respectively. Because ZD1839 has good oral bioavailability and demonstrated antitumor activity in a broad range of mouse xenograft models, it was selected for clinical development (9). Phase I trials of ZD1839 in patients with solid tumors refractory to standard chemotherapeutic agents have shown good tolerability and evidence of antitumor activity against such tumors (10, 11). These successes have led to additional clinical development, and additional clinical trials are currently underway to better determine its potential in the treatment of cancer patients.

We have studied the molecular basis for the sensitivity of some tumors to ZD1839. We find that, although this agent selectively inhibits EGFR activity, tumors with HER2 overexpression are particularly sensitive to it. Treatment of HER2-overexpressing tumors with ZD1839 results in dephosphorylation of HER2 and profound down-regulation of the PI3k/Akt signaling pathway attributable to dephosphorylation of HER3. These results indicate that the clinical potential of this agent is not limited to tumors with EGFR overexpression and, in fact, suggest that it may represent a novel modality in the treatment of patients with HER2-overexpressing tumors.

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<sup>3</sup>The abbreviations used are: RTK, receptor tyrosine kinase; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; mAb, monoclonal antibody; PI3k, phosphatidylinositol 3'-kinase; RIPA, radioimmunoprecipitation assay; MAP, mitogen-activated protein.

<sup>4</sup>A. Wakeling, personal communication.

## MATERIALS AND METHODS

Tumor cell lines were obtained from the American Type Culture Collection maintained in 1:1 mixture of DME:F12 medium supplemented with 100 units/ml penicillin, 100  $\mu\text{g/ml}$  streptomycin, 4 mM glutamine, and 10% heat-inactivated fetal bovine serum, and incubated at 37°C in 5% CO<sub>2</sub>. DU4475 cells were grown in RPMI 1640 with similar supplements. MCF10A cells were grown in DME:F12 supplemented with 5% donor horse serum, 0.5  $\mu\text{g/ml}$  hydrocortisone, 10  $\mu\text{g/ml}$  insulin, and 3 nM EGF. For growth assays, cells were seeded in six-well clusters at 20–50,000 cells/well. Later (24 h), the cells were placed in fresh medium containing ZD1839 and allowed to grow for 4–10 days, depending on the cell line. When control wells were near semiconfluent, cells were harvested by trypsinization and counted using a Coulter counter.

Western blots were performed by harvesting total cellular lysates in RIPA buffer [10 mM sodium phosphate (pH 7.2), 150 mM NaCl, 0.1% SDS, 1% NP-40, 1% sodium deoxycholate, and protease inhibitors], separating 50  $\mu\text{g}$  of each lysate by SDS-PAGE, and transferring to membrane. Immunoblot analyses were performed using antibodies specific for EGFR (Trans. Labs; E12020), HER2 (Neomarkers; Ab-15), HER3 (Neomarkers; Ab-2), HER4 (Neomarkers; Ab-2), and phospho-Akt (Cell Signaling; 9271), and visualized by enhanced chemiluminescence and autoradiography. Quantitative analysis of Akt activity was performed by imaging the autoradiograms in a Bio-Rad GelDoc 2000 in white light and quantitating relative band densities using Quantity One software (Bio-Rad).

Transfections were performed as follows. SkBr3 cells were seeded at 2 million cells/10-cm dish and were transfected with 2  $\mu\text{g}$  of pSG5-P110 $\alpha$ CAAX (kindly provided by Julian Downward, ICRF, London, United Kingdom) the following day or pSG5 control using Lipofectin reagent (Life Technologies, Inc.). After 24 h, cells were placed in medium containing indicated concentrations of ZD1839 for 30 min, and total lysates were harvested and analyzed by Western blotting as described above.

Receptor phosphorylation assays were performed as follows. RIPA lysates were immunoprecipitated with the relevant antibodies, separated on SDS-PAGE, transferred to membrane, and immunoblotted using antiphosphotyrosine antibodies (Santa Cruz; PY99). Immunoprecipitations were performed using monoclonal anti-EGFR (Calbiochem; GR13), monoclonal anti-HER2 (Neomarkers; Ab-4), and monoclonal anti-HER3 (Neomarkers; Ab-4) antibodies. All of these antibodies were against epitopes within the extracellular domains of the receptors, and previous titrating experiments had established the amount of lysate and antibody that would maximally immunoprecipitate the target protein.

## RESULTS

**Sensitivity of EGF-dependent Growth to ZD1839.** Because ZD1839 is a potent inhibitor of EGFR *in vitro*, we initially studied its efficacy in inhibiting EGFR-dependent cell growth. MCF10A cells are nontransformed breast epithelial cells that require EGF to proliferate. The monolayer growth of these EGF-driven untransformed cells is inhibited by ZD1839 with an IC<sub>50</sub> of 20 nM, similar to its IC<sub>50</sub> *in vitro* for EGFR and consistent with effective inhibition of EGFR *in vivo*. However, higher concentrations (0.5–20  $\mu\text{M}$ ) are required for growth inhibition in tumor cell lines, with considerable differences between different tumor cell types. This suggests that the inhibition of tumor growth may involve additional mechanisms. To better understand the differences in the sensitivity of tumors to ZD1839, we studied the effects of ZD1839 in a panel of human epithelial tumors.

**Sensitivity of HER2-overexpressing Tumors to ZD1839.** Because the function of EGFR is closely linked to the function of its family members HER2, HER3, and HER4, the expression of the HER family of RTKs in these cells was correlated with their sensitivity to ZD1839. Overexpression of EGFR and HER2 is seen commonly in breast cancers, so we initially studied the antitumor effects of ZD1839 in a panel of 10 breast cancer cell lines. In these cells we find that HER2-overexpressing tumors (SkBr3, BT474, MDA-MB-361, and MDA-MB-453) are more sensitive to ZD1839 than others, with IC<sub>50</sub>s under 10  $\mu\text{M}$ , and among these the tumors with the highest HER2 expression (SkBr3 and BT474) are the most sensitive, with IC<sub>50</sub>s of

0.8 and 0.3  $\mu\text{M}$  (Fig. 1, *Lanes 1–10*). Interestingly, the cell line with amplification and overexpression of EGFR (MDA-MB-468) is not as sensitive to ZD1839, with an IC<sub>50</sub> of 13  $\mu\text{M}$ . To determine whether the correlation between HER2 overexpression and ZD1839 sensitivity holds in other tumor types, we extended our analysis to additional tumor cell lines of different origins. This analysis included the HER2-overexpressing ovarian cancer cells SkOV3, which are sensitive to ZD1839, with an IC<sub>50</sub> of 2  $\mu\text{M}$  and confirms that HER2-overexpressing tumors are particularly sensitive to ZD1839 (Fig. 1, *Lanes 12–18*). Unlike MDA-MB-468 cells, the EGFR-overexpressing tumor A431 is sensitive to ZD1839 with an IC<sub>50</sub> of 0.7  $\mu\text{M}$  making it difficult to establish a definite correlation between EGFR overexpression and drug sensitivity. However, it is evident from MDA-MB-468 cells that EGFR overexpression is not sufficient to determine sensitivity to ZD1839. There is no apparent correlation between the expression of HER3 or HER4 with tumor sensitivity in these cells.

**Receptor Dephosphorylation.** The four members of the HER kinase family are closely related. Although ZD1839 is a relatively selective inhibitor of EGFR *in vitro*, it may have broader effects in cells. Therefore, we studied the effects of ZD1839 on the tyrosine phosphorylation of each receptor family member in the drug-sensitive SkBr3 cells, which are growth-inhibited with an IC<sub>50</sub> of 0.8  $\mu\text{M}$ . At concentrations >1  $\mu\text{M}$ , and in <30 min, ZD1839 reduces the basal phosphorylation of EGFR but also of HER2 and HER3 (Fig. 2A).

**Down-Regulation of the PI3K/Akt Pathway.** EGFR and HER2 activate a number of cytoplasmic signal transduction pathways of which the Ras/MAP kinase pathway has been the best defined. We have studied whether inhibition of this pathway may be mediating the antitumor effects of ZD1839. However, in MCF-7 and MDA-MB-468 cells, 1  $\mu\text{M}$  of ZD1839 inhibits EGFR-induced MAP kinase signaling (data not shown) yet has no effects on cell growth at this concentration. Thus far we have not found a correlation between MAP kinase inhibition and ZD1839 sensitivity.

Another downstream signaling event initiated by HER kinase stimulation is recruitment and activation of PI3k at the cell membrane leading to activation of the cytosolic serine/threonine kinase Akt. ZD1839 down-regulates Akt activity within 30 min in some tumor cell lines. This rapid down-regulation of Akt activity is predominantly seen in tumors that are sensitive to ZD1839 such that down-regulation of Akt activity is a marker of tumor response to ZD1839 (Figs. 1 and 3). In fact, Akt down-regulation is a better correlate of tumor sensitivity than HER2 overexpression, because A431 cell growth and Akt activity are very sensitive to ZD1839, yet these cells do not overexpress HER2. DU145 cells are also partially sensitive to ZD1839 and show a decline in Akt activity but no overexpression of HER2. There is no decline in total Akt expression in these experiments (data not shown).

To determine whether ZD1839 treatment down-regulates Akt activity through down-regulation of PI3k activity, we transfected the ZD1839-sensitive SkBr3 cells with an activated PI3k construct. P110-CAAX is a modified p110 that has a COOH-terminal CAAX motif. This motif signals post-translation prenylation leading to membrane localization and activation. Expression of constitutively active PI3k prevents the down-regulation of Akt activity by ZD1839 supporting the hypothesis that ZD1839 down-regulates Akt activity through inhibition of PI3k activity (Fig. 4). Although the resistance to ZD1839 in p110-CAAX-transfected cells appears incomplete here, this represents only the subset of cells that were successfully transfected, because these are transient transfections.

The activation of PI3k by HER family proteins is thought to occur predominantly through association of the SH2 domain of the p85 regulatory subunit of PI3k with several tyrosine-phosphorylated binding sites on the cytoplasmic domain of HER3. Because ZD1839 treatment of HER2-overexpressing tumors results in HER3 dephos-

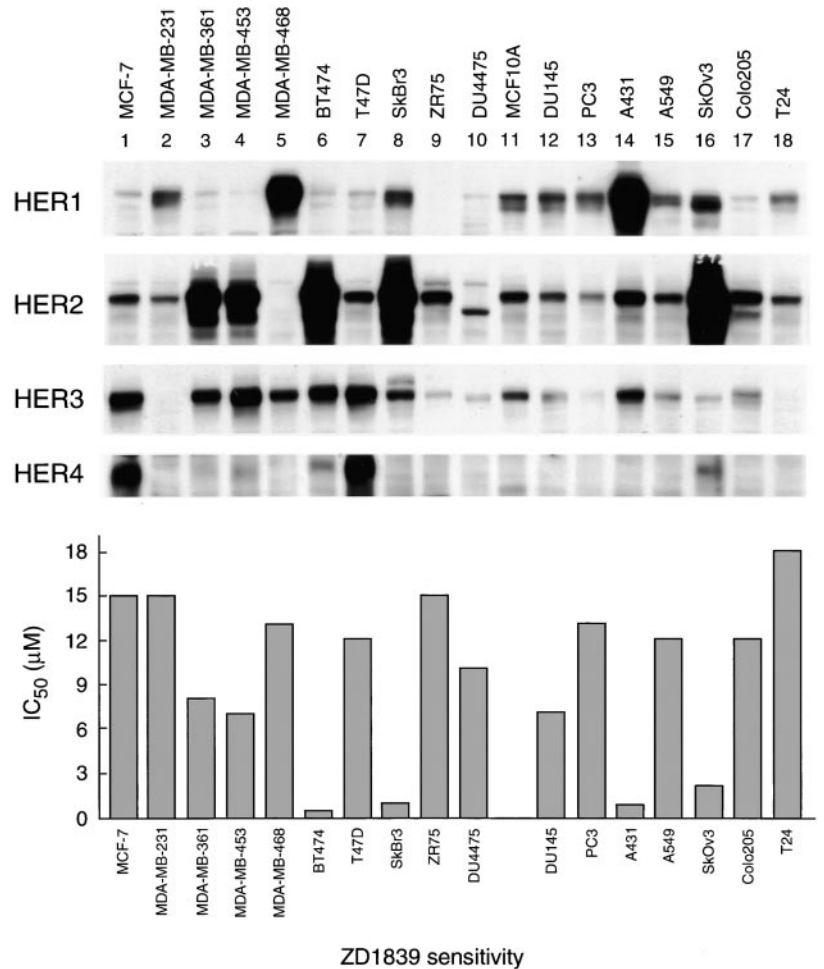


Fig. 1. Correlation of ZD1839 sensitivity with expression of HER family kinases. Growth inhibition assays were performed as described in "Materials and Methods" in various concentrations of ZD1839. Cells were incubated and counted after 4–10 days depending on the particular cell line. All experiments were performed in duplicate and averaged. Expression of HER family kinases was performed by Western blotting using 50 µg of total cellular RIPA lysates of each cell type as described in "Materials and Methods."

phorylation, we hypothesized that this would lead to loss of HER3 association with p85, and our experiments corroborate this hypothesis. Immunoprecipitation of EGFR, HER2, and HER3 from the HER2-overexpressing SkBr3 cells results in coimmunoprecipitation of p85 with HER3 but not with EGFR or HER2, and this association is lost at concentrations of ZD1839 >1 µM in parallel with the reduction in the phosphorylation of HER3 (Fig. 2B).

#### Combination Treatment of HER2-overexpressing Tumor Cells.

Because ZD1839 may have clinical application in the treatment of patients with HER2-overexpressing breast cancers, and such patients may benefit from combination therapies, we tested the efficacy of monoclonal anti-HER2 antibodies (trastuzumab [Herceptin]) in combination with ZD1839 in cell culture. Treatment of SkBr3 cells with trastuzumab and ZD1839 shows that the growth inhibitory activities of these two agents are additive (Fig. 5).

## DISCUSSION

Selective inhibition of tyrosine kinase activity is a promising strategy for cancer treatment. ZD1839 is one of the first tyrosine kinase inhibitors to be tested in patients with advanced solid tumors. Initial studies have shown promising results, and additional clinical trials are in progress to additionally define the activity and toxicities of this new treatment modality. We have been interested in how this agent inhibits the growth of tumor cells and which subset of tumors may be most effectively treated by this agent. In a survey of human tumor cell lines, we find that tumors with HER2 overexpression are particularly sensitive to ZD1839. There is no correlation with HER3 or HER4

expression. Although *in vitro* this agent shows the highest activity against EGFR, it is apparent from our analysis of two EGFR-overexpressing tumor types, MDA-MB-468 and A431, that overexpression of EGFR is not sufficient to determine sensitivity to ZD1839. It remains possible that the consequences of EGFR overexpression are modified by other molecular lesions in the tumor. For example, MDA-MB-468 cells may be driven by overexpression and overactivity of EGFR, but their relative resistance to ZD1839 may be attributable to the failure to down-regulate Akt activity, because these cells have a deletion of the PTEN tumor suppressor protein (12). Thus, the

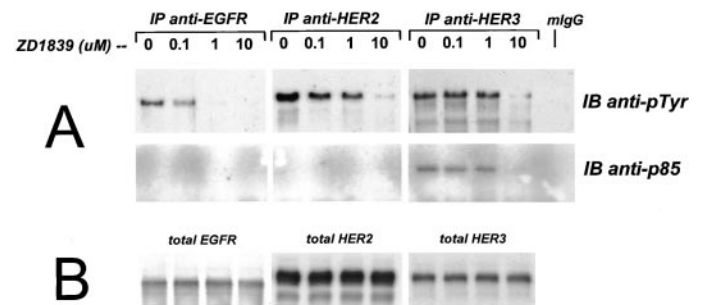


Fig. 2. Effects of ZD1839 on receptor phosphorylation and association with PI3K. SKBr3 cells were treated for 30 min with indicated concentrations of ZD1839 and rapidly harvested in RIPA lysis buffer. HER family receptors were immunoprecipitated using anti-EGFR, anti-HER2, and anti-HER3 mAbs as well as mouse IgG control. Immunoprecipitates were separated on SDS-PAGE and immunoblotted using antiphosphotyrosine antibodies and antibodies against the p85 regulatory subunit of PI3K (A). Total EGFR, HER2, and HER3 expression was analyzed by Western blotting (B).

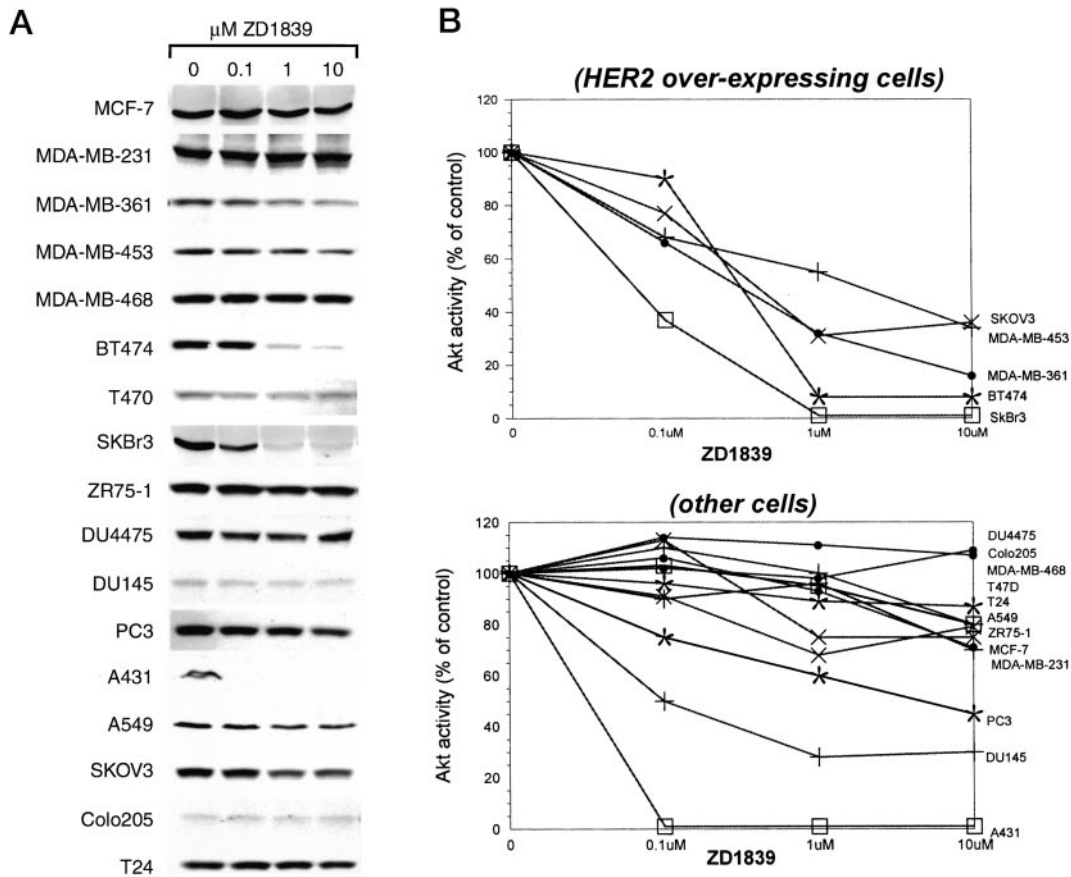


Fig. 3. Cells were placed in medium containing 0, 0.1, 1, or 10  $\mu\text{M}$  of ZD1839 for 30 min and then harvested rapidly on ice in RIPA buffer. Total cellular lysates were separated on SDS-PAGE, transferred to membrane, and blotted with anti-phospho-Akt (Ser473) antibodies (Cell Signaling). Akt phosphorylation was assayed as a correlate of Akt activity. It should be noted that the baseline expression of Akt and phospho-Akt varies considerably among the different cell lines, and to enable analysis of declines in Akt activity, increased total lysates and longer exposures were used for cells with low signal. Therefore, these experiments are designed to compare Akt activity within each cell line and not for comparison between cell lines. Quantitative analysis of Akt activity was performed on the immunoblots as described in "Materials and Methods" and plotted for each cell line using the 0  $\mu\text{M}$  arm as control.

assumption that EGFR overexpression *per se* will determine sensitivity to EGFR-targeted therapies such as ZD1839 may turn out to be over-simplistic.

Although ZD1839 shows relative selectivity for EGFR *in vitro*, our data show that in tumors it leads to reduced basal phosphorylation of EGFR, HER2, and HER3. The reduction in phosphorylation of these receptors occurs at low  $\mu\text{M}$  concentrations and correlates with the antitumor activity of this agent. At these concentrations, ZD1839 is not selective for EGFR *in vitro* and, in fact, inhibits the HER2 kinase domain *in vitro* with an  $\text{IC}_{50} = 1.2\text{--}3.7 \mu\text{M}$ .<sup>4</sup> Therefore, it is difficult to know, based on these studies alone, whether ZD1839 inhibits tumors through the inhibition of EGFR or HER2, or perhaps both. Because HER2-overexpressing tumors are thought to be driven by overactivity of HER2, the sensitivity of these tumors to ZD1839 would suggest that the inhibition of HER2 is most important in these tumors, and this remains a distinct possibility based on these studies. However, because HER2 functions preferentially as a heterodimer with other HER family members including EGFR resulting in receptor transphosphorylation EGFR may play an important role in the growth of HER2-overexpressing tumors. HER2 readily dimerizes with EGFR *in vivo* (2), and cells transfected with EGFR and HER2 have increased basal phosphorylation of these two receptors (13). In transgenic models of breast cancer driven by HER2 and transforming growth factor  $\alpha$  overexpression, inhibition of EGFR by a selective tyrosine kinase inhibitor suppresses tumor formation (14). It is possible that the oncogenic activity of HER2 requires phosphorylation by EGFR. Understanding the precise role and importance of the EGFR in human HER2-overexpressing breast cancers requires additional studies.

EGFR and HER2 affect the activity of a number of cytoplasmic signal transduction pathways, and it remains unclear which pathways are most important for maintaining the transformed phenotype in HER2-overexpressing tumors. Numerous effector molecules associate with phosphotyrosine residues within the cytoplasmic domains of HER kinases. These include Grb2, Shc, Shp1, PLC $\gamma$ , Cbl, p85 subunit of PI3k, and others that associate selectively with specific members of the HER kinases on specific phosphotyrosine residues (reviewed in Ref. 1). We have shown that the down-regulation of the PI3k/Akt pathway correlates with the antitumor effects of ZD1839. This suggests that the PI3k/Akt pathway may be important in driving tumor growth, especially in HER2-overexpressing tumors. Additional studies are required to determine the effects of ZD1839 on other effector molecules and signaling pathways. Although ZD1839 is effective in inhibiting the EGFR-Ras-MAP kinase pathway, this is not sufficient for inhibition of growth in some tumor cells. Future studies will attempt to define the role of this pathway in maintaining tumor growth.

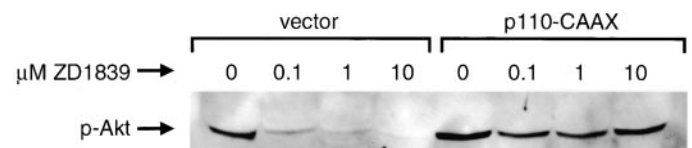


Fig. 4. SkBr3 cells were transfected with an activated PI3k construct (p110-CAAX) or vector control. After transfection (24 h), cells were treated with increasing concentrations of ZD1839 for 30 min, harvested, total protein quantitated, and Akt activity assayed by Western blotting as described previously using anti-phospho-Akt antibodies.

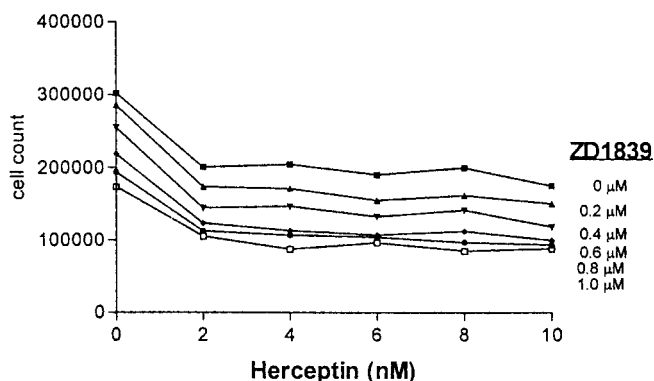


Fig. 5. SkBr3 cells were seeded in six-well clusters and the following day placed in medium containing increasing concentrations of ZD1839 and trastuzumab. Cells were allowed to grow and counted on day 5, and results shown reflect the average of duplicate wells.

Inhibition of Akt activity correlates well with and is a marker of sensitivity of tumor cells to ZD1839. Akt down-regulation correlates better with tumor sensitivity than HER2 overexpression, as demonstrated by two cell types that do not overexpress HER2. A431 cells are very sensitive to ZD1839, which inactivates their Akt activity. DU145 prostate cancer cells are partially sensitive to ZD1839, which partially inhibits their Akt activity. It is difficult to make broad conclusions regarding the importance of Akt down-regulation in ZD1839 sensitivity in different tumor types, because the signaling pathways that drive the growth of different types of cancer are probably different.

Our data suggest that the down-regulation of Akt activity by ZD1839 is mediated through down-regulation of PI3k activity. This is consistent with current models of HER family signaling through PI3k. PI3k is activated after EGF stimulation, and this activity is seen in EGFR immunoprecipitates in certain studies (15). However, the SH2 domain recognition sequence for the p85 regulatory subunit of PI3k is not found on EGFR or HER2, and additional analysis has shown that p85 associates directly with HER3, which has seven repeats of the p85 recognition sequence (16). Therefore, activation of PI3k by EGFR or HER2 occurs through the phosphorylation of HER3. EGFR may also activate PI3k through additional intermediaries of which several are currently known including HER4, Ras, the platelet-derived growth factor  $\beta$  receptor (17), and the docking protein Gab1 (18). In our survey, HER3 is expressed in most of the tumor cell lines that are sensitive to ZD1839 and may be driving the PI3k activity in these cells. The one exception is SkOV3 cells, which have low HER3 expression. However, these cells express HER4, which contains a p85 binding site (19) and which may also be recruiting PI3k.

The findings presented here have significant clinical relevance. Although ZD1839 was developed because of its *in vitro* selectivity for inhibition of EGFR, it is apparent from our studies that its clinical testing should not be confined to patients with EGFR-overexpressing tumors. Moreover, our data suggest that the clinical efficacy of ZD1839 should also be tested in patients with HER2-overexpressing tumors. HER2 overexpression is commonly seen in breast cancers, and these tumors are associated with a particularly poor prognosis. Considerable efforts have focused on the development of treatments for this tumor subtype. In fact the use of therapeutic mAbs against the extracellular domain of HER2 in patients with HER2-overexpressing tumors has resulted in significantly improved survival (20). Additional therapies targeted toward HER2 signaling pathways may con-

tinue to improve the outcome of these patients, and combination therapies of active agents may prove even more efficacious. In our preliminary analysis, ZD1839 shows activity in cell culture models of HER2-overexpressing tumors and additive activity with therapeutic anti-HER2 antibodies. The clinical relevance of these findings awaits clinical trials of ZD1839 in patients with HER2-overexpressing tumors.

## REFERENCES

- Olayioye, M. A., Neve, R. M., Lane, H. A., and Hynes, N. E. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J.*, *19*: 3159–3167, 2000.
- Tzahar, E., Waterman, H., Chen, X., Levkowitz, G., Karunakaran, D., Lavi, S., Ratzkin, B. J., and Yarden, Y. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol. Cell. Biol.*, *16*: 5276–5287, 1996.
- Guy, C. T., Webster, M. A., Schaller, M., Parsons, T. J., Cardiff, R. D., and Muller, W. J. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc. Natl. Acad. Sci. USA*, *89*: 10578–10582, 1992.
- Muller, W. J., Sinn, E., Pattengale, P. K., Wallace, R., and Leder, P. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. *Cell*, *54*: 105–115, 1990.
- Matsui, Y., Halter, S. A., Holt, J. T., Hogan, B. L., and Coffey, R. J. Development of mammary hyperplasia and neoplasia in MMTV-TGF  $\alpha$  transgenic mice. *Cell*, *61*: 1147–1155, 1990.
- Wilks, A. F. Protein tyrosine kinase growth factor receptors and their ligands in development, differentiation, and cancer. *Adv. Cancer Res.*, *60*: 43–73, 1993.
- Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science (Wash. DC)*, *235*: 177–182, 1987.
- Stebbing, J., Copson, E., and O'Reilly, S. Herceptin (trastuzumab) in advanced breast cancer. *Cancer Treat. Rev.*, *26*: 287–290, 2000.
- Woodburn, J. R. The epidermal growth factor receptor and its inhibition in cancer therapy. *Pharmacol. Ther.*, *82*: 241–250, 1999.
- Ferry, D., Hammond, L., Ranson, M., Kris, M. G., Miller, V., Murray, A., Tullo, A., Feyereislova, A., Averbuch, S., and Rowinsky, E. Intermittent oral ZD1839 (Iressa), a novel epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI), shows evidence of good tolerability and activity: final results from a Phase I study. *Proc. Am. Soc. Clin. Oncol.*, *19*: 3a, 2000.
- Kris, M. G., Ranson, M., Ferry, D., Hammond, L., Averbuch, S., Ochs, J., and Rowinsky, E. Phase I study of oral ZD1839, a novel inhibitor of epidermal growth factor receptor tyrosine kinase (EGFR-TK): evidence of good tolerability and activity. *Clin. Cancer Res.*, *5*: 3749s, 1999.
- Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S. I., Puc, J., Miliareis, C., Rodgers, L., McCombie, R., Bigner, S. H., Giovannella, B. C., Littmann, M., Tycko, B., Hibshoosh, H., Wigler, M. H., and Parsons, R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science (Wash. DC)*, *275*: 1943–1947, 1997.
- Riese, D. J., van Raaij, T. M., Plowman, G. D., Andrews, G. C., and Stern, D. F. The cellular response to neuregulins is governed by complex interactions of the erbB receptor family. *Mol. Cell. Biol.*, *15*: 5770–5776, 1995.
- Lenferink, A. E., Simpson, J. F., Shawler, L. K., Coffey, R. J., Forbes, J. T., and Arteaga, C. L. Blockade of the epidermal growth factor receptor tyrosine kinase suppresses tumorigenesis in MMTV/Neu + MMTV/TGF- $\alpha$  bigenic mice. *Proc. Natl. Acad. Sci. USA*, *97*: 9609–9614, 2000.
- Bjorge, J. D., Chan, T. O., Antczak, M., Kung, H. J., and Fujita, D. J. Activated type I phosphatidylinositol kinase is associated with the epidermal growth factor (EGF) receptor following EGF stimulation. *Proc. Natl. Acad. Sci. USA*, *87*: 3816–3820, 1990.
- Soltoff, S. P., Carraway, K. L., III, Prigent, S. A., Gullick, W. G., and Cantley, L. C. ErbB3 is involved in activation of phosphatidylinositol 3-kinase by epidermal growth factor. *Mol. Cell. Biol.*, *14*: 3550–3558, 1994.
- Habib, A. A., Hognason, T., Ren, J., Stefansson, K., and Ratan, R. R. The epidermal growth factor receptor associates with and recruits phosphatidylinositol 3-kinase to the platelet-derived growth factor  $\beta$  receptor. *J. Biol. Chem.*, *273*: 6885–6891, 1998.
- Rodrigues, G. A., Falasca, M., Zhang, Z., Ong, S. H., and Schlessinger, J. A novel positive feedback loop mediated by the docking protein Gab1 and phosphatidylinositol 3-kinase in epidermal growth factor receptor signaling. *Mol. Cell. Biol.*, *20*: 1448–1459, 2000.
- Elenius, K., Choi, C. J., Paul, S., Santiestevan, E., Nishi, E., and Klagsbrun, M. Characterization of a naturally occurring ErbB4 isoform that does not bind or activate phosphatidylinositol 3-kinase. *Oncogene*, *18*: 2607–2615, 1999.
- Norton, L., Slamon, D. J., Leyland-Jones, B., Wolter, J., Fleming, T., Eirmann, W., Baselga, J., Mendelsohn, J., Bajamonde, A., Ash, M., and Shak, S. Overall survival advantage to simultaneous chemotherapy plus the humanized anti-Her2 monoclonal antibody Herceptin in Her2-overexpressing metastatic breast cancer. *Proc. Amer. Soc. Clin. Oncol.*, *18*: 127, 1999.