PD153035, a Tyrosine Kinase Inhibitor, Prevents Epidermal Growth Factor Receptor Activation and Inhibits Growth of Cancer Cells in a Receptor Number-dependent Manner

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ABSTRACT

PD153035 is reported to be a specific and potent inhibitor of the epidermal growth factor (EGF) receptor tyrosine kinase and, to a lesser degree, of the closely related HER2/neu receptor. We show that PD153035 inhibits EGF-dependent EGF receptor phosphorylation and suppresses the proliferation and clonogenicity of a wide panel of EGF receptor-overexpressing human cancer cell lines. EGF receptor autophosphorylation in response to exogenous EGF was completely inhibited at PD153035 concentrations of >75 nM in cells overexpressing the EGF receptor. In contrast, PD153035 only reduced heregulin-dependent tyrosine phosphorylation in HER2/neu-overexpressing cell lines at significantly higher concentrations (1400–2800 nM). PD153035 exposure did not affect the expression of either EGF receptors or HER2/neu. PD153035 caused a dose-dependent growth inhibition of EGF receptor-overexpressing cell lines at low micromolar concentrations, and the IC50 in monolayer cultures was less than 1 μM in most cell lines tested. At doses of up to 2.5 μM, the IC50 for HER2/neu-overexpressing cells was not reached. In colony-forming assays, the PD153035 growth-inhibitory activity in cultures driven by endogenous (autocrine) ligand was correlated with EGF receptor number, with higher activity in cells expressing higher numbers of EGF receptors and only minimal activity in cells expressing normal numbers of EGF receptors but high HER2/neu levels. PD153035 also abolished all growth effects mediated by the addition of exogenous EGF; this condition could be reversed upon removal of the compound. Cotreatment with C225, an anti-EGF receptor-blocking monoclonal antibody, further enhanced the antitumor activity of PD153035, suggesting mechanisms of action for C225 other than competition with ligand binding. This latter finding also suggests that combined anti-EGF receptor strategies may be of enhanced benefit against tumors with high levels of EGF receptor expression.

INTRODUCTION

The EGF receptor is a Mr 170,000 plasma membrane glycoprotein composed of an extracellular ligand-binding domain, a transmembrane lipophilic segment, and an intracellular protein tyrosine kinase domain with a regulatory COOH-terminal segment (1). Following ligand binding, EGF receptor dimerization occurs, which results in high-affinity ligand binding, activation of the intrinsic protein tyrosine kinase activity, and tyrosine autophosphorylation (1). These events lead to activation of a cascade of biochemical and physiological responses that are involved in the mitogenic signal transduction of cells (2).

The EGF receptor has been proposed as a potential target for new anticancer agents for a variety of reasons: coexpression of high levels of EGF receptor and its ligands leads to a transformed cellular phenotype (3, 4); the expression of EGF receptors is elevated in many epithelial tumors and tumor-derived cell lines (4, 5); and this overexpression correlates with a poor clinical outcome in a number of malignancies (6). Furthermore, MAbs that block ligand-induced activation of the EGF receptor tyrosine kinase inhibit the growth of human tumor xenografts (7–9).

Recently, the quinazolones have been identified as a new class of tyrosine kinase inhibitors (10). One of these compounds, PD153035, is a reversible inhibitor of the EGF receptor tyrosine kinase and, to a lesser degree, of the closely related HER2/neu receptor (11). Prior studies with cell lysates had shown that PD153035 inhibited the isolated tyrosine kinase activity of the EGF receptor at picomolar concentrations but had little effect against a panel of non-EGF receptor tyrosine kinase receptors, except for the closely related HER2/neu receptor. PD153035 inhibited EGF receptor autophosphorylation in mouse fibroblasts and A431 human epidermoid cells at nanomolar concentrations (11). PD153035 was also capable of blocking EGF-mediated mitogenesis and oncogenic transformation in fibroblasts overexpressing EGF receptors (11).

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The abbreviations used are: EGF, epidermal growth factor; MAb, monoclonal antibody; FBS, fetal bovine serum.
Here, we have studied the capacity of PD153035 to inhibit ligand-induced EGF receptor phosphorylation and cell growth in a panel of human carcinoma cell lines with various levels of EGF receptor and/or HER2/neu receptor overexpression. We report that PD153035-mediated inhibition is dependent upon the level of expressed EGF receptor numbers, with less activity against cells that have lower receptor number and only HER2/neu receptor overexpression. Interestingly, cotreatment with C225, an anti-EGF receptor MAb, further enhances the antitumor activity of PD153035, suggesting that combined anti-EGF receptor strategies may be of benefit against tumors with high levels of EGF receptor expression.

MATERIALS AND METHODS

Compounds and Antibodies. Tyrosine kinase inhibitor PD153035 and anti-EGF receptor MAbs 225 and 528 have been described previously (7, 8, 11). A human-murine chimeric version of MAb 225 (C225) was used for these experiments (12). Rabbit polyclonal antibody RK-2, against the COOH-terminal portion of the EGF receptor, was a gift from Dr. J. Schlessinger (New York University Medical Center, New York, NY). Anti-HER2/neu Ab2 and Ab3 MAbs were from Oncogene Science (Cambridge, MA). EGF was from Collaborative Research (Wal-tham, MA). Antiphosphotyrosine antibody 4G10 was from Up-state Biotechnology Inc. (Lake Placid, NY). Heregulin, a ligand that phosphorylates HER2/neu, was a gift from Dr. M. Sklowski (Genentech Inc., South San Francisco, CA).

Cell Lines and Culture Media. For the present study, cell lines with various levels of either EGF receptor or HER2/neu expression were chosen. Vulvar squamous carcinoma cells (A431), breast adenocarcinoma cells (BT474, MDA-MB-231, MDA-MB-453, MDA-MB-468, and SK-BR-3), colon adenocarcinoma cells (SW620), prostate adenocarcinoma cells (DU145), and cervical squamous carcinoma cell lines (C4i, ME180, and SiHa) were obtained from the American Type Culture Collection (Rockville, MD). Difi colon adenocarcinoma cells were kindly provided by Dr. B. Boman (Creighton University, Omaha, NE). The breast adenocarcinoma cell line MCF7/HER-2, transfected with HER2/neu, was a gift of Dr. C. Benz (University of California, San Francisco, CA). The numbers of EGF receptors per cell in the studied cell lines were as follows: Difi, 4.8-5 × 106 receptors/cell (13); A431, 2 × 106 receptors/cell (14); MDA-MB-468, 2 × 106 receptors/cell (15); DU145, 2.5 × 106 receptors/cell (16); MDA-MB-231, 2.5 × 106 receptors/cell (17); BT474, 1.4 × 106 receptors/cell (18); SK-BR-3, 1 × 105 receptors/cell (17); ME-180, 8.6 × 105 receptors/cell (19); SiHa, 5 × 104 receptors/cell (19); and C4i, 2.2 × 104 receptors/cell (19). SW620, MDA-MB-453, and MCF7/HER-2 cells were considered to have low EGF receptor levels because the receptor could not be detected by Western blot analysis, a method that correlates well with receptor number (17). HER2/neu-overexpressing cell lines MD-MB-453, BT474, SK-BR-3, SW620, and MCF7/HER-2 all had HER2/neu levels of >40 ng/mg protein (Ref. 20 and data not shown). The CMK megakaryoblastic leukemia cell line, which lacks EGF receptors and HER2/neu, was kindly provided by Dr. H. Avraham (Beth Israel Deaconess Medical Center, Boston, MA).

A431, MDA-MB-231, MDA-MB-453, MDA-MB-468, SK-BR-3, DU145, and Difi cells were grown in monolayer culture with DMEM and Ham’s F-12 medium (1:1), with 10% FBS. BT474 cells were grown in DMEM/Ham’s F-12 medium (1:1) with 10% FBS, 300 mg/liter l-glutamine, and 10 μg/ml human insulin. SW620, C4i, SiHa, and ME180 cells were grown in RPMI 1640 with 10% FBS, 300 mg/liter l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. MCF7/HER-2 cells were cultured in DMEM/H16 medium (1 g/liter glucose), with 10% FBS, 100 units/ml penicillin, 100 units/ml streptomycin, and 400 μg/ml G-418. CMK cells were cultured in Iscove’s modified Dulbecco’s medium with 10% FBS, 300 μg/ml l-glutamine, 100 μg/ml ampicillin, and 50 μg/ml kanamycin. All cells were grown at 37°C and 5% CO2.

Western Immunoblotting. Selected cell lines were grown in 100-mm dishes until subconfluence and then incubated in serum-free medium for 18 h. The monolayers were exposed to various concentrations of PD153035 for 2 h and then treated with either EGF (20 nm) for 10 min or heregulin (20 nm) for 15 min. Then, the medium was removed, the cells were washed twice with cold PBS, and the monolayer was scraped into 1 ml of ice-cold lysis buffer [50 mM Heps (pH = 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 5 mM EDTA, 1 mM MgCl2, 25 mM NaF, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 25 μg/ml aprotinin, 50 μg/ml leupeptin, and 1% bovine albumin]. The lysate was transferred to a microfuge tube, placed on ice for 15 min, and centrifuged 5 min at 10,000 g. The supernatant was transferred to a clean microfuge tube, and the protein concentration was determined. Precipitates were resuspended in 30 μl of Laemmli buffer, heated to 100°C for 5 min, and centrifuged to obtain the supernatant. Samples were loaded onto a 6% SDS polyacrylamide gel, followed by overnight transfer to polyvinylidene difluoride membranes. Membranes were blocked with a solution containing 150 mM NaCl, 1% BSA, 0.1% Tween 20, and 20 mM Tris (pH 7.4) for 1 h, then incubated for an additional hour with either the antiphosphotyrosine antibody (1:15,000), anti-EGF receptor RK-2 antibody (1:2,000), or anti-HER2/neu Ab3 (1:7,500). Membranes were washed four times for 5 min each in the same solution and incubated for 45-60 min with a 1:10,000 dilution of horseradish peroxidase-conjugated secondary antibody. Subsequently, membranes were vigorously washed four times for 5 min each in the same solution, followed by a 1-min incubation with a luminol-based solution and chemiluminescent detection.

Monolayer Growth Assay. Cells were seeded in six-well plates (model 3046; Falcon, Lincoln Park, NJ) at 104 cells/well. The next day, cells were changed to medium containing 0.5% FBS for 18 h, and then PD153035 was added at various concentrations to the cultures. After 72 h of treatment, cells were washed once with PBS, harvested with 0.1% human trypsin-1 mM EDTA in PBS, and counted with a Coulter counter. The CMK cells grow in suspension and, therefore, did not require trypsinization.

Soft Agar Colony-forming Assay. To perform soft agar assays, a bottom layer of 1 ml of the corresponding culture medium containing 0.7% agar (Difco Laboratories, Detroit, MI)
and 10% FBS was prepared in 35-mm six-well plates (model 3046; Falcon). After the bottom layer was solidified, 20,000 cells per dish were added in 1.5 ml of culture medium containing the sample, 0.35% agar, and 10% FBS. The additions also contained PD153035 at different concentrations. Triplicates were performed for every condition. Cells were incubated for 11–14 days at 37°C in 5% CO2 atmosphere. Colonies with more than 25 cells were then counted manually. The experiments were repeated, and the results were comparable.

**RESULTS**

Inhibition of Ligand-induced Receptor Phosphorylation. First, the inhibitory potency of PD153035 was tested by analysis of receptor phosphorylation against a panel of cancer cell lines overexpressing various levels of EGF receptor and HER2/neu. With EGF receptor-overexpressing cell lines, PD153035 caused a dose-dependent decrease in ligand-induced EGF receptor phosphorylation. In cell lines with the highest level of EGF receptor overexpression, Difi, A431, and MDA-MB-468, drug concentrations of ≥75 nM resulted in complete inhibition of EGF receptor phosphorylation. Bottom, HER2/neu-overexpressing cell lines SK-BR-3, SW620, and MCF7/HER-2 were treated with PD153035 at various concentrations for 2 h and then with 20 nM EGF for 10 min (top). PD153035 treatment at concentrations of >75 nM resulted in complete inhibition of EGF receptor phosphorylation. Bottom, HER2/neu-overexpressing cell lines SK-BR-3, SW620, and MCF7/HER-2 were treated with PD153035 at various concentrations for 2 h and then with 20 nM heregulin for 15 min. PD153035 only inhibited HER2/neu phosphorylation at the highest concentrations tested (1400–2800 nM). B, increasing concentrations of PD153035 did not affect the expression of EGF receptor protein in Difi cells, as shown in Western blotting assays with antibody RK2 (top). Likewise, PD153035 did not affect HER2/neu protein levels in SK-BR-3 cells, as determined by Western blotting with antibody Ab3 (bottom).

**Table 1** Inhibitory effects of PD153035 in cultures of EGF receptor versus HER-2/neu-overexpressing human cancer cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC50 (μM)</th>
<th>0.125 μM</th>
<th>0.25 μM</th>
<th>0.5 μM</th>
<th>2.5 μM</th>
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<td>EGF receptor</td>
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<tr>
<td>A431</td>
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<td>25</td>
<td>22</td>
<td>3</td>
<td>22</td>
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<tr>
<td>Difi</td>
<td>0.125</td>
<td>23</td>
<td>28</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>DU145</td>
<td>0.125</td>
<td>24</td>
<td>28</td>
<td>3</td>
<td>22</td>
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<tr>
<td>MDA-MB-468</td>
<td>0.125</td>
<td>25</td>
<td>28</td>
<td>3</td>
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</tr>
<tr>
<td>ME180</td>
<td>0.125</td>
<td>25</td>
<td>28</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>C4i</td>
<td>0.125</td>
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<td>28</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>SiHa</td>
<td>0.125</td>
<td>25</td>
<td>28</td>
<td>3</td>
<td>22</td>
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</tbody>
</table>

**HER-2/neu**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC50 (μM)</th>
<th>0.125 μM</th>
<th>0.25 μM</th>
<th>0.5 μM</th>
<th>2.5 μM</th>
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</thead>
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<tr>
<td>SK-BR-3</td>
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<tr>
<td>MDA-MB-453</td>
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<td>MCF7/HER-2</td>
<td>0.125</td>
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<td>SW620</td>
<td>0.125</td>
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<td>Control'</td>
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<td>25</td>
<td>22</td>
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</tbody>
</table>

*Results are expressed as a percentage of the cell number in untreated cell cultures from the same cell line.

NR, not reached.

' The non-EGF receptor/non-HER-2/neu-bearing leukemia cell line CMK was used as a control.
Inhibition of Cell Proliferation in Monolayers. To assess whether this specific EGF receptor tyrosine kinase inhibitory effect resulted in growth inhibition in monolayer cell culture, different EGF receptor-overexpressing cell lines (A431, Difl, MDA-MB-468, MDA-MB-231, DU145, SiHa, C4i, and ME180) were treated with PD153035 at increasing concentrations of 0.125-2.5 μM, as described in the "Materials and Methods." Leukemia cell line CMK, which lacks EGF receptors and HER2/neu, was used as a control. PD153035 caused a dose-dependent growth inhibition of EGF receptor-positive cell lines, beginning at less than micromolar concentrations, and the IC_{50} was less than 1 μM in most cases (Table 1). In contrast, in the HER2/neu-overexpressing cell lines (SK-BR-3, MDA-MB-453, and MCF7/HER-2), the growth-inhibitory effect of PD153035 was very modest and almost comparable with its effect on the CMK control cells, except at the highest concentration (Table 1).

Inhibition of Growth in Soft Agar. To further characterize the growth-inhibitory capacity of PD153035 on the panel of EGF receptor- and HER2/neu-expressing human cancer cell lines, a series of clonogenic growth assays was conducted. Cells were plated and treated with PD153035 at concentrations from 0.125 to 2.5 μM. Fig. 2A shows the dose-dependent, growth-inhibitory effect of PD153035 on the clonogenic growth of EGF receptor-overexpressing cell lines. A431 cells were not tested because they do not grow well in soft agar. The inhibition of clonal growth was clearly more pronounced in EGF receptor-overexpressing cancer cell lines than it was in HER2/neu-overexpressing cell lines (Fig. 2B). The relationship between EGF receptor number and response to PD153035 was further analyzed by plotting the IC_{50} of PD153035 for cell lines versus their EGF receptor numbers (Fig. 3). We found an exponential relationship between the number of EGF receptor and growth-inhibitory effects of PD153035, with increasing PD153035 sensitivity for cell lines expressing higher numbers of receptors (Fig. 3). The only exception was the HER2/neu-overexpressing cell line SK-BR-3, which also expresses a high number of EGF receptors (1 × 10^5 receptors/cell). In SK-BR-3 cells, PD153035...
induced only a modest inhibition of growth both in monolayer as well as in soft agar (Figs. 2 and 3). Fig. 4 illustrates the morphological changes of Difi cells grown in the absence of PD153035. Higher drug concentrations resulted in a decrease in the number as well as the size of the colonies.

**Abrogation of EGF-mediated Growth Effects and Reversibility.** In the cell cultures studied above, proliferation was driven by exogenous (autocrine) ligand. We evaluated whether PD153035 was also capable of preventing the growth effects of exogenous EGF. A431 cells are growth stimulated by EGF at picomolar concentrations but inhibited at higher concentrations (21), whereas SiHa cells are growth stimulated by all concentrations of EGF (19). These cells were grown in monolayer culture in the absence or presence of PD153035 at concentrations of 0.25 or 1 μM. After 24 h of exposure to PD153035, 200 picomolar to 100 nM EGF was added. Treatment with PD153035 resulted in a complete abolition of EGF-induced growth effects upon A431 cells (Fig. 5A) and SiHa cells (Fig. 5B). The data suggest that inhibition of EGF receptor tyrosine kinase by PD153035 results in unresponsiveness to the growth-regulatory activity of exogenous EGF and that this effect is complete and cannot be reversed by high EGF concentrations.

The growth-inhibitory capacity and the insensitivity to EGF-induced growth-regulation observed with PD153035 is reversible upon removal of the compound. In the experiment shown in Fig. 6, PD153035-containing medium of the EGF-treated SiHa cells was replaced with fresh medium, in the absence of EGF or containing 2 or 20 nM EGF. Upon removal of PD153035, cells regained their capacity to grow and to respond to exogenous EGF. Similar data were obtained with other cell lines (data not shown).

**Combined Therapy with PD153035 and the Anti-EGF Receptor-blocking Antibody MAb C225.** To investigate whether a combined anti-EGF receptor strategy would be more efficacious than PD153035 alone, we performed a series of studies with PD153035 given in combination with the EGF receptor-blocking MAb C225. This human-murine chimeric MAb, currently under clinical evaluation (22), is derived from our well-characterized murine MAb 225, which binds to the receptor with an affinity comparable to that of the natural ligand (Ka = 2 nM), competes with EGF binding, blocks the activation of receptor tyrosine kinase by EGF or transforming growth factor α, and inhibits the growth of cell lines expressing high level of EGF receptors (7–9, 12).

PD153035 was added to A431 cell monolayer cultures, and MAb C225 was added 24 h later. After 72 h cells were counted, an additive growth inhibitory effect was found for every treatment point (Fig. 7). This additive effect was observed with all concentrations of PD153035, including concentrations (0.25 and 0.5 μM) higher than those required to achieve complete tyrosine kinase inhibition in our assay (see Fig. 1A). Thus, even in a situation of complete inhibition of EGF receptor tyrosine kinase by PD153035, C225 can further inhibit cell growth, suggesting the possibility of an alternative mechanism of action.

**DISCUSSION**

Here, using a large panel of human cancer cell lines expressing various levels of the EGF receptor and/or the closely related HER2/neu receptor, we have shown that the quinazolone PD153035 is a highly specific and potent inhibitor of ligand-dependent EGF receptor phosphorylation. In addition, PD153035 suppresses proliferation and clonogenicity with greater efficacy in cell lines expressing greater numbers of receptors. Our studies also show that concentrations that were active against cells expressing high levels of EGF receptors did not significantly affect proliferation of cells expressing high levels of HER2/neu and normal levels of EGF receptors. Fry et al. (11) have previously shown that PD153035 inhibited EGF receptor tyrosine kinase at picomolar concentrations and HER2/neu receptor at micromolar concentrations. At concentrations as high as 50 μM, PD153035 had little effect against isolated recombinant platelet-derived growth factor receptors, fibroblastic growth factor receptors, colony-stimulating factor-1 receptors, insulin receptors, or src tyrosine kinases. At nanomolar concentrations, PD153035 inhibited EGF- but not basic fibroblastic growth factor- or platelet-derived growth factor-induced receptor phosphorylation in Swiss 3T3 fibroblasts and EGF receptor autophosphorylation in human A431 epidermoid carcinoma cells. In our studies with a panel of EGF receptor-expressing cell lines, we have observed that autophosphorylation was completely inhibited in overexpressing cancer cells at concentrations of ≥75 nm. We also found that PD153035 did not alter the expression of the EGF

![Fig. 4](https://clincancerres.aacrjournals.org)
PD153035 Treatment of Cancer Cells

Fig. 5 PD153035 prevents EGF-mediated growth effects. A, SiHa cells, which are growth stimulated by EGF, were grown in monolayer culture in the presence or absence of various concentrations of PD153035 (● 0 μM; ■ 0.25 μM; ○ 1 μM). After 24 h, EGF at 200 pm, 2 nm, 20 nm, and 100 nm was added. Mean cell numbers of triplicate experiments were assayed after 72 h. Data points, percentages of cell numbers compared with the same cell line grown in the absence of both PD153035 and EGF. B, A431 cells, which are growth stimulated by picomolar concentrations of EGF but inhibited by higher doses, were cultured under the same conditions as SiHa cells, as described above. PD153035 addition resulted in a complete inhibition of both growth-stimulatory and -inhibitory effects of EGF in A431 cells.

Fig. 6 Reversibility of PD153035 growth inhibition. After 72 h of treatment with PD153035 at 1 μM, medium from SiHa cells was removed and replaced with fresh medium containing EGF at 2 nm (■) or 20 nm (○) or media alone (●, control). Cells were counted daily for 3 days. Data points, percentages of the cell number observed at the time PD153035 was removed.

PD153035 induced a marked inhibition of growth both in monolayer and soft agar with cells expressing high levels of EGF receptors but not with cells bearing normal levels of EGF receptors and high levels of HER2/neu. The only exception was observed in SK-BR-3 cells, which overexpress both EGF receptor and HER2/neu and were not significantly growth inhibited by PD153035 despite inhibition of EGF receptor phosphorylation (data not shown). These findings are in contrast with the inhibition of growth observed in BT474 cells, which express levels of both receptors similar to those expressed by SK-BR-3 cells (Fig. 3). A possible explanation for this discrepancy is that SK-BR-3 cells have a potential but not obligatory EGF receptor autocrine loop because they are also resistant to anti-EGF receptor blocking MAbs (23) and other EGF receptor-targeted treatment strategies (17).

The degree of growth inhibition observed with PD153035 was dependent on the number of EGF receptors per cell, with increased efficacy in cells with higher receptor numbers. This dependency was better observed in soft agar assays, a more stringent test of mitogenic capacity because several cycles of cell division are required to form a detectable colony (Fig. 3). Prior studies with EGF-receptor-blocking antibodies have shown that they inhibit the growth of nearly all cells that have an active EGF receptor autocrine pathway, independent of the number of receptors (6). Although all of the cells that were inhibited by PD153035 are known to produce ligand (19, 24), the clear relationship between increased receptor number and improved response to treatment with PD153035 may be an indication that an increased receptor number could result in a higher capacity for receptor autophosphorylation by additional mechanisms of receptor activation, such as spontaneous dimerization of receptors.

The observation that PD153035 completely prevented ef-
Cells. Cell number was determined after 72 h. Columns, cell growth expressed as percentages of the cell number in untreated cultures of A431 cells (control).

Effects of exogenous EGF upon SiHa and A431 cells is important because it shows that the antigrowth effects of this compound cannot be rescued by supraphysiological amounts of ligand. Presumably, this is a consequence of the location of the receptor kinase downstream from the ligand-binding site. As expected, this effect is different from our observations with anti-EGF receptor antibodies because carefully titrated amounts of the ligand can reverse the growth-inhibitory capacity of the MAb (25). Our studies also show that PD153035 is cytostatic and that cells regain their growth capacity and ligand sensitivity upon drug removal. These findings suggest that continuous drug exposure will be required to achieve clinical efficacy and that further preclinical studies should take this fact into consideration. In vivo studies with another tyrosine kinase inhibitor have also shown lack of antitumor activity when drug concentrations fell below the optimal in vitro dose, further supporting the need for continuous drug exposure (26).

Combined treatment of A431 cells with PD153035 and antireceptor MAb C225 caused an additive inhibitory effect, despite the fact that EGF receptor tyrosine activity was completely blocked with PD153035 concentrations higher than 75 nM (Fig. 1) and could not be rescued with high concentrations of EGF (Fig. 5A). Furthermore, treatment of cells with normal number of EGF receptors with MAb 225 could inhibit cell growth in our earlier studies (for review see Ref. 6). This suggests that MAb C225 might have effects other than competing with ligand binding and, thereby, preventing tyrosine kinase activation. Studies by Fan et al. (27) have shown that MAb 225 produces antibody-mediated EGF receptor dimerization, resulting in receptor down-regulation. Down-regulation of receptors by the antibody appears to be an important factor in its growth-inhibitory capacity because a monovalent 225 Fab' fragment, which retains capacity to block binding and tyrosine kinase activation of receptors by exogenous ligand, was unable to induce receptor dimerization and down-regulation and had weaker antiproliferative capacity (27). In contrast, we have not observed EGF receptor down-regulation with PD153035 (Fig. 1B). Thus, combined antireceptor treatment strategies with a tyrosine kinase inhibitor and agents that down-regulate the receptor may offer a therapeutic advantage.

In conclusion, the data presented here demonstrates the efficacy and specificity of PD153035 as an anticancer agent against EGF receptor-overexpressing human tumor cell lines. Further in vivo studies with this class of specific EGF tyrosine kinase inhibitors are justified and may provide the basis for their future clinical development.

REFERENCES

PD153035, a tyrosine kinase inhibitor, prevents epidermal growth factor receptor activation and inhibits growth of cancer cells in a receptor number-dependent manner.

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