

Antitumor Effect and Potentiation of Cytotoxic Drugs Activity in Human Cancer Cells by ZD-1839 (Iressa), an Epidermal Growth Factor Receptor-selective Tyrosine Kinase Inhibitor¹

Fortunato Ciardiello,² Rosa Caputo, Roberto Bianco, Vincenzo Damiano, Grazia Pomatico, Sabino De Placido, A. Raffaele Bianco, and Giampaolo Tortora

Cattedra di Oncologia Medica, Dipartimento di Endocrinologia e Oncologia Molecolare e Clinica, Università degli Studi di Napoli Federico II, 5-80131 Naples, Italy

ABSTRACT

Transforming growth factor α (TGF- α) is an autocrine growth factor for human cancer. Overexpression of TGF- α and its specific receptor, the epidermal growth factor receptor (EGFR), is associated with aggressive disease and poor prognosis. The EGFR has been proposed as a target for anticancer therapy. Compounds that block ligand-induced EGFR activation have been developed. ZD-1839 (Iressa) is a p.o.-active, quinazoline derivative that selectively inhibits the EGFR tyrosine kinase and is under clinical development in cancer patients. The antiproliferative activity of ZD-1839 alone or in combination with cytotoxic drugs differing in mechanism(s) of action, such as cisplatin, carboplatin, oxaliplatin, paclitaxel, docetaxel, doxorubicin, etoposide, topotecan, and raltitrexed, was evaluated in human ovarian (OVCAR-3), breast (ZR-75-1, MCF-10A *ras*), and colon cancer (GEO) cells that coexpress EGFR and TGF- α . ZD-1839 inhibited colony formation in soft agar in a dose-dependent manner in all cancer cell lines. The antiproliferative effect was mainly cytostatic. However, treatment with higher doses resulted in a 2–4-fold increase in apoptosis. A dose-dependent supra-additive increase in growth inhibition was observed when cancer cells were treated with each cytotoxic drug and ZD-1839. The combined treatment markedly enhanced apoptotic cell death induced by single-agent treatment. ZD-1839 treatment of nude mice bearing established human GEO colon cancer xenografts revealed a reversible dose-dependent inhibition of tumor growth be-

cause GEO tumors resumed the growth rate of controls at the end of the treatment. In contrast, the combined treatment with a cytotoxic agent, such as topotecan, raltitrexed, or paclitaxel, and ZD-1839 produced tumor growth arrest in all mice. Tumors grew slowly for approximately 4–8 weeks after the end of treatment, when they finally resumed a growth rate similar to controls. GEO tumors reached a size not compatible with normal life in all control mice within 4–6 weeks and in all single agent-treated mice within 6–8 weeks after GEO cell injection. In contrast, 50% of mice treated with ZD-1839 plus topotecan, raltitrexed, or paclitaxel were still alive 10, 12, and 15 weeks after cancer cell injection, respectively. These results demonstrate the antitumor effect of this EGFR-selective tyrosine kinase inhibitor and provide a rationale for its clinical evaluation in combination with cytotoxic drugs.

INTRODUCTION

Growth factors of the EGF³ gene family, such as TGF- α , are potent mitogens for several human epithelial cell types including breast, colon, ovary, kidney, prostate, and lung, and have been implicated in cancer development and progression through autocrine and paracrine pathways (1). TGF- α binds to the extracellular domain of the EGFR and activates its intracellular tyrosine kinase domain (1). The EGFR is a M_r 170,000 transmembrane glycoprotein with an external binding domain and an intracellular tyrosine kinase domain (1). Ligand binding induces dimerization of the EGFR and its autophosphorylation on several tyrosine residues in the intracellular domain, creating a series of high-affinity binding sites for various transducing molecules that are involved in transmitting the mitogenic signaling through the *ras/raf/mitogen-activated protein kinase* pathway (2). Enhanced expression of TGF- α and/or EGFR has been detected in the majority of human carcinomas (1) and has been associated with poor prognosis in several human tumor types, such as breast cancer (3). EGFR overexpression has been also found in human cancer cell lines that are resistant to different cytotoxic drugs (4). For these reasons, the blockade of the TGF- α /EGFR autocrine pathway has been proposed as a potential therapeutic modality (5–7). Several pharmacological and biological approaches have been developed for blocking EGFR activation and/or function in cancer cells. In the past 10 years, various anti-EGFR blocking MABs, recombinant proteins containing TGF- α or EGF fused to toxins, and tyrosine kinase inhibitors have been generated and characterized for their bio-

Received 11/19/99; revised 2/14/00; accepted 2/16/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported by grants from the Associazione Italiana per la Ricerca sul Cancro and the Consiglio Nazionale delle Ricerche Target Project on Biotechnologies.

² To whom requests for reprints should be addressed, at Cattedra di Oncologia Medica, Dipartimento di Endocrinologia e Oncologia Molecolare e Clinica, Università degli Studi di Napoli Federico II, Via S. Pansini, 5-80131 Naples, Italy. Phone: 39-081-7462061; Fax: 39-081-7462066; E-mail: fortunatociardiello@yahoo.com.

³ The abbreviations used are: EGF, epidermal growth factor; EGFR, EGF receptor; TGF, transforming growth factor; MAB, monoclonal antibody.

logical and potentially therapeutic properties (8–15). One of these agents, MAb C225, a chimeric human-mouse IgG1 MAb, has recently started Phase II and Phase III clinical evaluation in cancer patients (8, 16–19). Several compounds that block the ligand-induced activation of the EGFR tyrosine kinase and, in some cases, to a lesser extent that of the closely related *c-erbB-2* receptor have been developed (15, 20–29). Among these, various quinazoline-derived agents have been synthesized and tested as anticancer agents *in vitro* and in preclinical models (6, 15). ZD-1839, an anilinoquinazoline, is a p.o.-active, selective EGFR-tyrosine kinase inhibitor that blocks signal transduction pathways implicated in cancer cell proliferation and other host-dependent processes promoting cancer growth (26, 27). ZD-1839 is currently under clinical evaluation in early clinical trials in cancer patients (30).

In the present study, we tested the antiproliferative activity of ZD-1839 in four human cancer cell lines of different histotypes, including ovarian, breast, and colon cancer, that express both EGFR and TGF- α . Furthermore, a large body of experimental and clinical evidence has been recently accumulated on the enhanced antitumor activity of some chemotherapeutic agents, such as doxorubicin, cisplatin, paclitaxel, or topotecan, in combination with specific MABs that selectively block either the EGFR or the closely related *c-erbB-2* receptor (31–42). For this purpose, we also evaluated whether ZD-1839 has cooperative effect with cytotoxic drugs with different mechanism(s) of action and with antitumor activity in a variety of human malignancies, in which EGFR is generally overexpressed and a TGF- α /EGFR autocrine pathway is operative.

MATERIALS AND METHODS

Materials. Clinical grade ZD-1839 (Iressa) and raltitrexed (Tomudex) were provided by AstraZeneca Pharmaceuticals (Macclesfield, United Kingdom). Doxorubicin, etoposide, cisplatin, carboplatin, and paclitaxel were purchased from Sigma Chemical Co. (St. Louis, MO). Docetaxel (Taxotere) was a gift of Rhone-Poulenc Rorer Italia. Topotecan was kindly provided by SmithKline Beecham Italia. Oxaliplatin was provided by Sanofi Italia.

Cell Lines. GEO human colon cancer, OVCAR-3 human ovarian cancer, and ZR-75-1 human breast cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD). MCF-10A Ha-*ras* cells have been obtained by cotransfection of human nontransformed MCF-10A cells with an expression vector plasmid containing the human activated c-Ha-*ras* proto-oncogene and an expression vector plasmid containing the neomycin resistance gene (43). GEO, OVCAR-3, and ZR-75-1 cells were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 20 mM HEPES (pH 7.4), 100 UI/ml penicillin, 100 μ g/ml streptomycin, and 4 mM glutamine (ICN, Irvine, United Kingdom) in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. MCF-10A Ha-*ras* cells were grown in a 1:1 (v/v) DMEM and Ham's F12 mixture, supplemented with 5% heat-inactivated horse serum, 20 mM HEPES (pH 7.4), 4 mM glutamine, 0.5 μ g/ml hydrocortisone (Sigma), 10 ng/ml EGF, 10 μ g/ml insulin (Collaborative Research Products, Bedford, MA), 100 units/ml penicillin, and 100

μ g/ml streptomycin in a humidified atmosphere of 95% air and 5% CO₂ at 37°C.

Growth in Soft Agar. Cells (10⁴ cells/well) were suspended in 0.5 ml of 0.3% Difco Noble agar (Difco, Detroit, MI) supplemented with complete culture medium. This suspension was layered over 0.5 ml of 0.8% agar-medium base layer in 24 multiwell cluster dishes (Becton Dickinson, Lincoln Park, NJ) and treated with different concentrations of ZD-1839 alone and/or in combination with the indicated concentrations of cytotoxic drugs. After 10–14 days, cells were stained with nitro blue tetrazolium (Sigma), and colonies >0.05 mm were counted as described previously (33).

Immunoprecipitation and Western Blot Analysis. Total cell lysates were obtained as described previously (44) from serum-starved MCF-10A Ha-*ras* cells that were treated for 3 h with the indicated concentrations of ZD-1839, followed by the addition of complete medium containing EGF (50 ng/ml) for 15 min. Proteins were immunoprecipitated with MAb C225 anti-EGFR monoclonal antibody (kindly provided by Dr. H. Waksal, ImClone Systems, New York, NY), as reported previously (44). For Western blot analysis, immunoprecipitates were resolved by a 7.5% SDS-PAGE and probed with either an antihuman EGFR monoclonal antibody (Transduction Laboratories, Lexington, KY) or the PY20 anti-P-tyrosine monoclonal antibody (Transduction Laboratories). Immunoreactive proteins were visualized by enhanced chemiluminescence (Amersham International, Buckinghamshire, England), as described previously (44).

Apoptosis Assay. The induction of programmed cell death was determined as described previously (45) by the Cell Death Detection ELISA Plus Kit (Boehringer Mannheim, Indianapolis, IN). Briefly, 5 \times 10⁴ cells/well were seeded into six multiwell cluster dishes. After appropriate treatment, the cells were washed once with PBS, and 0.5 ml lysis buffer was added. After a 30-min incubation, the supernatant was recovered and assayed for DNA fragments as recommended by the manufacturer at 405 nm using a Microplate Reader Model 3550-UV (Bio-Rad, Milan, Italy). Each treatment was performed in quadruplicate. Additional plates identically treated were analyzed for cell number with a hemocytometer to normalize the values for cell numbers, and the results are expressed relative to untreated control samples.

GEO Xenografts in Nude Mice. Female BALB/c athymic (*nu+ / nu+*) mice, 4–6 weeks of age, were purchased from Charles River Laboratories (Milan, Italy). The research protocol was approved, and mice were maintained in accordance with the institutional guidelines of the University of Naples Animal Care and Use Committee. Mice were acclimatized at the University of Naples Medical School Animal Facility for 1 week prior to being injected with cancer cells. Mice were injected s.c. with 10⁷ GEO cells that had been resuspended in 200 μ l of Matrigel (Collaborative Biomedical Products, Bedford, MA). After 7 days, when established tumors of approximately 0.2–0.3 cm³ in diameter were detected, 10 mice/group were treated i.p. on days 1–5 of each week for 4 weeks with ZD-1839 at the indicated daily doses. To determine the effects of the combination of ZD-1839 and cytotoxic drugs, additional groups of 10 mice were treated i.p. on days 1–5 of each week for 4 weeks with ZD-1839, 2.5

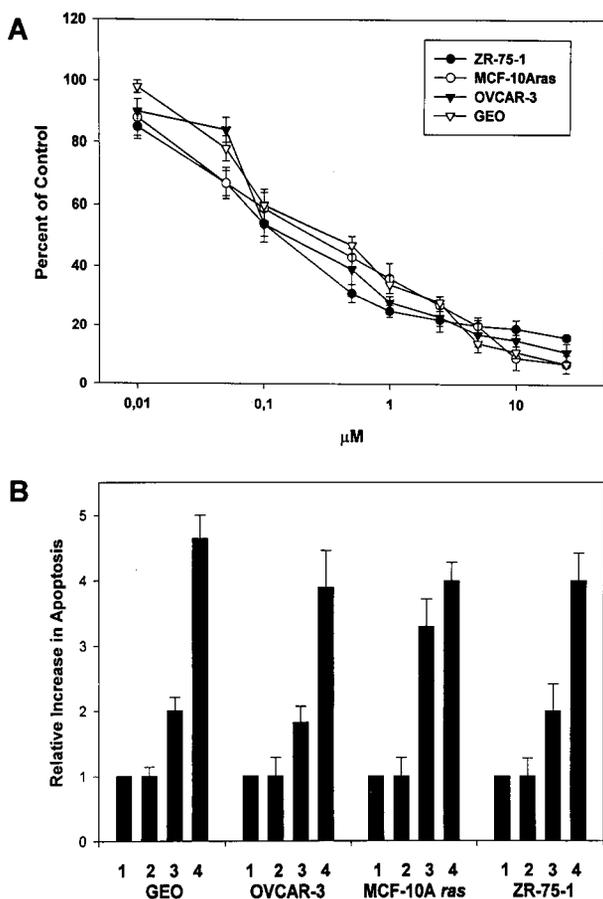


Fig. 1 A, dose-dependent growth-inhibitory effects of ZD-1839 on the soft agar growth of human ZR-75-1, MCF-10A *ras*, OVCAR-3, and GEO cell lines. Cells were treated with the indicated concentrations of ZD-1839 each day for 5 consecutive days. Colonies were counted after 10–14 days. Data represent the averages of three different experiments, each performed in triplicate; bars, SD. B, dose-dependent induction of programmed cell death by treatment with ZD-1839 in human ZR-75-1, MCF-10A *ras*, OVCAR-3, and GEO cell lines. Cells were treated each day for 3 days with the following doses of ZD-1839: columns 1, untreated control; columns 2, 0.05 μM ; columns 3, 0.1 μM ; and columns 4, 1 μM . Analysis of apoptosis was performed 4 days after the beginning of treatment. Data represent the averages of quadruplicate determinations; bars, SD.

mg/mouse/day, alone or in combination with paclitaxel, 20 mg/kg on day 1 of each week for 4 weeks; with topotecan, 2 mg/kg on day 1 of each week for 4 weeks; or with raltitrexed, 12.5 mg/kg on day 1 of each week for 4 weeks. Tumor size was measured using the formula $\pi/6 \times \text{larger diameter} \times (\text{smaller diameter})^2$, as reported previously (33).

Statistical Analysis. The Student's *t* test (46) and the Mantel-Cox log-rank test (47) were used to evaluate the statistical significance of the results. All *P*s represent two-sided tests of statistical significance. All analyses were performed with the BMDP New System statistical package version 1.0 for Microsoft Windows (BMDP Statistical Software, Los Angeles, CA).

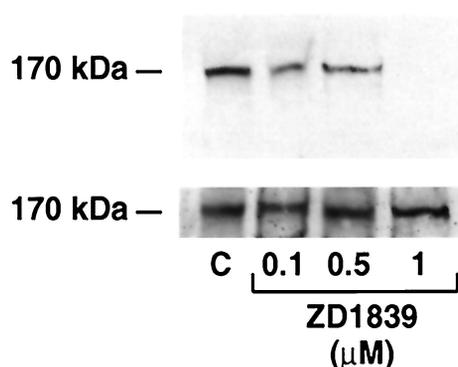


Fig. 2 Dose-dependent inhibition of EGF-induced EGFR autophosphorylation by ZD-1839 in human MCF-10A *ras* cells. Serum-starved MCF-10A Ha-*ras* cells were treated for 3 h with the indicated concentrations of ZD-1839, followed by addition of complete medium containing EGF (50 ng/ml) for 15 min. Protein extracts were then immunoprecipitated with the MAb C225 anti-EGFR monoclonal antibody, resolved by a 7.5% SDS-PAGE and probed with either the PY20 anti-P-tyr monoclonal antibody (*top*) or an antihuman EGFR monoclonal antibody (*bottom*). Immunoreactive proteins were visualized by enhanced chemiluminescence.

RESULTS

We have evaluated the effect of ZD-1839 on the growth of various human epithelial cancer cell lines in soft agar. For this purpose, we selected GEO colon cancer, ZR-75-1 and MCF-10A Ha-*ras* breast cancer, and OVCAR-3 ovarian cancer cell lines. All of these cell lines express functional EGFR, ranging from approximately 20,000 (ZR-75-1) to 40,000 (GEO), 150,000 (OVCAR-3), and 250,000 (MCF-10A Ha-*ras*) EGF binding sites/cell and secrete high levels of TGF- α (33). Treatment with ZD-1839 determined a dose-dependent inhibition of colony formation in soft agar with an IC_{50} ranging between 0.2 and 0.4 μM in all cancer cell lines tested (Fig. 1A). It has been shown that treatment with agents that selectively inhibit the EGFR, such as anti-EGFR blocking MAbs, have a cytostatic effect generally with cell cycle arrest in the G_1 phase (5). However, in some cancer cell lines, inhibition of the EGFR function also caused apoptotic cell death (34, 48). We, therefore, determined whether the ZD-1839 antiproliferative effect was accompanied by the induction of programmed cell death. As shown in Fig. 1B, ZD-1839 treatment induced a dose-dependent 2–4-fold increase in apoptosis in all cancer cell lines tested with a maximum effect between 0.1 and 1 μM . Treatment with ZD-1839 resulted also in a dose-dependent inhibition of EGF-induced tyrosine autophosphorylation of the EGFR in MCF-10A Ha-*ras* cells (Fig. 2) as well as in the other three cancer cell lines (data not shown).

A series of experiments was performed to evaluate the potential combined antiproliferative effect of treatment with ZD-1839 and a wide variety of cytotoxic drugs with different mechanism(s) of action that are currently used in the treatment of human epithelial cancers. The effects on the anchorage-independent growth of all four cancer cell lines of three different platinum-derived compounds (cisplatin, carboplatin, and oxaliplatin), two taxanes (paclitaxel and docetaxel), two topoisomerase II inhibitors (doxorubicin and etoposide), a topoisomerase I

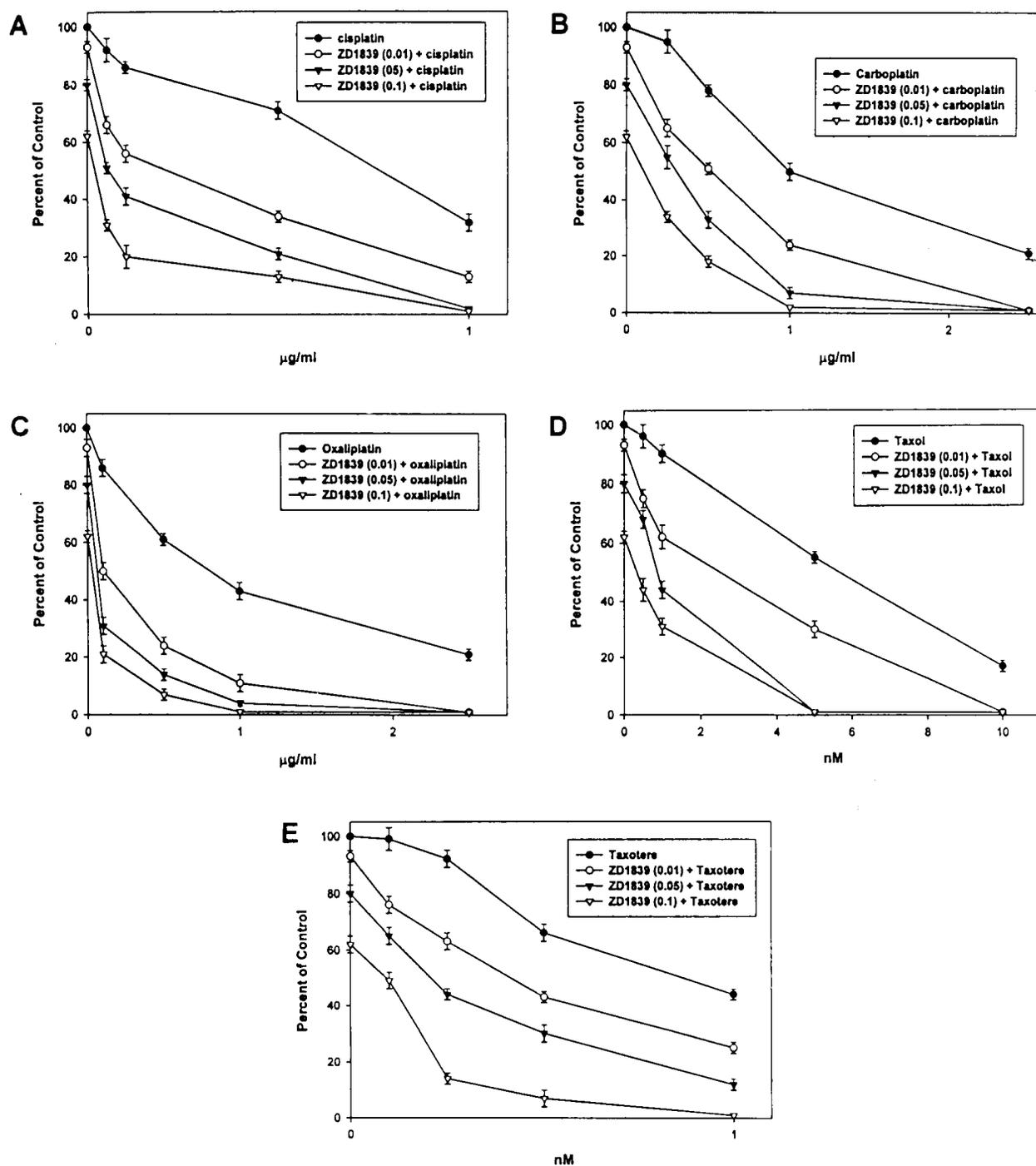


Fig. 3 Growth-inhibitory effects of treatment with ZD-1839 (0.01, 0.05, and 0.1 μM) in combination with cisplatin (A), carboplatin (B), oxaliplatin (C), paclitaxel (Taxol; D), or docetaxel (Taxotere; E) on the soft agar growth of OVCAR-3 cells. Cells were treated with the indicated concentrations of cytotoxic drug on day 1, followed by the indicated concentrations of ZD-1839 on each day from days 2 to 6. Colonies were counted after 10–14 days. Data represent the averages of three different experiments, each performed in triplicate; bars, SD.

inhibitor (topotecan), and a thymidylate synthase inhibitor (raltitrexed) were tested in combination with the EGFR tyrosine kinase inhibitor ZD-1839. A supra-additive, growth-inhibitory effect was observed with all doses of ZD-1839 and each cyto-

toxic drug tested in OVCAR-3 cells (Figs. 3 and 4). Similar results were obtained in GEO, MCF-10A Ha-*ras*, and ZR-75-1 cells (data not shown). Furthermore, when combinations of lower doses of ZD-1839 and cytotoxic drugs were used, the

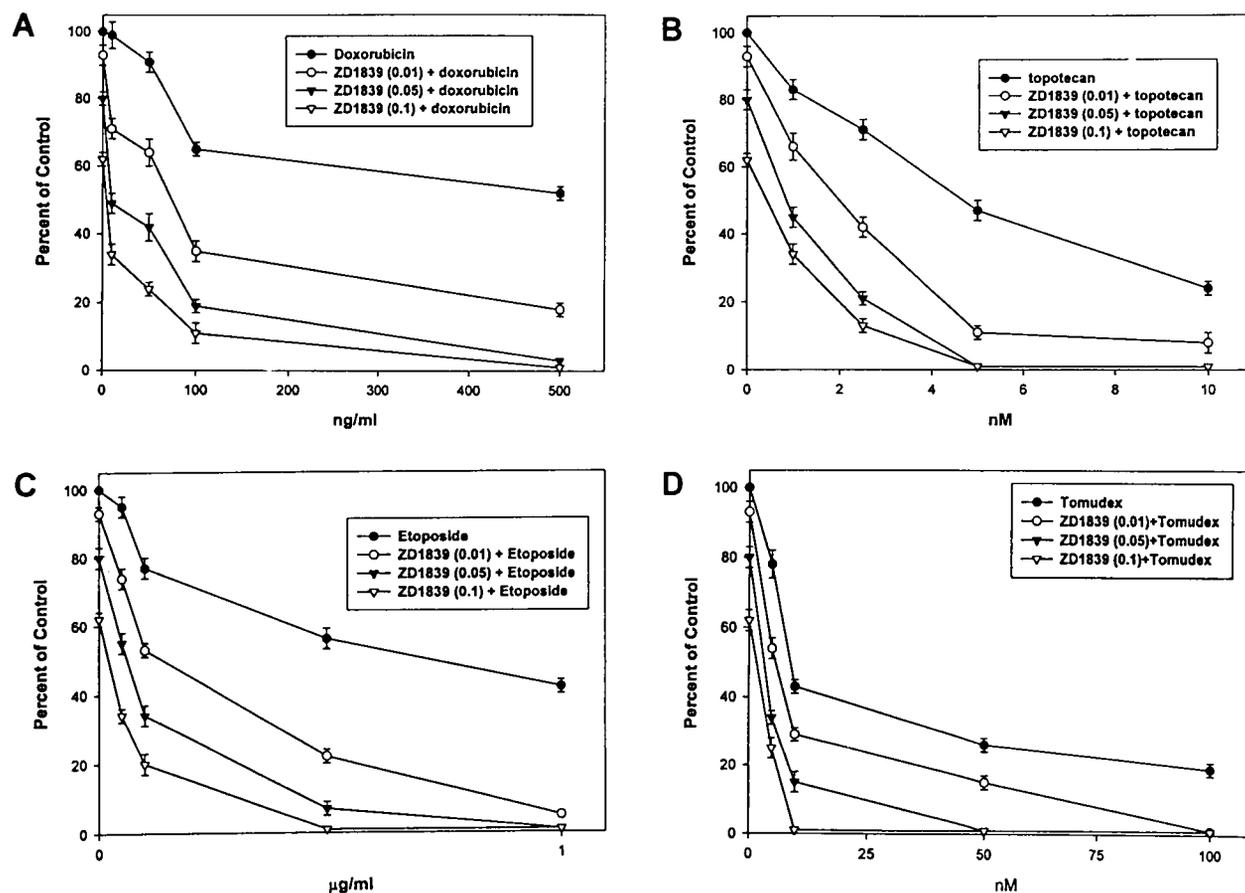


Fig. 4 Growth-inhibitory effects of treatment with ZD-1839 (0.01, 0.05, and 0.1 μM) in combination with doxorubicin (A), topotecan (B), etoposide (C), or raltitrexed (Tomudex; D) on the soft agar growth of OVCAR-3 cells. Cells were treated with the indicated concentrations of cytotoxic drug on day 1, followed by the indicated concentrations of ZD-1839 on each day from days 2 to 6. Colonies were counted after 10–14 days. Data represent the averages of three different experiments, each performed in triplicate; bars, SD.

antiproliferative effect was clearly cooperative in all cell lines examined. For example, the cooperativity quotient of the combined treatment, defined as the ratio between the actual growth inhibition obtained with the combination of a cytotoxic drug (paclitaxel, topotecan, doxorubicin, oxaliplatin, or raltitrexed) and ZD-1839 and the sum of the growth inhibition achieved by each agent was between 1.5 and 2.5 in all cancer cell lines tested (Fig. 5).

We also determined whether the cooperative growth-inhibitory effect of cytotoxic drugs and ZD-1839 could involve induction of programmed cell death in cancer cells. GEO and OVCAR-3 cells were treated with different concentrations of a cytotoxic drug (doxorubicin, paclitaxel, topotecan, raltitrexed, or oxaliplatin) alone or in combination with ZD-1839 (Fig. 6). Treatment with each cytotoxic drug increased apoptotic cell death in a dose-dependent manner in both GEO and OVCAR-3 cells. Addition of ZD-1839 at low doses that induce little or no apoptosis alone potentiated cytotoxic-induced apoptosis in both cell lines by approximately 2–3.5-fold (Fig. 6).

We have tested previously the effect of different novel therapeutic agents in nude mice bearing GEO colon cancer xenografts (33, 49, 50). GEO cells form moderately differenti-

ated adenocarcinomas that express both the EGFR and TGF α , when injected s.c. in immunodeficient mice (48). GEO cells (10^7) were injected s.c. into the dorsal flank of nude mice. After 1 week, when established GEO xenografts were palpable with a tumor size of $\sim 0.25\text{ cm}^3$, mice were treated i.p. on days 1–5 of each week for 4 weeks with different concentrations of ZD-1839 (Fig. 7A). ZD-1839 treatment produced a dose-dependent inhibition of GEO tumor growth that was almost completely suppressed in mice treated with the 5 mg of daily dose. This effect was cytostatic rather than cytotoxic. In fact, GEO tumors resumed a growth rate comparable with controls within 1–2 weeks by the termination of treatment (data not shown). ZD-1839 treatment was generally well tolerated by mice with no signs of acute or delayed toxicity. However, an approximately 5–10% reduction in body weight that was reversible upon cessation of treatment was observed at the end of the treatment period in mice receiving the highest ZD-1839 dose. Although GEO tumor growth was only delayed, mouse survival duration was significantly increased in the ZD-1839 (5 mg/dose)-treated group ($P < 0.001$), as shown in Fig. 7B.

We next evaluated whether the cooperative growth-inhibitory effect of ZD-1839 and cytotoxic drugs observed

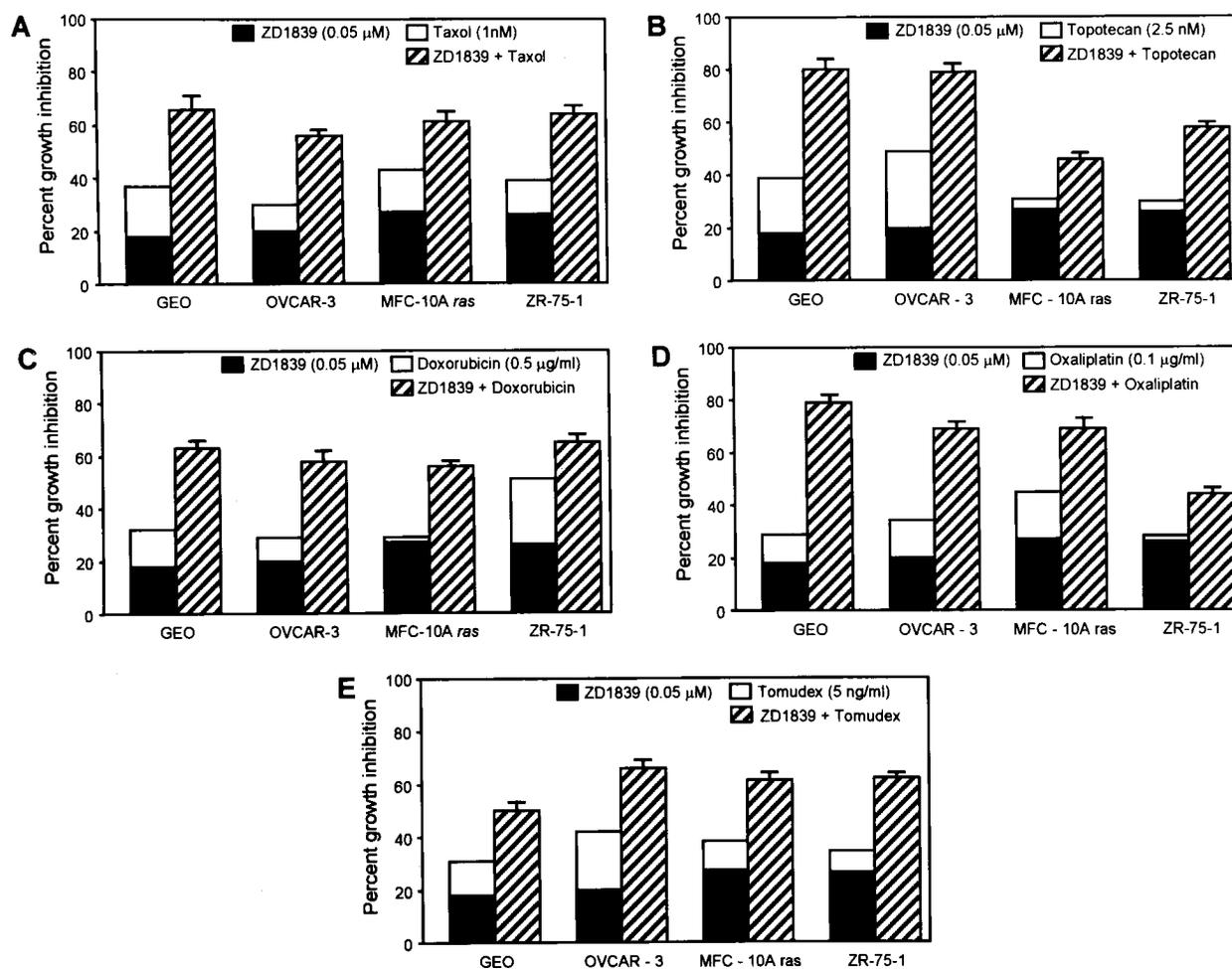


Fig. 5 Growth-inhibitory effects of the combined treatment with ZD-1839 and paclitaxel (Taxol; **A**), topotecan (**B**), doxorubicin (**C**), oxaliplatin (**D**), or raltitrexed (Tomudex; **E**) on the soft agar growth of GEO, OVCAR-3, MCF-10A *ras*, and ZR-75-1 cell lines. Cells were plated in soft agar and treated with the indicated concentration of cytotoxic drug on day 1 and with 0.05 μM ZD-1839 on days 2–6 or with each agent alone. Colonies were counted after 10–14 days. Data are expressed as the percentage of growth inhibition as compared with the growth of untreated control cells. For each pair of columns, the height of the columns on the left represents the sum of the effect of each agent and, therefore, the expected growth inhibition if their effect is additive when used in combination. The total height of the columns on the right indicates the observed growth inhibition when the agents are used in combination. The difference between the heights of the paired columns reflects the magnitude of cooperativity on growth inhibition. Data represent the averages of three different experiments, each performed in duplicate; bars, SD.

in vitro could be also obtained *in vivo*. For the combined treatment, a 2.5-mg/dose of ZD-1839 was selected, because this induced an ~50% inhibition of tumor growth after 4 weeks of treatment and was very well tolerated. This dose of ZD-1839 was given for 4 weeks in combination with four weekly administrations of either paclitaxel, topotecan, or raltitrexed to nude mice bearing established GEO tumors. The maximum tolerated doses of paclitaxel, topotecan, or raltitrexed using this schedule were selected. Treatment of mice with each agent significantly inhibited GEO tumor growth *in vivo* as compared with control untreated mice. However, shortly after the end of the treatment with ZD-1839, paclitaxel, topotecan, or raltitrexed, GEO tumors resumed the growth rate of untreated tumors (Fig. 8). A cooperative antitumor effect was observed when ZD-1839 was

used in combination with each cytotoxic drug tested with a significant suppression of tumor growth at the end of the 4 weeks of treatment in all mice as compared with untreated mice or to single agent-treated mice. As shown in Fig. 8, GEO tumors reached a size not compatible with normal life in all untreated mice within 4–6 weeks and in all single agent-treated mice within 6–8 weeks after GEO cell injection. The delayed GEO tumor growth in the ZD-1839 plus chemotherapy-treated groups was accompanied by a prolonged life span of mice that was significantly different from that of untreated controls or single agent-treated groups. This effect was more pronounced with the paclitaxel plus ZD-1839 combination. In fact, 40% of mice treated with this combination were the only mice alive 16 weeks after tumor cell injection (Fig. 8B). Combined treatments with ZD-1839

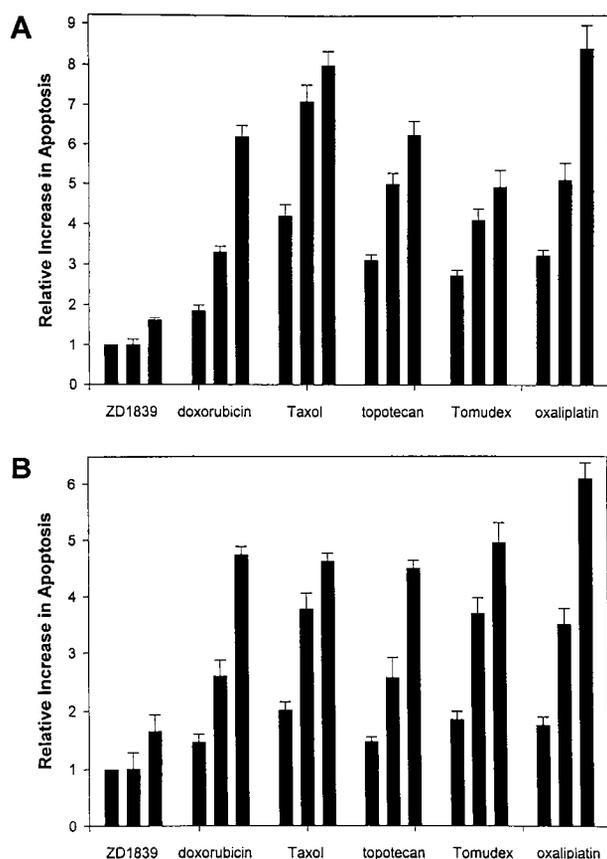


Fig. 6 Induction of apoptosis by treatment with ZD-1839 in combination with the indicated cytotoxic drugs in GEO (A) or OVCAR-3 (B) cells. Cells were treated with ZD-1839 alone (0, 0.05, or 0.1 μM each day for 3 days); with doxorubicin (0.5 $\mu\text{g}/\text{ml}$ on day 1), alone or in combination with ZD-1839 (0.05 or 0.1 μM each day for 3 days); with paclitaxel (Taxol; 5 nM on day 1), alone or in combination with ZD-1839 (0.05 or 0.1 μM each day for 3 days); with topotecan (5 nM on days 1 and 2), alone or in combination with ZD-1839 (0.05 or 0.1 μM each day for 3 days); with raltitrexed (Tomudex; 0.05 μM on days 1 and 2), alone or in combination with ZD-1839 (0.05 or 0.1 μM each day for 3 days); or with oxaliplatin (1 $\mu\text{g}/\text{ml}$ on day 1), alone or in combination with ZD-1839 (0.05 or 0.1 μM each day for 3 days). Analysis of apoptosis was performed 4 days after the beginning of treatment. Data represent the averages of quadruplicate determinations; bars, SD.

and each cytotoxic drug at the dose and schedule tested were well tolerated by mice, with no weight loss or other signs of acute or delayed toxicity observed.

DISCUSSION

In the last 10 years, a large body of experimental studies have been performed to develop novel antitumor agents that are able to selectively inhibit important pathways that control cancer cell proliferation. In this respect, the blockade of the EGFR-activated mitogenic pathway is a promising novel therapeutic strategy in the control of human cancer, as demonstrated by the preclinical and clinical development of the human-mouse chimeric anti-EGFR C225 MAb (8). An alternative approach for blocking EGFR function in cancer cells has been the development of small molecules that are able to interfere with the

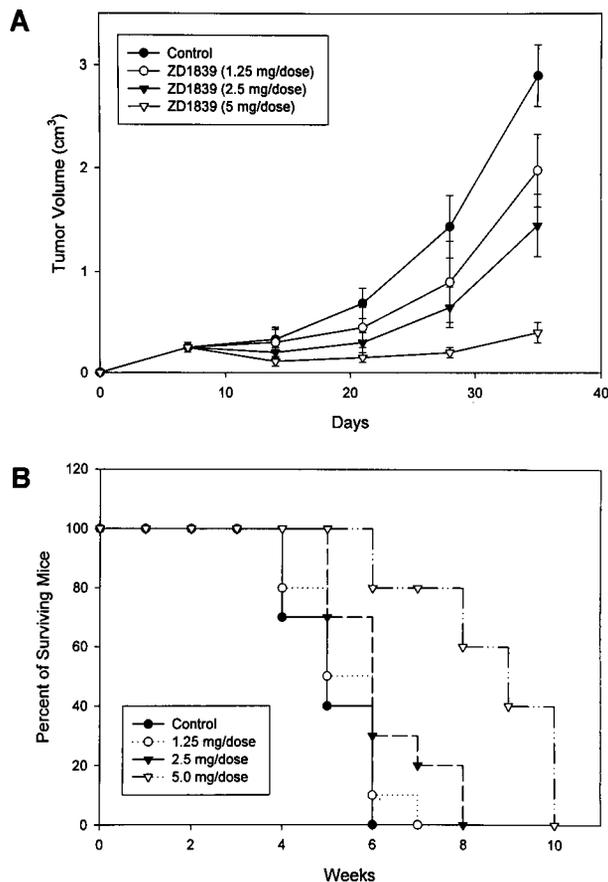


Fig. 7 A, antitumor activity of ZD-1839 treatment on established GEO human colon carcinoma xenografts. Mice were injected s.c. into the dorsal flank with 10^7 GEO cells. After 7 days (average tumor size, 0.25 cm^3), the mice were treated i.p. on days 1 to 5 of each week for 4 weeks with ZD-1839 at the indicated daily doses. Each group consisted of 10 mice. Data represent the averages; bars, SD. Student's *t* test was used to compare tumor sizes among different treatment groups at day 35 after GEO cell injection. ZD-1839, 1.25-mg/dose, versus control (two-sided $P = 0.04$); ZD-1839, 2.5-mg/dose, versus control (two-sided $P = 0.01$); and ZD-1839, 5-mg/dose, versus control (two-sided $P < 0.001$) are shown. B, effects of ZD-1839 treatment on the survival of GEO tumor-bearing mice. Ten mice/group were monitored for survival. Differences in animal survival among groups were evaluated using the Mantel-Cox log-rank test. The survival of mice was significantly different between: the ZD-1839 (5 mg/dose)-treated group and the control group ($P < 0.001$); the ZD-1839 (5 mg/dose)-treated group and the ZD-1839 (2.5 mg/dose)-treated group ($P < 0.001$); and the ZD-1839 (5 mg/dose)-treated group and the ZD-1839 (1.25 mg/dose)-treated group ($P < 0.001$).

enzymatic activity of the ligand-activated EGFR (15). Among the novel EGFR tyrosine kinase inhibitors, quinazoline-derived drugs have been synthesized as potential anticancer drugs (6, 15). ZD-1839, an anilinoquinazoline, is a potent and selective inhibitor of the EGFR tyrosine kinase *in vitro* and *in vivo* (27).

In the present study, we analyzed the effects of ZD-1839 on cell proliferation, induction of apoptosis, and antitumor activity in several human cancer cell lines with a functional EGFR-driven autocrine pathway. ZD-1839 treatment produced a dose-dependent growth inhibition *in vitro* in all human cancer cell

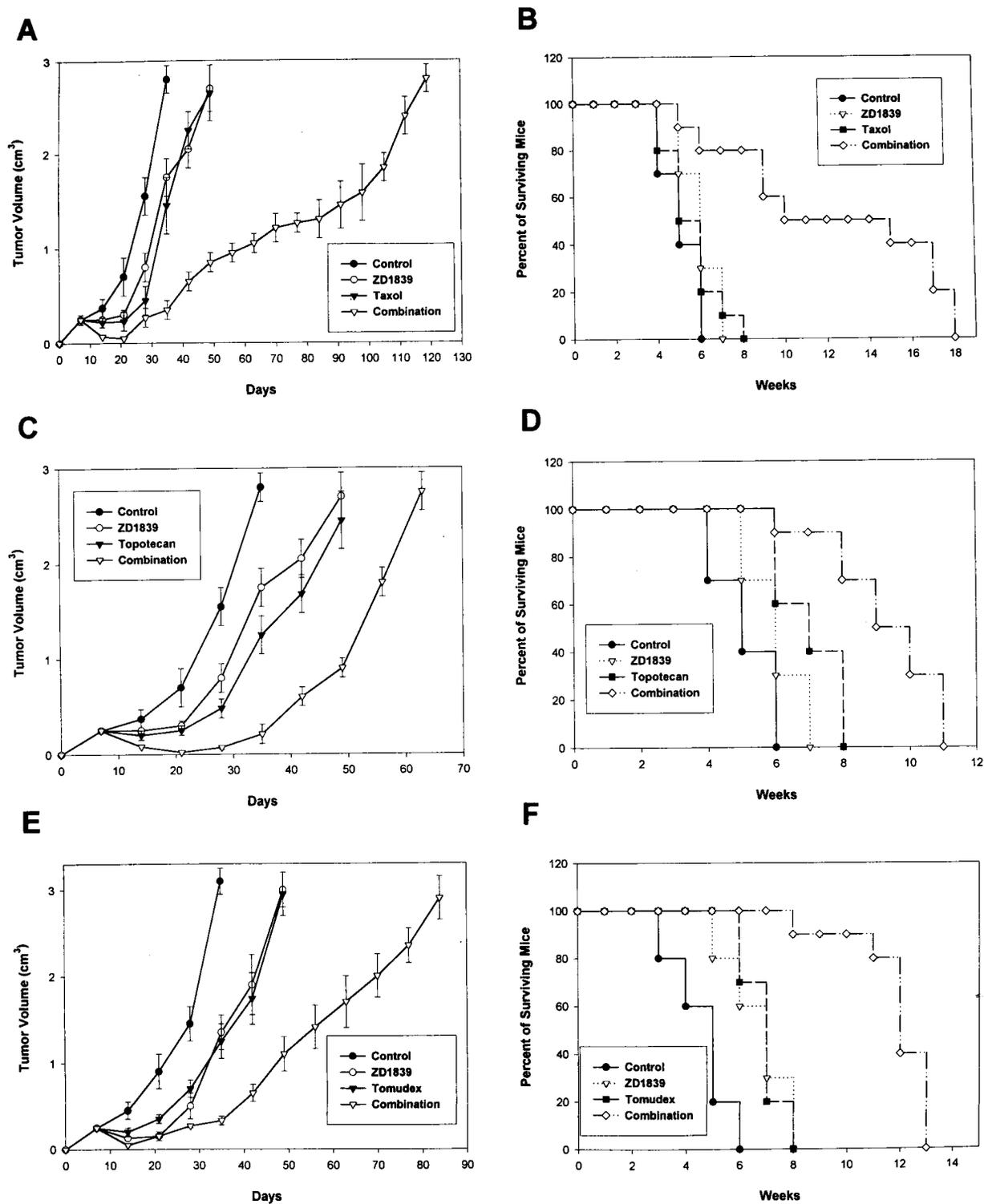


Fig. 8 Antitumor activity of ZD-1839 treatment in combination with cytotoxic drugs on established GEO human colon carcinoma xenografts. Mice were injected s.c. in the dorsal flank with 10^7 GEO cells. Three different experiments with a total of 40 mice for each experiment were performed. In each experiment, each group consisted of 10 mice. Data represent the averages; bars, SD. In each experiment, after 7 days (average tumor size, 0.2–0.3 cm³), the mice were treated i.p. on days 1–5 of each week for 4 weeks with ZD-1839, 2.5 mg/dose, alone or in combination with paclitaxel (Taxol; A), 20 mg/kg/dose, on day 1 of each week for 4 weeks; with topotecan (C), 2 mg/kg/dose, on day 1 of each week for 4 weeks; or with raltitrexed (Tomudex; E), 12.5 mg/kg/dose, on day 1 of each week for 4 weeks. For each experiment, the Student's *t* test was used to compare tumor sizes among different treatment groups at day 35 after GEO cell injection. Tumor sizes were significantly different between: ZD-1839 and control (two-sided $P = 0.01$); paclitaxel (Taxol) and control (two-sided $P = 0.01$); topotecan and control (two-sided $P = 0.01$); raltitrexed (Tomudex) and

lines tested. The growth inhibition induced by ZD-1839 was mainly cytostatic, although an increase in programmed cell death was observed with higher doses. Furthermore, ZD-1839 treatment had antitumor activity *in vivo* against established GEO cancer xenografts in immunodeficient mice. This effect was also dose dependent. At the end of a 4-week treatment period with the highest dose tested (5 mg/dose), GEO tumor growth was almost completely suppressed. ZD-1839 was well tolerated *in vivo*, because it could be administered to mice for at least 4 weeks without relevant toxicity. A reversible mild reduction in body weight was the only side effect observed with the highest dose used. However, the antitumor effect of ZD-1839 *in vivo* was reversible upon cessation of treatment. These results were similar to those obtained with the treatment of established human solid tumor xenografts with other types of anti-EGFR agents, such as EGFR blocking MAbs (13, 31–33).

We also evaluated the potential cooperative antiproliferative effect of the combined treatment with ZD-1839 and cytotoxic drugs. There was a significant dose-dependent potentiation of the proapoptotic effect and of the growth-inhibitory activity of all cytotoxic drugs tested by the EGFR tyrosine kinase inhibitor. Furthermore, mice were treated with weekly injections of cytotoxic drugs and ZD-1839 for 4 weeks in an attempt to develop treatment schedules of combined treatment suitable for testing in cancer patients. With this treatment schedule, an enhancement of antitumor activity was observed *in vivo* in mice bearing established GEO cancer xenografts when treated with ZD-1839 in combination with paclitaxel, topotecan, or raltitrexed. This effect was accompanied by significantly increased survival in the combined therapy group as compared with the groups treated with a single agent. In fact, in established GEO human colon carcinoma xenografts, treatment with each agent alone transiently inhibited tumor growth as the tumors resumed the growth rate of untreated controls after cessation of therapy. In contrast, the combined treatment with each cytotoxic agent and ZD-1839 produced tumor growth arrest in all mice; tumors grew slowly for approximately 4–8 weeks after the end of treatment, when they finally resumed a growth rate similar to controls. GEO tumors reached a size not compatible with normal life in all control mice within 4–6 weeks and in all single agent-treated mice within 6–8 weeks after GEO cell injection. In contrast, 50% of mice treated with ZD-1839 plus topotecan, raltitrexed, or paclitaxel were still alive 10, 12, and 15 weeks after cancer cell injection, respectively. The combined treatment with these selected doses of ZD-1839 and paclitaxel, topotecan,

or raltitrexed was well tolerated by mice without signs of toxicity. Therefore, in an attempt to increase mice survival and possibly to eradicate established tumors, it is conceivable that additional cycles of combined therapy could be administered.

The increase in cytotoxicity and in antitumor efficacy obtained by the blockade of EGFR activation with ZD-1839 treatment seems to be independent of the mechanism(s) of action of the chemotherapeutic agents used in combination. In fact, an enhanced effect was found with structurally and functionally different drugs, such as topoisomerase I- or topoisomerase II-selective inhibitors, taxanes, platinum-derived agents, and a thymidylate synthase inhibitor. These results are in agreement with and extend those of previous reports that have shown a cooperative antitumor activity for different cytotoxic drugs such as cisplatin, doxorubicin, paclitaxel, or topotecan when combined with antibodies generated against the EGFR or the closely related *c-erbB-2* type I tyrosine kinase receptor (31–42). Taken together, these studies support the hypothesis that cellular damage by chemotherapy can convert EGFR ligands from growth factors into survival factors for cancer cells that express functional EGFR (51). In this situation, the blockade of EGFR mitogenic signaling in combination with cytotoxic drugs could cause irreparable cancer cell damage, leading to programmed cell death (51).

The potentiation of the antitumor activity of cytotoxic drugs by interfering with EGFR activation may have important clinical implications. In fact, it has been proposed that it is possible to enhance anticancer activity by treatment with maximum tolerated doses of cytotoxic drugs in combination with signal transduction inhibitors instead of increasing chemotherapy doses to supertoxic levels that require complex medical support, including hematopoietic rescue (51). In this respect, the feasibility and antitumor activity of the combined treatment of cisplatin and MAb C225 in patients with advanced head and neck or lung carcinomas has been demonstrated (18). Similarly, blockade of *c-erbB-2* signaling by treatment with a recombinant humanized anti-*c-erbB-2* MAb (Herceptin) enhances the antitumor activity of cisplatin in metastatic breast cancer patients (40). Furthermore, a randomized Phase III trial has recently demonstrated that the addition of Herceptin in advanced breast cancer patients treated with paclitaxel or doxorubicin increases the activity of chemotherapy alone (42).

In summary, the results of the present study demonstrate the antitumor activity of ZD-1839 and provide a rationale for the evaluation of the anticancer activity of this EGFR-specific ty-

control (two-sided $P = 0.01$); ZD-1839 plus paclitaxel (Taxol) and control (two-sided $P < 0.001$); ZD-1839 plus paclitaxel (Taxol) and paclitaxel (Taxol) alone (two-sided $P = 0.01$); ZD-1839 plus paclitaxel (Taxol) and ZD-1839 alone (two-sided $P = 0.01$); ZD-1839 plus topotecan and control (two-sided $P < 0.001$); ZD-1839 plus topotecan and topotecan alone (two-sided $P = 0.01$); ZD-1839 plus topotecan and ZD-1839 alone (two-sided $P = 0.01$); ZD-1839 plus raltitrexed (Tomudex) and control (two-sided $P < 0.001$); ZD-1839 plus raltitrexed (Tomudex) and raltitrexed (Tomudex) alone (two-sided $P = 0.01$); and ZD-1839 plus raltitrexed (Tomudex) and ZD-1839 alone (two-sided $P = 0.01$). The effects of ZD-1839 treatment in combination with paclitaxel (Taxol; *B*), with topotecan (*D*), or with raltitrexed (Tomudex; *F*) on the survival of GEO tumor-bearing mice were also determined. Differences in animal survival among groups were evaluated using the Mantel-Cox log-rank test. The survival of mice was significantly different between: ZD-1839 plus Taxol and control (two-sided $P < 0.001$); ZD-1839 plus paclitaxel (Taxol) and paclitaxel (Taxol) alone (two-sided $P < 0.001$); ZD-1839 plus paclitaxel (Taxol) and ZD-1839 alone (two-sided $P < 0.001$); ZD-1839 plus topotecan and control (two-sided $P < 0.001$); ZD-1839 plus topotecan and topotecan alone (two-sided $P = 0.05$); ZD-1839 plus topotecan and ZD-1839 alone (two-sided $P = 0.02$); ZD-1839 plus raltitrexed (Tomudex) and control (two-sided $P < 0.001$); ZD-1839 plus raltitrexed (Tomudex) and raltitrexed (Tomudex) alone (two-sided $P = 0.01$); and ZD-1839 plus raltitrexed (Tomudex) and ZD-1839 alone (two-sided $P = 0.01$).

rosine kinase inhibitor alone and in combination with cytotoxic drugs in cancer patients with epithelial tumors that express functional EGFR. Preliminary results of two independent Phase I studies have been reported recently (52, 53). These studies have shown the feasibility of oral administration of ZD-1839 to cancer patients for prolonged periods with no major toxic effects at doses that allowed us to obtain steady-state plasma concentrations that are within the biologically effective concentrations demonstrated in the present study.

ACKNOWLEDGMENTS

We thank Dr. Steven Averbuch, AstraZeneca Pharmaceuticals, for the generous gift of ZD-1839 and for helpful discussions.

REFERENCES

- Salomon, D. S., Brandt, R., Ciardiello, F., and Normanno, N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit. Rev. Oncol. Hematol.*, *19*: 183–232, 1995.
- Pawson, T. Protein modules and signaling networks. *Nature (Lond.)*, *373*: 573–579, 1994.
- Fox, S. B., Smith, K., Hollyer, J., Greenall, M., Hastrich, D., and Harris, A. L. The epidermal growth factor receptor as a prognostic marker: results of 370 patients and review of 3009 patients. *Breast Cancer Res. Treat.*, *29*: 41–49, 1994.
- Wosikowski, K., Schuurhuis, D., Kops, G. J. P. L., Saceda, M., and Bates, S. E. Altered gene expression in drug-resistant human breast cancer cells. *Clin. Cancer Res.*, *3*: 2405–2414, 1997.
- Fan, Z., and Mendelsohn, J. Therapeutic application of anti-growth factor receptor antibodies. *Curr. Opin. Oncol.*, *10*: 67–73, 1998.
- Strawn, L. M., and Shawver, L. K. Tyrosine kinases in disease: overview of kinase inhibitors as therapeutic agents and current drugs in clinical trials. *Exp. Opin. Investig. Drugs*, *7*: 553–573, 1998.
- Baselga, J., and Mendelsohn, J. Type I receptor tyrosine kinases as targets for therapy in breast cancer. *J. Mammary Gland Biol. Neopl.*, *2*: 165–174, 1997.
- Mendelsohn, J. Epidermal growth factor receptor inhibition by a monoclonal antibody as anticancer therapy. *Clin. Cancer Res.*, *3*: 2703–2707, 1997.
- Masui, H., Kamrath, H., Apell, G., Houston, L. L., and Mendelsohn, J. Cytotoxicity against human tumor cells mediated by the conjugate of anti-epidermal growth factor receptor monoclonal antibody to recombinant ricin A chain. *Cancer Res.*, *49*: 3482–3488, 1989.
- Arteaga, C. L., Hurd, S. D., Dugger, T. C., Winnier, A. R., and Robertson, J. B. Epidermal growth factor receptors in human breast carcinoma cells: a potential selective target for transforming growth factor α -*Pseudomonas* exotoxin 40 fusion protein. *Cancer Res.*, *54*: 4703–4709, 1994.
- Kirk, J., Carmichael, J., Stratford, I. J., and Harris, A. L. Selective toxicity of TGF- α -PE40 to EGFR-positive cell lines: selective protection of low EGFR-expressing cell lines by EGF. *Br. J. Cancer*, *69*: 988–994, 1994.
- Schmidt, M., and Wels, W. Targeted inhibition of tumor cell growth by a bispecific single-chain toxin containing an antibody domain and TGF α . *Br. J. Cancer*, *74*: 853–862, 1996.
- Aboud-Pirak, E., Hurwitz, E., Pirak, M. E., Bellot, F., Schlessinger, J., and Sela, M. Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma *in vitro* and in nude mice. *J. Natl. Cancer Inst.*, *80*: 1605–1611, 1988.
- Modjtahedi, H., Eccles, S., Box, G., Styles, J., and Dean, C. Immunotherapy of human tumour xenografts overexpressing the EGF receptor with rat antibodies that block growth factor-receptor interaction. *Br. J. Cancer*, *67*: 254–261, 1993.
- Fry, D. W. Inhibition of the epidermal growth factor receptor family of tyrosine kinases as an approach to cancer chemotherapy: progression from reversible to irreversible inhibitors. *Pharmacol. Ther.*, *82*: 207–218, 1999.
- Goldstein, N. I., Prewett, M., Zuklys, K., Rockwell, P., and Mendelsohn, J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin. Cancer Res.*, *1*: 1311–1318, 1995.
- Anderson, V., Cooper, M., O'Chei, E., Gilly, J., Falcey, J., and Waksal, H. W. Dose dependent pharmacokinetics of a chimerized monoclonal antibody (C225) against EGFR. *Proc. Am. Assoc. Cancer Res.*, *39*: 3561, 1998.
- Mendelsohn, J., Shin, D. M., Donato, N., Khuri, F., Radinsky, R., Glisson, B. S., Shin, H. I., Metz, E., Pfister, D., Perez-Soler, R., Lawhorn, K., Matsumoto, T., Gunnett, K., Falcey, J., Waksal, H., and Hong, W. K. A Phase I study of chimerized anti-epidermal growth factor receptor (EGFr) monoclonal antibody, C225, in combination with cisplatin (CDDP) in patients (PTS) with recurrent head and neck squamous cell carcinoma (SCC). *Proc. Am. Soc. Clin. Oncol.*, *18*: 1502, 1999.
- Gunnett, K., Motzer, R., Amato, R., Todd, M., Poo, W.-J., Cohen, R., Baselga, J., Cooper, M., Robert, F., Falcey, J., and Waksal, H. Phase II study of anti-epidermal growth factor receptor (EGFr) antibody C225 alone in patients (pts) with metastatic renal cell carcinoma (RCC). *Proc. Am. Soc. Clin. Oncol.*, *18*: 1309, 1999.
- Fry, D. W., Kraker, A. J., McMichael, A., Ambrosio, L. A., Nelson, J. M., Leopold, W. R., Connors, R. W., and Bridges, A. J. A specific inhibitor of the epidermal growth factor receptor tyrosine kinase. *Science (Washington DC)*, *265*: 1093–1095, 1994.
- Buchdunger, E., Mett, H., Trinks, U., Regenass, U., Muller, M., Meyer, T., Beilstein, P., Wirz, B., Schneider, P., Traxler, P., and Lydon, N. 4,5-Bis(4-fluoroanilino)phthalimide: a selective inhibitor of the epidermal growth factor receptor signal transduction pathway with potent *in vivo* antitumor activity. *Clin. Cancer Res.*, *1*: 813–821, 1995.
- Moyer, J. D., Barbacci, E. G., Iwata, K. K., Arnold, L., Boman, B., Cunningham, A., DiOrto, C., Doty, J., Morin, M., Moyer, M. P., Neveu, M., Pollack, V. A., Pustilnik, L. R., Reynolds, M. M., Sloan, D., Theleman, A., and Miller, P. Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. *Cancer Res.*, *57*: 4838–4848, 1997.
- Bos, M., Mendelsohn, J., Kim, Y.-M., Albanell, J., Fry, D. W., and Baselga, J. PD153035, a tyrosine kinase inhibitor, prevents epidermal growth factor receptor activation and inhibits growth of cancer cells in a receptor number-dependent manner. *Clin. Cancer Res.*, *3*: 2099–2106, 1997.
- Baguley, B. C., Marshall, E. S., Holdaway, K. M., Rewcastle, G. W., and Denny, W. A. Inhibition of growth of primary human tumour cell cultures by a 4-anilinoquinazoline inhibitor of the epidermal growth factor receptor family of tyrosine kinases. *Eur. J. Cancer*, *34*: 1086–1090, 1998.
- Simpson, B. J., Bartlett, J. M., Macleod, K. G., Rabiasz, G., Miller, E. P., Rae, A. L., Gordge, P., Leake, R. E., Miller, W. R., Smyth, J., and Langdon, S. P. Inhibition of transforming growth factor α (TGF- α)-mediated growth effects in ovarian cancer cell lines by a tyrosine kinase inhibitor ZM 252868. *Br. J. Cancer*, *79*: 1098–1103, 1999.
- Wakeling, A. E., Barker, A. J., Davies, D. H., Brown, D. S., Green, L. R., Cartledge, S. A., and Woodburn, J. R. Specific inhibition of epidermal growth factor receptor tyrosine kinase by 4-anilinoquinazolines. *Breast Cancer Res. Treat.*, *38*: 67–73, 1996.
- Woodburn, J. R., Barker, A. J., Gibson, K. H., Ashton, S. E., Wakeling, A. E., Curry, B. J., Scarlett, L., and Henthorn, L. R. ZD1839, an epidermal growth factor receptor tyrosine kinase inhibitor selected for clinical development. *Proc. Am. Assoc. Cancer Res.*, *38*: 4251, 1997.
- Sun, Y., Fry, D. W., Vincent, P., Nelson, J. M., Elliott, W., and Leopold, W. R. Growth inhibition of nasopharyngeal carcinoma cells by EGF receptor tyrosine kinase inhibitors. *Anticancer Res.*, *19*: 919–924, 1999.
- Dinney, C. P. N., Parker, C., Dong, Z., Fan, D., Eve, B. Y., Bucana, C., and Radinsky, R. Therapy of human transitional cell carcinoma of the bladder by oral administration of the epidermal growth factor re-

- ceptor protein tyrosine kinase inhibitor 4,5-dianilinophthalimide. *Clin. Cancer Res.*, 3: 161–168, 1997.
30. Hammond, L., Ranson, M., Ferry, D., Kris, M., Kelly, H., Ochs, J., Averbuch, S., and Rowinsky, E. ZD1839, an oral epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor: first Phase I, pharmacokinetic (PK) results in patients. *Proc. Am. Soc. Clin. Oncol.*, 18: 1500, 1999.
 31. Fan, Z., Baselga, J., Masui, H., and Mendelsohn, J. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus *cis*-diamminedichloroplatinum on well established A431 cell xenografts. *Cancer Res.*, 53: 4637–4642, 1992.
 32. Baselga, J., Norton, L., Masui, H., Pandiella, A., Coplan, K., Miller, W. H., and Mendelsohn, J. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. *J. Natl. Cancer Inst.*, 85: 1327–1333, 1993.
 33. Ciardiello, F., Bianco, R., Damiano, V., De Lorenzo, S., Pepe, S., De Placido, S., Fan, Z., Mendelsohn, J., Bianco, A. R., and Tortora, G. Antitumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225. *Clin. Cancer Res.*, 5: 909–916, 1999.
 34. Tortora, G., Caputo, R., Pomato, G., Pepe, S., Bianco, A. R., Agrawal, S., Mendelsohn, J., and Ciardiello, F. Cooperative inhibitory effect of novel mixed backbone oligonucleotide targeting protein kinase A in combination with docetaxel and anti-EGF-receptor antibody on human breast cancer cell growth. *Clin. Cancer Res.*, 5: 875–881, 1999.
 35. Hoffmann, T., Hafner, D., Ballo, H., Haas, I., and Bier, H. Antitumor activity of anti-epidermal growth factor receptor monoclonal antibodies and cisplatin in ten human head and neck squamous cell carcinoma lines. *Anticancer Res.*, 17: 4419–4425, 1997.
 36. Hancock, M. C., Langton, B. C., Chan, T., Toy, P., Monhanan, J. J., Mischak, R. P., and Shawver, L. K. A monoclonal antibody against the c-erbB-2 protein enhances the cytotoxicity of *cis*-diamminedichloroplatinum against human breast and ovarian tumor cell lines. *Cancer Res.*, 51: 4574–4580, 1991.
 37. Arteaga, C. L., Winnier, A. R., Poirer, M. C., Lopez-Larrazza, D. M., Shawer, L. K., Hurd, S. D., and Stewart, S. J. p185c-erbB-2 signaling enhances cisplatin-induced cytotoxicity in human breast carcinoma cells: association between an oncogenic receptor tyrosine kinase and drug-induced DNA repair. *Cancer Res.*, 54: 3758–3765, 1994.
 38. Pietras, R. J., Fendly, B. M., Chazin, V. R., Pegram, M. D., Howell, S. B., and Slamon, D. J. Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene*, 9: 1829–1838, 1994.
 39. Pietras, R. J., Pegram, M. D., Finn, R. S., Maneval, D. A., and Slamon, D. J. Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. *Oncogene*, 17: 2235–2249, 1998.
 40. Baselga, J., Norton, L., Albanell, J., Kim, Y.-M., and Mendelsohn, J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/*neu* overexpressing human breast cancer xenografts. *Cancer Res.*, 58: 2825–2831, 1998.
 41. Pegram, M. D., Lipton, A., Hayes, D. F., Weber, B. L., Baselga, J., Tripathy, D., Baly, D., Baughman, S. A., Twaddell, T., Glaspy, J. A., and Slamon, D. J. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185^{HER2}/*neu* monoclonal antibody plus cisplatin in patients with HER2/*neu*-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J. Clin. Oncol.*, 16: 2659–2671, 1998.
 42. Slamon, D. J., Leyland-Jones, B., Shak, S., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Volter, J., Baselga, J., and Norton, L. Addition of Herceptin (humanized anti-HER2 antibody) to first line chemotherapy for HER2 overexpressing metastatic breast cancer markedly increases anticancer activity: a randomized multinational controlled Phase III trial. *Proc. Am. Soc. Clin. Oncol.*, 17: 377, 1998.
 43. Ciardiello, F., McGeady, M. L., Kim, N., Basolo, F., Hynes, N. E., Langton, B., Yokozaki, H., Saeki, T., Elliott, J., Masui, H., Mendelsohn, J., Soule, H., Russo, J., and Salomon, D. S. Transforming growth factor- α expression is enhanced in human mammary epithelial cells transformed by an activated c-Ha-*ras* protooncogene but not by the c-*neu* protooncogene, and overexpression of the transforming growth factor- α complementary DNA leads to transformation. *Cell Growth Differ.*, 1: 407–420, 1990.
 44. Tortora, G., Damiano, V., Bianco, C., Baldassarre, G., Bianco, A. R., Lanfrancone, L., Pelicci, P. G., and Ciardiello, F. The RI α subunit of protein kinase A (PKA) binds to Grb2 and allows PKA interaction with the activated EGF-receptor. *Oncogene*, 14: 923–928, 1997.
 45. De Luca, A., Casamassimi, A., Selvam, M. P., Losito, S., Ciardiello, F., Agrawal, S., Salomon, D. S., and Normanno, N. EGF-related peptides are involved in the proliferation and survival of MDA-MB-468 human breast carcinoma cells. *Int. J. Cancer*, 80: 589–594, 1999.
 46. Fleiss, J. L. *Statistical Methods for Rates and Proportions*, Ed. 2. New York: John Wiley & Sons, Inc., 1981.
 47. Mantel, N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother. Rep.*, 50: 163–170, 1966.
 48. Wu, X., Fan, Z., Masui, H., Rosen, N., and Mendelsohn, J. Apoptosis induced by an anti-epidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin. *J. Clin. Investig.*, 95: 1897–1905, 1995.
 49. Ciardiello, F., Damiano, V., Bianco, R., Bianco, C., Fontanini, G., De Laurentiis, M., De Placido, S., Mendelsohn, J., Bianco, A. R., and Tortora, G. Antitumor activity of combined blockade of epidermal growth factor receptor and protein kinase A. *J. Natl. Cancer Inst.*, 88: 1770–1776, 1996.
 50. Tortora, G., Caputo, R., Damiano, V., Bianco, R., Pepe, S., Bianco, A. R., Jiang, Z., Agrawal, S., and Ciardiello, F. Synergistic inhibition of human cancer cell growth by cytotoxic drugs and mixed backbone antisense oligonucleotide targeting protein kinase A. *Proc. Natl. Acad. Sci. USA*, 94: 12586–12591, 1997.
 51. Mendelsohn, J., and Fan, Z. Epidermal growth factor receptor family and chemosensitization. *J. Natl. Cancer Inst.*, 89: 341–343, 1997.
 52. Baselga, J., LoRusso, P., Herbst, R., Rischin, D., Ranson, M., Maddox, A.-M., Feyereislova, A., and Averbuch, S. A pharmacokinetic/pharmacodynamic trial of ZD1839 (“Iressa”), a novel oral epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitor, in patients with five selected tumor types (a Phase I/II trial of continuous once-daily treatment). AACR-NCI-EORTC Meeting on Molecular Targets and Cancer Therapeutics, abstract 29. *Clin. Cancer Res.*, 5: 3735s, 1999.
 53. Kris, M., Ranson, M., Ferry, D., Hammond, L., Averbuch, S., Ochs, J., and Rowinsky, E. Phase I study of oral ZD1839 (“Iressa”), a novel inhibitor of epidermal growth factor receptor tyrosine kinase (EGFR-TK): evidence of good tolerability and activity. AACR-NCI-EORTC Meeting on Molecular Targets and Cancer Therapeutics, abstract 99. *Clin. Cancer Res.*, 5: 3749s–3750s, 1999.

Clinical Cancer Research

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

Fortunato Ciardiello, Rosa Caputo, Roberto Bianco, et al.

Clin Cancer Res 2000;6:2053-2063.

Updated version Access the most recent version of this article at:
<http://clincancerres.aacrjournals.org/content/6/5/2053>

Cited articles This article cites 48 articles, 18 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/6/5/2053.full#ref-list-1>

Citing articles This article has been cited by 100 HighWire-hosted articles. Access the articles at:
<http://clincancerres.aacrjournals.org/content/6/5/2053.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://clincancerres.aacrjournals.org/content/6/5/2053>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.