

Pulsatile Administration of the Epidermal Growth Factor Receptor Inhibitor Gefitinib Is Significantly More Effective than Continuous Dosing for Sensitizing Tumors to Paclitaxel

David B. Solit,^{1,2} Yuhong She,² Jose Lobo,²
Mark G. Kris,¹ Howard I. Scher,¹ Neal Rosen,^{1,2}
and Frank M. Sirotnak²

Departments of ¹Medicine and ²Molecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center, New York, New York

ABSTRACT

Purpose: Gefitinib is an inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase. Continuous inhibition of EGFR signaling is thought necessary for optimal inhibition of tumor cell proliferation. We hypothesized that continuous gefitinib may antagonize the effects of cytotoxics that inhibit tumor cells in other phases of the cell cycle. Furthermore, we hypothesized that intermittent dosing would allow for dose escalation and greater inhibition of EGFR-dependent antiapoptotic pathways.

Experimental Design: To test these assertions, we compared combinations of paclitaxel and gefitinib using either intermittent or continuous dosing schedules in mice.

Results: We found that when used in combination with paclitaxel, pulsatile gefitinib was significantly superior to continuous dosing. When gefitinib was administered for one or two consecutive days before paclitaxel, much higher doses could be given safely. Two days of gefitinib treatment before paclitaxel was most effective, causing significantly greater mean tumor regression and a higher percentage of complete responses than other schedules.

Conclusions: The results suggest that the dose and schedule of an EGFR inhibitor required to effectively inhibit proliferation may differ from that required to stimulate apoptosis or to induce other effects. The former may require continuous EGFR inhibition to maintain cell cycle arrest, whereas sensitization to apoptosis may be optimally induced

by profound but temporary inhibition of survival pathways. Our data suggest that the effects of receptor inhibition vary as a function of dose and schedule and that continuous administration of tyrosine kinase inhibitors may not be the best schedule with which to combine such agents with taxanes.

INTRODUCTION

The HER family of receptor tyrosine kinases, which includes epidermal growth factor receptor (EGFR), HER2, HER3, and HER4, is often overexpressed, amplified, or mutated in human epithelial malignancies and probably plays a pathogenic role in some tumors (1, 2). HER kinase activation may deregulate growth, desensitize cells to apoptotic stimuli, and regulate angiogenesis (3, 4).

Gefitinib (Iressa, ZD1839) is an orally active EGFR tyrosine kinase inhibitor (5). At higher concentrations than those that inhibit EGFR, gefitinib also inhibits HER2. Gefitinib inhibits the growth of several cancer cell lines and xenografts but its clinical activity does not correlate with EGFR expression (4–9). In phase I/II trials of this agent, significant tumor regressions were observed in 10% to 20% of patients with chemotherapy-refractory non-small cell lung cancer (10, 11). Based on these observations, gefitinib was approved by the Food and Drug Administration for use in this setting. Recently, somatic mutations in the EGFR gene have been identified in tumors of patients with non-small cell lung cancer who responded to gefitinib (12, 13). Preliminary data suggest that these mutations increase the affinity of gefitinib for the receptor and that they correlate with response to this agent. A second common EGFR mutation formed by an in-frame deletion and insertion of glycine at the fusion junction of the extracellular domain (EGFRvIII) is found in patients with glioblastoma (14). This mutation leads to constitutive receptor dimerization and, therefore, kinase activation. Although clinical activity has been reported with gefitinib in patients with glioblastoma, the predictive value of the EGFRvIII mutation for response to this agent remains unknown (15).

Anti-EGFR (cetuximab, Erbitux) and anti-HER2 (trastuzumab, Herceptin) antibodies have been shown to enhance the activity of cytotoxic chemotherapies in animal models (16, 17). These preclinical findings prompted clinical studies in which Herceptin was combined with paclitaxel (breast cancer) and Erbitux with irinotecan (colon cancer). These studies show that the trastuzumab/paclitaxel combination is significantly more effective than either agent alone in the treatment of breast cancer patients with HER2 amplification and the cetuximab/irinotecan combination is active in patients with irinotecan-refractory colon cancer (18, 19).

Received 7/8/04; revised 11/18/04; accepted 12/7/04.

Grant support: NIH grant P50-CA92629-01, William H. Goodwin and Alice Goodwin Foundation for Research, the MSKCC Experimental Therapeutics Program, and American Society of Clinical Oncology Career Development Award (D.B. Solit).

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.

Note: D.B. Solit, Y. She, N. Rosen, and F.M. Sirotnak contributed equally to this work.

Requests for reprints: David B. Solit, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: 646-422-4459; Fax: 253-423-3415; E-mail: solitd@mskcc.org.

©2005 American Association for Cancer Research.

In mice, gefitinib has also been shown to synergize with cytotoxic chemotherapies (7). Based on these results, two large phase III clinical trials of gefitinib and chemotherapy were initiated in patients with non–small cell lung cancer, but in both cases, these studies did not show an advantage of the combination over chemotherapy alone (20, 21). Studies of the EGFR inhibitor erlotinib (Tarceva) and chemotherapy also failed to show any advantage of the combination over single-agent therapy (22). Because gefitinib causes G₁ growth arrest of EGFR-dependent tumor cells, we hypothesized that continuous administration of this agent may attenuate the effects of tubulin inhibitors such as paclitaxel that arrest cells in mitosis. We therefore treated xenograft-bearing mice with the paclitaxel/ gefitinib combination using both pulsatile and continuous dosing schedules. We found that the maximally tolerated dose (MTD) of gefitinib was higher when administered intermittently and that pulsatile dosing was significantly more active than continuous therapy.

MATERIALS AND METHODS

Materials. Gefitinib (ZD1839, Iressa) was obtained from AstraZeneca Pharmaceuticals (Cheshire, United Kingdom). For cell culture studies, the drug was dissolved in DMSO and stored at –20°C. The following antibodies were used for immunoblotting: Akt, P-Akt (Cell Signaling, Beverly, MA), EGFR, HER2, and HER3 (Santa Cruz Biotechnology, Santa Cruz, CA).

Cell Culture. The human cancer cell lines BT-474, A431, and SK-LC-16 (American Type Culture Collection, Manassas, VA) and CWR22rv1 (Dr. Thomas Pretlow, Case Western Reserve University, Cleveland, OH) were maintained in a 1:1 mixture of DMEM:F-12 or RPMI supplemented with 2 mmol/L glutamine, 50 units/mL penicillin, 50 units/mL streptomycin, and 10% heat-inactivated fetal bovine serum (Gemini Bioproducts, Calabasa, CA) and incubated at 37°C in 5%CO₂.

Animal Studies. Four- to six-week-old *nu/nu* athymic female mice were obtained from the National Cancer Institute-Frederick Cancer Center and maintained in ventilated caging. Experiments were carried out under an Institutional Animal Use and Care Committee–approved protocol and institutional guidelines for the proper and humane use of animals in research were followed. BT-474 tumors were generated by injecting 1×10^7 BT-474 tumor cells together with reconstituted basement membrane (Matrigel, Collaborative Research, Bedford, MA). Before tumor cell inoculation, 0.72 mg/d 17β-estradiol pellets (Innovative Research of America, Sarasota, FL) were inserted s.c. in the left flank. All other tumor models were maintained by s.c. transplantation as follows: after growth of a seed tumor, a cell suspension in RPMI medium was prepared from the excised tumor and centrifuged for 5 minutes at $1000 \times g$, and the pellet was mixed with Matrigel and aliquots of tumor cell suspension were then implanted in a group of mice.

When tumors reached 5 to 6 mm in diameter, mice were randomized among control and the various treatment groups. A stock solution of paclitaxel was prepared in a 1:1 solution of Cremophor EL (Sigma, St. Louis, MO) and ethanol at a concentration of 10 mg/kg. This solution was held at –4°C for

no longer than 2 weeks and was diluted with saline before injection. Paclitaxel was given weekly by i.p. injection. Gefitinib was dissolved in distilled water with 0.5% lactic acid (85%, Fisher Scientific, Pittsburgh, PA) to prepare a 40 mg/mL stock solution, which was stored until use at 4°C. Gefitinib was given by p.o. gavage. Mice were sacrificed by CO₂ euthanasia. The average tumor diameter (two perpendicular axes of the tumor were measured) was measured in control and treated groups by caliper. The data are expressed as the increase or decrease in tumor volume in cubic millimeters ($\text{mm}^3 = 4/3\pi r^3$). Differences between treatment arms were analyzed using the Wilcoxon rank sum test using the SAS 8.2 program.

Immunoblotting. To prepare lysates from xenograft tumors, flash-frozen tumor tissue was homogenized in modified radioimmunoprecipitation assay buffer (0.1% SDS, 1% NP40, 1% sodium deoxycholate, 10% glycerol, 1 mmol/L EDTA, 1 mmol/L β-glycerolphosphate, 2.5 mmol/L sodium pyrophosphate, 2.5 mmol/L sodium orthovanadate, 10 mmol/L phenylmethylsulfonyl fluoride, and 10 μmol/L each leupeptin, aprotinin, and soybean trypsin inhibitor). Lysates were cleared by centrifugation, separated by SDS-PAGE, transferred to membrane, and immunoblotted using specific primary and secondary antibodies.

Immunohistochemistry. For immunohistochemical studies, xenograft tumors were washed with PBS and then fixed overnight in paraformaldehyde followed by dehydration in graded ethanols. Tissues were embedded in paraffin and sectioned at a thickness of 5 to 8 μm. To optimize antigen retrieval, slides were treated with 10 mmol/L citric acid at 100°C for 15 minutes. Immunohistochemical staining was done with antibodies directed against the following antigens: cleaved caspase 3 (Cell Signaling Technology, Beverly, MA) at 1 μg/mL and phospho-histone H3 (Upstate Cell Signaling Solutions, Lake Placid, NY) at 5 μg/mL.

RESULTS

To test whether intermittent gefitinib treatment was more effective than continuous therapy, we treated mice bearing BT-474 and MX-1 breast cancer xenografts with gefitinib, paclitaxel, or the combination in one of several possible sequences. The BT-474 cell line was chosen as the initial cell line for these experiments because this cell line is sensitive to gefitinib (IC₅₀ of 0.3 μmol/L) and exposure to gefitinib causes them to undergo arrest in G₁ (ref. 5; Fig. 1). At the time these studies were initiated, EGFR mutations in patients with non–small cell lung cancer had not yet been identified (12, 13). We have since tested and confirmed that the EGFR in BT-474 is wild-type and does not contain the EGFR mutations that are associated with response to gefitinib alone in patients with non–small cell lung cancer (23).³

In some tumors with overexpressed or amplified EGFR or HER2, G₁ progression is regulated by cyclin D/cdk4/6 activity in a HER kinase–dependent manner (24). We therefore assessed by immunoblot whether treatment with gefitinib could cause down-regulation of cyclin D1 in tumor

³ W. Pao, personal communication.

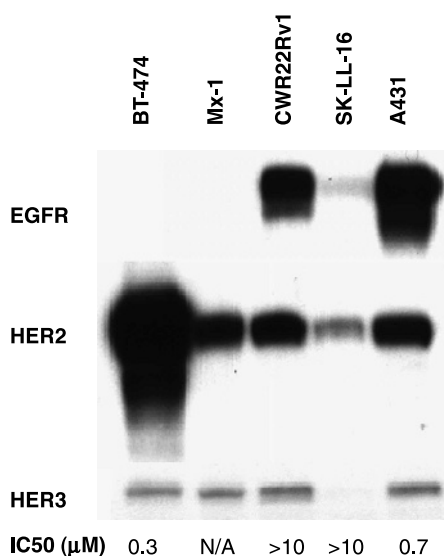


Fig. 1 HER kinase expression of BT-474, MX-1, CWR22rv1, SK-LC-16, and A431 xenograft tumors. BT-474 and A431 are sensitive to gefitinib in cell culture with IC₅₀ values <1 µmol/L, whereas both CWR22rv1 and SK-LC-16 are highly resistant with IC₅₀ values >10 µmol/L. MX-1 grows only as xenografts.

xenografts. Twelve hours after treatment with a single dose, gefitinib (200 or 500 mg/kg) caused inactivation of mitogen-activated protein kinase and Akt and down-regulation of cyclin D1 (Fig. 2; data not shown). Inhibition of these pathways was temporary and, by 24 hours, levels of P-Akt, P-MAPK and cyclin D had returned to baseline (Fig. 2). Retreatment with a second dose of gefitinib at 24 hours led to a more prolonged effect on Akt activity and cyclin D1 expression (data not shown). HER2 expression in addition to activity was also found to be down-regulated after gefitinib treatment (Fig. 2). Down-regulation of HER2 expression in cell culture model systems by HER kinase inhibitors has been reported previously and is the result of enhanced ubiquitylation and proteasomal degradation (25).

For efficacy studies, we used as our standard arm gefitinib (65 mg/kg) for five consecutive days each week for 3 weeks with paclitaxel weekly on day 3. Using this schedule, we previously reported that gefitinib enhanced the activity of several cytotoxic agents in a broad range of tumor types (7). Due to enhanced toxicity, the dose of gefitinib needed to be reduced from 150 to 65 mg/kg when this agent was used in combination with paclitaxel (7). Comparing this schedule to gefitinib given at the same dose (65 mg/kg) either pre- (days -1 and -2) or postpaclitaxel (days +1 and +2), we found that pretreatment with gefitinib was the most effective (Table 1). The improved efficacy of 2 days of pretreatment with gefitinib before paclitaxel was observed despite the lower total dose given (130 versus 325 mg/wk). A further increase in efficacy, including the induction of complete regressions (25%), was achieved by increasing the dose of gefitinib to 150 mg/kg × 2 days before paclitaxel (total weekly dose, 300 mg/kg). No benefit over paclitaxel alone was seen in mice treated with paclitaxel first followed by 2 days of gefitinib (Table 1).

Similar results were observed using a second breast cancer xenograft model (MX-1), which also expresses wild-type EGFR. Using the MX-1 tumor model, we again observed that pretreatment with gefitinib using an intermittent dosing schedule was superior to continuous dosing and that gefitinib dose escalation using the intermittent schedule was well tolerated and resulted in even greater activity (Table 2).

To determine the full benefit of intermittent dosing, we sought to define the MTD of gefitinib when dosed either once or twice weekly in combination with paclitaxel. We found that when used alone, intermittent gefitinib was less toxic than continuous dosing and that doses of up to 300 mg/kg twice weekly were well tolerated (Fig. 3). Doses of 500 mg/kg twice weekly and higher resulted in unacceptable weight loss or death. When combined with paclitaxel, the MTD of gefitinib (300 mg/kg) once or twice weekly was 4–5 fold higher than the MTD when given daily (65 mg/kg).

We examined whether the increased dose of gefitinib allowed by the intermittent schedule conferred an advantage in tumor models with intermediate (CWR22rv1, prostate) and high (SK-LC-16, non-small cell lung cancer) levels of paclitaxel resistance. SK-LC-16 expresses wild-type EGFR and is resistant to gefitinib alone in cell culture and *in vivo*. Gefitinib was given at the schedule-specific MTDs (65 mg/kg for the continuous schedule and 250 mg/kg for the intermittent schedule). In contrast to BT-474 and MX-1, both SK-LC-16 and CWR22rv1 were highly resistant to gefitinib as a single agent. As was observed with MX-1 and BT-474, pretreatment with gefitinib using an intermittent schedule was much more effective than continuous gefitinib in enhancing the antitumor activity of paclitaxel, whereas as a single agent, gefitinib using either schedule was inactive in this model system (Fig. 4; data not shown). The improvement with pulsatile dosing was

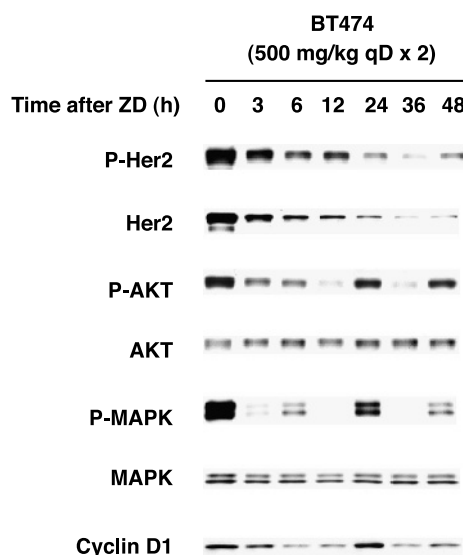


Fig. 2 Gefitinib inhibits MAP kinase and Akt activity and causes down-regulation of cyclin D1 expression. Mice with established xenograft tumors were treated with a single dose of gefitinib (500 mg/kg) and sacrificed from 3 to 24 hours later. The mice sacrificed at 36 and 48 hours were treated with two doses of gefitinib at time 0 and a second dose 24 hours after the first.

Table 1 Treatment with paclitaxel and ZD1839 given on different schedules in nude mice bearing BT-474 human mammary carcinoma xenografts

Treatment, MTD	Schedule for ZD1839	Average weight change (%)	Average tumor diameter* (mm), mean \pm SE	Average tumor volume (mm ³)	Complete regressions, n/total
Control		-3	10.6 \pm 2	629	0/8
ZD 150	qd \times 5 \times 3	-6	8.8 \pm 2	357	0/8
PTXL35		-5	6.0 \pm 1	113	0/8
PTXL35 + ZD65	qd \times 5 \times 3	-6	5.0 \pm 1.3	66 (-39%)	0/8
PTXL35 + ZD65	Day -1, 2 \times 3	-4	4.3 \pm 1	40 (-63%)	0/8
PTXL35 + ZD150	Day -1, 2 \times 3	-4	3.3 \pm 1	19 (-86%)	2/8
PTXL35 + ZD65	Day +1, 2 \times 3	-3	6.4 \pm 1.4	137	0/8

NOTE. The data reflect two experiments of four mice per group. Paclitaxel given qw \times 3 i.p. ZD1839 given p.o. The data presented are at the combination nadir, which represented the first measurement after completion of drug therapy.

Abbreviations: ZD, ZD1839; PTXL, paclitaxel; qd, every day.

*Initial tumor diameter = 5.9 \pm 1 (109 mm³).

manifested as both greater mean absolute tumor regressions in the pulsatile arm (SK-LC-16: 70% mean tumor regression with pulsatile gefitinib versus 460% mean tumor growth for continuous, $P < 0.0001$) and by an increase in the fraction of tumor-free mice after treatment (SK-LC-16: 5 of 13 complete responses with pulsatile gefitinib versus 1 of 13 for continuous, $P = 0.01$).

Because SK-LC-16 was highly resistant to gefitinib alone using all schedules, we did additional studies of gefitinib alone using BT-474 to determine whether pulsatile dosing as a single agent was more effective than continuous dosing in a gefitinib-sensitive model. We found that when used as a single agent, pulsatile gefitinib (400 mg/kg on days 1 and 2 weekly \times 3 weeks) was no more effective than continuous dosing (150 mg/kg 5 \times a week \times 3 weeks) with average treatment to control volume ratios of 0.65 for pulsatile dosing and 0.59 for continuous dosing (day 22 after initiation of treatment, $P > 0.1$).

Immunohistochemical analysis was done on tumors from mice sacrificed 12 and 24 hours following the week 2 paclitaxel treatment. In paclitaxel- and paclitaxel plus gefitinib-treated mice, evidence of mitotic block was observed with an increase in staining for phosphorylated histone H3 compared with control tumors or tumors treated with gefitinib alone. Large areas of necrosis were observed in tumors from animals treated with paclitaxel alone or with the combination, although no significant

increase in apoptosis was observed at this particular time point. As we have reported previously, gefitinib enhancement did not correlate with the expression of EGFR or other HER kinases (Fig. 1). In addition, enhanced activity with the combination was observed in tumor models sensitive (BT-474 and MX-1) and in those resistant (SK-LC-16 and CWR22rv1) to gefitinib as a single agent.

DISCUSSION

We show in this article that pulsatile administration of high doses of the EGFR tyrosine kinase inhibitor gefitinib (Iressa, ZD1839) before paclitaxel has significantly greater antitumor activity compared with either drug alone or to the combination of daily gefitinib and paclitaxel. In contrast, when gefitinib is administered as a single agent, the antitumor activity of the pulsatile schedule is not superior. These data suggest that by whatever mechanism, the dose and schedule required to elicit antiproliferative effects with this drug are different from those required to sensitize tumor cells to paclitaxel. We have begun a clinical trial based on this concept to determine its therapeutic relevance.

When used in combination with paclitaxel, intermittent treatment may be more effective for several reasons. First, intermittent therapy alters the toxicity profile of the drug, thus allowing for gefitinib dose escalation. Given the role of EGFR

Table 2 Treatment with PTXL and ZD1839 given using different schedules in nude mice bearing MX-1 human mammary carcinoma xenografts

Treatment, MTD	Schedule for ZD1839	Average weight change (%)	Average tumor diameter* (mm), mean \pm SE	Average tumor volume (mm ³)	Complete regressions, n/total
Control		+2	15.1 \pm 2	1602	0/12
ZD 150	qd \times 5 \times 3	-2	11.4 \pm 2	780	0/12
PTXL35		-3	5.6 \pm 1	52	0/12
PTXL35 + ZD65	qd \times 5 \times 3	-4	2.1 \pm 0.5	5	5/12
PTXL35 + ZD65	Day -1, 2 \times 3	-3	<1	<1	9/12
PTXL35 + ZD65	Day -1 \times 3	-2	3.4 \pm 1	21	1/4
PTXL35 + ZD65	Day +1, 2 \times 3	-4	3.5 \pm 1	23	2/8
PTXL35 + ZD150	Day -1 \times 3	-3	<1	<1	3/4

NOTE. Three experiments of four mice per group. Paclitaxel given qw \times 3 i.p. ZD1839 given p.o. The data presented are at the combination nadir, which represented the first measurement following completion of drug therapy.

*Initial tumor diameter = 6.4 \pm 0.4 (127 mm³).

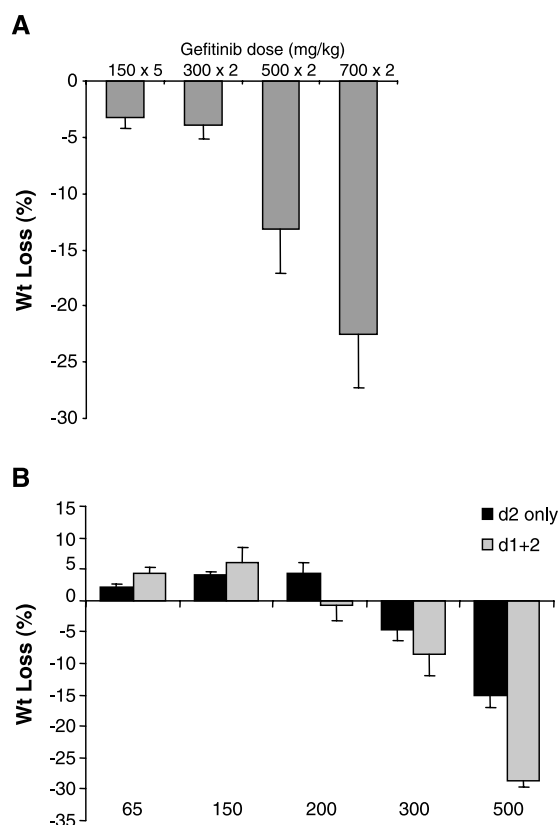


Fig. 3 Tolerability of gefitinib as a function of dose and schedule. Gefitinib-associated toxicities included weight loss (*Wt Loss*), skin rash, and diarrhea.

in activating prosurvival/ antiapoptotic pathways, the increased dose intensity of gefitinib therapy may result in more profound inhibition of such pathways and, thus, greater sensitization to chemotherapy. Whereas inhibition of Ras/mitogen-activated protein kinase and Akt activity was seen in BT-474 and A431 tumors (data not shown) treated with gefitinib, such effects were not seen in all cell lines tested and therefore the particular downstream target responsible for sensitizing to chemotherapy remains unknown and may vary as a function of the complement of mutations found in different tumor types. Because all the xenograft models used in this study expressed wild-type EGFR, it is possible that gefitinib dose escalation is more relevant in such tumors and would provide little additional benefit in tumors with EGFR kinase domain mutations in which the binding affinity of gefitinib for EGFR is 6- to 10-fold higher (13).

Second, the improved efficacy with pulsatile gefitinib treatment may be in part due to antagonism between continuous gefitinib treatment and paclitaxel. In some tumor types, treatment with gefitinib leads to down-regulation of D cyclin expression and G_1 growth arrest. Paclitaxel exerts its antitumor effects by stabilizing microtubules, and thus its cytotoxic effects occur during mitosis (26). Therefore, pretreatment with a cytostatic agent such as gefitinib before paclitaxel may protect cells from paclitaxel-induced apoptosis by preventing their entry into the mitotic phase of the cell cycle. Such sequence-specific

antagonism has been reported in cell culture model systems with EGFR inhibitors and other biological agents that cause G_1 growth arrest (27, 28). It is likely that the contribution of such antagonism to the overall response to the paclitaxel-gefitinib combination is also cell line dependent. This is because sensitization was observed in tumors such as SK-LC-16 and CWR22rv1, which are highly resistant to gefitinib alone, and suggests that the optimal timing of gefitinib and paclitaxel administration may vary as a function of the sensitivity of an individual patient's tumor to gefitinib.

The improved efficacy of the dose-escalated intermittent gefitinib-paclitaxel combination may also be due to non-EGFR or non-tumor-cell-specific mechanisms. Specifically, higher doses of gefitinib may lead to inhibition of other targets within the cancer cell. Of particular interest would be HER2, which is inhibited with an IC_{50} ~10 times higher than that required to inhibit EGFR (5). Effects of the combination on tumor endothelia leading to impaired angiogenesis or to changes in paclitaxel transport associated with gefitinib treatment may also be contributing to the added benefit observed with dose escalation (4).

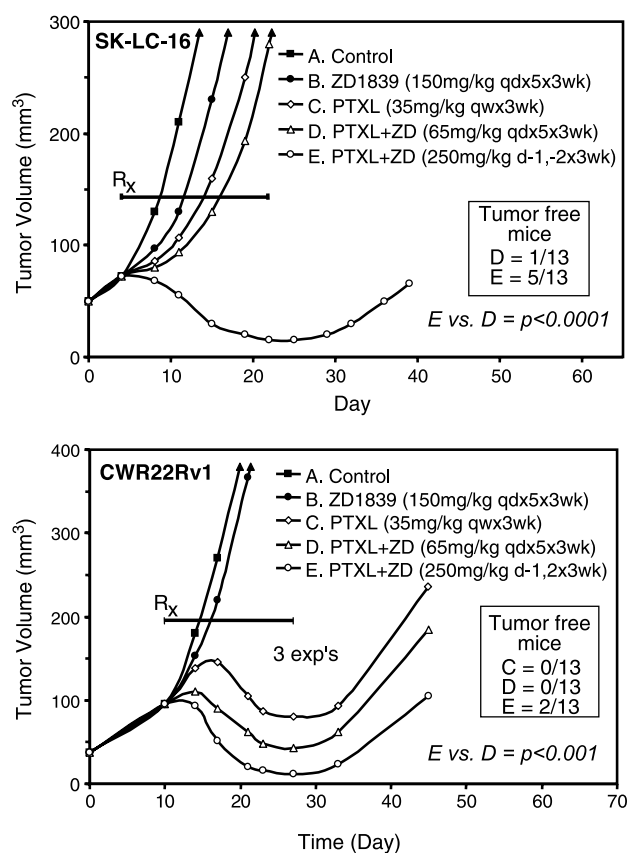


Fig. 4 Treatment of mice bearing SK-LC-16 (*top*) and CWR22rv1 (*bottom*) xenograft tumors with gefitinib alone and in combination with paclitaxel (PTXL). Gefitinib alone had minimal antitumor activity. Intermittent gefitinib dosing allowed for dose escalation from 65 to 250 mg/kg. Pretreatment with gefitinib on days -2 and -1 only was dramatically more effective than daily $\times 5$ treatment (days -2 through +2). This difference was statistically significant in both SK-LC-16 ($P < 0.0001$) and CWR22rv1 ($P < 0.01$). The data were analyzed by the SAS 8.2 program using Wilcoxon rank sum test.

Finally, it is important to state what we believe these findings do and do not suggest. They do not suggest that high-dose, pulsatile administration of signaling inhibitors or even of EGFR inhibitors is always better than continuous administration. Furthermore, they do not predict that this schedule is likely to yield improved sensitization to all forms of cytotoxic therapy. The data only speak of paclitaxel; experiments with other agents have not yet been done. The data simply generate the hypothesis that pulsatile, high-dose administration with an EGFR inhibitor before paclitaxel administration results in enhanced antitumor activity.

We believe that there are two general lessons of this work. First, dose and schedule matter and must be studied in preclinical models in light of the mechanism one is trying to elicit. Preferably, these studies will occur before initiation of large-scale clinical trials and the data obtained will be used to plan these trials. EGFR and other components of the signaling apparatus are pleiotypic regulators of proliferation, cell survival, angiogenesis, and other aspects of cell physiology. The dose and schedule required to optimally inhibit each of these processes may differ, as illustrated in this study and in the work of Kerbel and others, who have shown that low-dose continuous (chronotropic) administration of cytotoxics may have more antiangiogenic activity than traditional schedules (29).

Second, it is generally believed that mechanism-based therapies are more likely to be effective than traditional ones and that they will work best in combination. However, the mechanism whereby the combinations work is rarely explored. Instead, the chemotherapeutic agent used in the combination is chosen because it is frequently used in the particular disease and the dose and schedule of each agent is determined empirically or from single-agent studies. For example, in the large phase III studies in which gefitinib was combined with standard chemotherapy for lung cancer, each agent was given at schedules determined empirically in single-agent studies (20–22). We propose that realization of the potential of targeted therapies will require the testing in clinic of hypotheses generated in preclinical models that concern the biological basis for the antitumor activity of the combination.

ACKNOWLEDGMENTS

We thank Dr. Katia Manova and the staff of the immunohistochemistry core facility for their technical advice and support, Dr. William Pao and Harold Varmus for EGFR genotyping, and Qing Ye, Haiying Ju, and Wai Lin Wong for technical assistance.

REFERENCES

1. Sliwkowski MX, Lofgren JA, Lewis GD, Hotaling TE, Fendly BM, Fox JA. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin Oncol* 1999;26:60–70.
2. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J* 2000;19:3159–67.
3. Kumar R, Yarmand-Bagheri R. The role of HER2 in angiogenesis. *Semin Oncol* 2001;28:27–32.
4. Ciardiello F, Caputo R, Bianco R, et al. Inhibition of growth factor production and angiogenesis in human cancer cells by ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor. *Clin Cancer Res* 2001;7:1459–65.
5. Moasser MM, Basso A, Averbuch SD, Rosen N. The tyrosine kinase inhibitor ZD1839 (“Iressa”) inhibits her2-driven signaling and suppresses the growth of her2-overexpressing tumor cells. *Cancer Res* 2001;61:7184–8.
6. Moulder SL, Yakes FM, Muthuswamy SK, Bianco R, Simpson JF, Arteaga CL. Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2/neu (erbB2)-overexpressing breast cancer cells *in vitro* and *in vivo*. *Cancer Res* 2001;61:8887–95.
7. Sirotiak FM, Zakowski MF, Miller VA, Scher HI, Kris MG. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. *Clin Cancer Res* 2000;6:4885–92.
8. Ciardiello F, Caputo R, Bianco R, et al. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin Cancer Res* 2000;6:2053–63.
9. Sirotiak FM. Studies with ZD1839 in preclinical models. *Semin Oncol* 2003;30:12–20.
10. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2003;21:2237–46.
11. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;290:2149–58.
12. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1458–61.
13. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–39.
14. Moscatello DK, Holgado-Madruga M, Godwin AK, et al. Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. *Cancer Res* 1995;55:5536–9.
15. Rich JN, Reardon DA, Peery T, et al. Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* 2004;22:133–42.
16. Baselga J, Norton L, Masui H, et al. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. *J Natl Cancer Inst* 1993;85:1327–33.
17. Baselga J, Norton L, Albanell J, Kim YM, 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* 1998;58:2825–31.
18. Baselga J, Tripathy D, Mendelsohn J, et al. Phase II study of weekly intravenous trastuzumab (Herceptin) in patients with HER2/neu-overexpressing metastatic breast cancer. *Semin Oncol* 1999;26:78–83.
19. Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol* 2004;22:1201–8.
20. Herbst RS, Giaccone G, Schiller JH, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 2. *J Clin Oncol* 2004;22:785–94.
21. Giaccone G, Herbst RS, Manegold C, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 1. *J Clin Oncol* 2004;22:777–84.
22. Herbst RS, Prager D, Hermann R, et al. TRIBUTE—a phase III trial of erlotinib HCl (OSI-774) combined with carboplatin and paclitaxel (CP) chemotherapy in advanced non-small cell lung cancer (NSCLC). *Proc ASCO* 2004;Abst 7011:23, p. 617.
23. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated

- with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306–11.
24. Basso A, Solit D, Munster P, Rosen N. Ansamycin antibiotics inhibit Akt activation and tumor growth in human breast cancers that overexpress HER2. Oncogene 2002;21:1159–66.
25. Citri A, Alroy I, Lavi S, et al. Drug-induced ubiquitylation and degradation of ErbB receptor tyrosine kinases: implications for cancer therapy. EMBO J 2002;21:2407–17.
26. Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly *in vitro* by taxol. Nature 1979;277:665–7.
27. Davies AM, Pryde BJ, Kimura T, et al. EGFR inhibitor OSI-774 activity in non-small cell lung carcinoma (NSCLC) cells. Proc AACR 2003;44:Abst 3779.
28. Munster PN, Basso A, Solit D, Norton L, Rosen N. Modulation of Hsp90 function by ansamycins sensitizes breast cancer cells to chemotherapy induced apoptosis in an RB- and schedule-dependent manner. Clin Cancer Res 2001;7:2228–36.
29. Kerbel RS, Klement G, Pritchard KI, Kamen B. Continuous low-dose anti-angiogenic/ metronomic chemotherapy: from the research laboratory into the oncology clinic. Ann Oncol 2002;13:12–5.