Efficacy of Cytotoxic Agents against Human Tumor Xenografts Is Markedly Enhanced By Coadministration of ZD1839 (Iressa), an Inhibitor of EGFR Tyrosine Kinase[1]

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ABSTRACT

The blockade of epidermal growth factor receptor (EGFR) function with monoclonal antibodies has major antiproliferative effects against human tumors *in vivo*. Similar antiproliferative effects against some of these same tumors have also been observed with specific inhibitors of the EGFR-associated tyrosine kinase. One such inhibitor, the p.o. active ZD1839 (Iressa), has pronounced antiproliferative activity against human tumor xenografts. We now show that coadministration of ZD1839, as with anti-EGFR, will enhance the efficacy of cytotoxic agents against human vulvar (A431), lung (A549 and SK-LC-16 NSCL and LX-1), and prostate (PC-3 and TSU-PR1) tumors. Oral ZD1839 (five times daily × 2) and cytotoxic agents (i.p. every 3–4 days × 4) were given for a period of 2 weeks to mice with well-established tumors. On this schedule, the maximum tolerated dose (150 mg/kg) of ZD1839 induced partial regression of A431, a tumor that expresses high levels of EGFR, 70–80% inhibition among tumors with low but highly variable levels of EGFR expression (A549, SKLC-16, TSU-PR1, and PC-3), and 50–55% inhibition against the LX-1 tumor, which expresses very low levels of EGFR. ZD1839 was very effective in potentiating most cytotoxic agents in combination treatment against all of these tumors, irrespective of EGFR status, but dose reduction of ZD1839 below its single-agent maximum tolerated dose was required for optimum tolerance. The pronounced growth inhibitory action of the platinums, cisplatin and carboplatinum, as single agents against A431 vulvar, A549 and LX-1 lung, and TSU-PR1 and PC-3 prostate tumors was increased severalfold when ZD1839 was added, with some regression of A431 and PC-3 tumors. Although the taxanes, paclitaxel or docetaxel, as single agents markedly inhibited the growth of A431, LX-1, SK-LC-16, TSU-PR1, and PC-3, when combined with ZD1839, partial or complete regression was usually seen. Against A549, the growth inhibition of doxorubicin was increased 10-fold (>99%) with ZD1839. The folate analogue, edatrexate, was highly growth inhibitory against A549, LX-1, and TSU-PR1, whereas edatrexate combined with ZD1839 resulted in partial or complete regression in these tumors. Against the A431 tumor, paclitaxel alone either was highly growth inhibitory or induced some regression, but when combined with ZD1839, pronounced regression was obtained. Combination with gemcitabine neither added nor detracted from baseline cytotoxic efficacy, whereas ZD1839 combined with vinorelbine was poorly tolerated. Overall, these results suggest that potentiation of cytotoxic treatment with ZD1839 does not require high levels of EGFR expression in the target tumors. They also suggest significant clinical benefit from ZD1839 in combination with a variety of widely used cytotoxic agents.

INTRODUCTION

Two important autocrine regulatory pathways in many tumors are constituted by the epidermal growth factor (1, 2) and HER-2/neu (3, 4) receptors and their ligands. The blockade of EGFR or HER-2/neu function Mabs[3] has been shown to have marked antiproliferative effects against tumors in culture (5, 6) and in animals (7, 8). While targeting HER-2/neu, the same blocking Mabs brought about (9) significant tumor regression when used to treat patients with metastatic breast cancer that expressed this growth factor receptor. In other studies (10–14), coadministration of either anti-EGFR or anti-HER-2/neu antibodies has been found to potentiate the efficacy of cytotoxic agents as well as radiation therapy against human tumors *in vitro* and *in vivo*. As another extension of these studies (9, 15), anti-Her-2/neu antibody coadministered with CDDP or PTXL to patients with HER-2/neu overexpressing breast cancer resulted in a significant increase in the number of major responses over that obtained with PTXL alone.

More recent studies (16) in animal model systems have shown that the blockade of EGFR function and antiproliferative effects against tumors can also be achieved by the use of

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[3] The abbreviations used are: Mab, monoclonal antibody; MTD, maximum tolerated dose; CDDP, cisplatin; CBDCA, carboplatinum; DOX, doxorubicin; PTXL, paclitaxel; DTXL, docetaxel; EDX, edatrexate (10-ethyl-10-deazaaminopterin); GEM, gemcitabine; VNR, vinorelbine; IHC, immunohistochemistry; RT-PCR, reverse transcriptase-PCR; EGFR, epidermal growth factor receptor; qd, once daily.

Table 1 *EGFR* gene expression in various human tumors as determined by IHC and RT-PCR

Tumors were xenografted to NCR-nu mice and, after growth to an average diameter of 1 ± 0.2 cm, excised for *EGFR* gene expression analysis. Other experimental details are described in the text. Averages of two experiments are shown.

		Relative EGFR Gene Expression	
Tumor	Tumor type	IHC[a]	RT-PCR[b]
A431	Vulvar	10	10
SK-LC-16	NSCL[c]	2	2
A549	NSCL[c]	1	2
PC-3	Prostate	1	2
TSU-PR1	Prostate	±	1
LX-1	Lung	0	<1

[a] The intensity of the specific staining for each tumor was compared with that seen with A431.

[b] The amount of PCR product shown in Fig. 1 was compared with that obtained with A431.

[c] NSCL, non-small cell lung cancer.

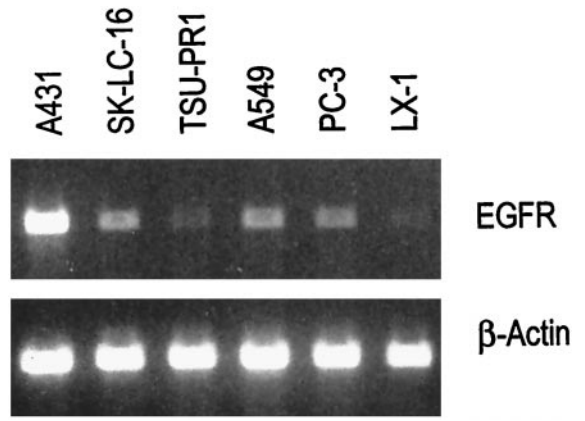

Fig. 1 Semiquantitative RT-PCR of *EGFR* gene expression in human tumor xenografts. *EGFR* gene expression was normalized to β-*actin* gene expression in these tumors after a 28-cycle PCR. The methodologies used and a description of the primers used are provided in the text. One of several replicates is shown.

specific inhibitors of its associated tyrosine kinase. One of these inhibitors, the p.o. active ZD1839, has marked effects against the growth of the A431 tumor, which exhibits high levels of expression of EGFR. Growth of a variety of other human tumors xenografted in mice was also inhibited by this agent (17). In the present report, we describe studies showing that coadministration of ZD1839 to mice with a variety of cytotoxic agents will enhance their efficacy against human lung and prostate tumors as well as the A431 vulvar tumor. Moreover, enhancement by ZD1839 could occur in tumors with very low levels of expression of EGFR. As in the case of combinations that use anti-EGFR and anti-HER-2/neu antibodies, ZD1839 enhanced the antitumor activity of DOX, platinum compounds, and taxanes. In addition, coadministration of ZD1839 enhanced the antitumor activity of the classical folate analogue, EDX, but not of the pyrimidine nucleoside analogue, GEM. The combination of ZD1839 with VNR was poorly tolerated and could not be adequately evaluated for efficacy. The results of these experiments are described below.

MATERIALS AND METHODS

Animal Studies. The tumors used during the *in vivo* studies were obtained from the National Cancer Institute, Developmental Therapeutics Program (LX-1 lung tumor) or the American Type Culture Collection (A431, A549 and SK-LC-16 NSCL, TSU-PR1, and PC-3 prostate tumors). These human tumors were maintained by s.c. transplantation in athymic NCR-nu mice. After tumor growth, a cell suspension in RPMI medium was prepared from the excised tumor and centrifuged for 5 min at 1000 × *g*, and the pellet was resuspended in RPMI complete medium with 10% FCS for implantation. For A549 and the prostate tumors, an equal volume of Matrigel (Becton-Dickinson, Franklin Lakes, NJ) was used to suspend the cell pellet. Aliquots of tumor cell suspension were implanted in a group of mice, and 3–5 days later the mice, now bearing tumors 5–6 mm in diameter, were randomized among control and the various treated groups. The MTDs of the various agents alone or in combination were determined in preliminary experiments comparing the effect of varying doses. ZD1839 was given qd × five for two successive weeks, and the cytotoxic agents were given on a schedule of every 3–4 days × 4. The doses eventually selected resulted in <10% weight loss and no toxic deaths. The average tumor diameter (two perpendicular axes of the tumor were measured) was measured in control and treated groups by caliper 2–20 days after cessation of treatment. The data are expressed as the increase or decrease in tumor volume in mm^3 ($mm^3 = 4/3\pi r^3$). Statistical analysis was carried out by the χ^2 method (18). Working solutions of EDX, DOX, GEM, CDDP, CBDCA, and VNR were prepared in 0.9% NaCl (pH 7). PTXL was prepared in a 1:1 solution of Cremaphor and ethanol, and DTXL was prepared in 13% ethanol in H_2O. ZD1839 was prepared as a lactate salt (pH 5.2). These solutions were held at −4°C for no longer than 2 weeks except in the case of GEM, which was always used immediately. These studies were performed in accordance with "Principles of Laboratory Animal Care" (NIH publication No. 85–23 revised 1985). EDX was provided by Novartis. ZD1839 was provided by AstraZeneca. PTXL was purchased from Hande Tech. GEM, DTXL, and VNR were obtained from the Memorial Sloan-Kettering Cancer Center Pharmacy. NCR-nu(AT) mice were purchased from Sprague Dawley (Madison, WI).

Semiquantitative RT-PCR. mRNA from the various human tumors used in these studies was reverse transcribed into cDNA using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD) and random hexamers in a final volume of 28 μl, according to the manufacturer's instructions. The reaction mixture was incubated at 26°C for 8 min, heated to 42°C for 45 min and 95°C for 5 min, and then held at 4°C in a programmable thermocycler (MJ Research, Watertown, MA). The amplification was performed with platinum Taq DNA polymerase (Life Technologies) using the recommended buffer, 10 pmol of the primers, 1 μl of cDNA reaction mixture, and 200 μmol deoxynucleotide triphosphate in a total volume of 50 μl. After the initial denaturation step of 3 min at 94°C, 25 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min

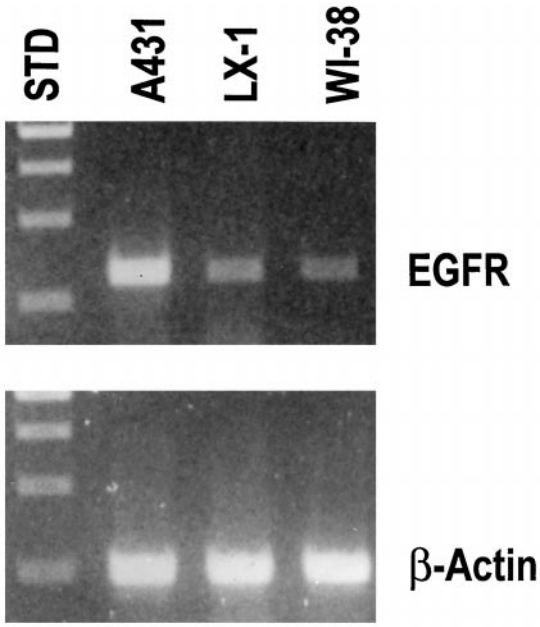

Fig. 2 Semiquantitative RT-PCR of *EGFR* gene expression in A431, LX-1, and WI-38 cells. *EGFR* gene expression was normalized to β-*actin* gene expression after a 32-cycle PCR. See text for a description of the methodologies and primers used. One of several replicates is shown in the figure.

at 72°C were used. The reaction was extended for 10 min at 72°C. Preliminary runs were performed to determine the maximum number of cycles that could be carried out with cDNA derived from the high-EGFR-expressing tumor in the linear range. This was the number of cycles selected for a comparison of EGFR sequence product with β-actin product among the different tumor-derived cDNAs. EGFR primers were 5′-GTGGCTGGTTATGTCCTCATTGCC-3′ and 5′ACACTTCT-TCAGGCAGGTGCCACC-3′ for a 637 bp product, and β-actin primers were 5′-GCTACGTCGCCCTGGACTTC-3′ and 5′-GTCATAGTCCGCCTAGAAGC-3′ for a 490 bp product.

IHC. Detection of EGFR in tumor specimens was carried out with anti-EGFR Mabs in a manner described previously (19).

RESULTS

Expression of EGFR in Human Tumor Xenografts. Preliminary to the antitumor studies of ZD1839 with and without cytotoxic agents described below, we determined the relative level of gene expression of EGFR among the target tumors used. Both IHC and RT-PCR measurements showed the same rank order in relative expression for these tumors (Table 1 and Fig. 1). As noted in previous studies (7, 10), *EGFR* gene expression in A431 was extremely high. In contrast, expression was 5–10-fold lower in SK-LC-16, A549, and PC-3 tumors and >10-fold lower in TSU-PR1 and LX-1 tumors. In the latter cases, *EGFR* gene expression was almost undetectable (TSU-PR1) or undetectable (LX-1) by IHC but could be detected by the more sensitive RT-PCR method. It was also determined (Fig. 2) by RT-PCR that the low level of expression of EGFR in

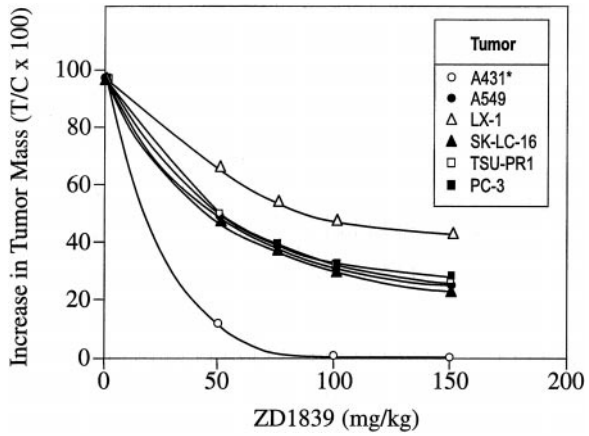

Fig. 3 Antitumor dose response for ZD1839 against several human tumor xenografts. The relative level of EGFR expression is also shown in the figure. Average of three experiments with SE of $<\pm 15\%$. *Partial regressions were observed at doses of 100 or 150 mg/kg.

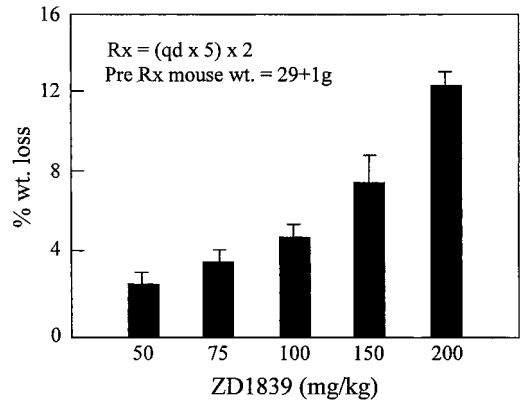

Fig. 4 Dose response for weight loss induced by ZD1839 in NCR-nu mice. Average of three experiments with SE given in the figure.

LX-1 was similar to the expression of this gene in nontransformed WI-38 human fibroblasts that are able to grow in culture. Such normal cell types characteristically exhibit (1, 2) low levels of expression of growth factor receptors.

Studies of ZD1839 as a Single Agent. Most of the original studies (7) documenting antitumor effects of Mabs that target EGFR were carried out with the A431 vulvar carcinoma, which markedly overexpresses EGFR. Early *in vivo* studies (17) with ZD1839 were also directed at this human tumor xenograft. As a baseline for comparing our own results obtained with the various human lung and prostate tumors, we also carried out studies with the A431 tumor. Single-agent activity of ZD1839 against a range of human tumor xenografts is presented in Fig. 3. ZD1839 had greatest activity against the A431 tumor, in which substantial inhibition of growth at the lowest dose (50 mg/kg) and partial regressions over the range of 100–150 mg/kg were observed. A similar dose response was observed against the other tumors. At the MTD (150 mg/kg), ZD1839 was 70–80% growth inhibitory against A549, SK-LC-16, TSU-PR1, and PC-R tumors and 50–55% growth inhibitory against the

Table 2 Dose dependency for lethal toxicity of ZD1839 administered with various cytotoxic agents to athymic NCR-nu mice

Cytotoxic compound	Agent MTD (mg/kg)	Toxic deaths						
		ZD1839 (mg/kg)[a]						
		0	50	75	100	150	200	MTD
			0/32	0/28	0/24	0/30	1/4	150
GEM	350	0/15	0/15	2/6	6/6	ND[b]	ND	50
EDX	45	0/24	ND	0/24	2/15	2/8	ND	75
PTXL	25	1/34	0/34	2/8	3/6	ND	ND	50
DTXL	20	0/18	0/18	1/8	4/6	ND	ND	50
VNR	2.5	0/18	4/6	6/6	6/6	ND	ND	<25
CDDP	1.5	0/22	0/6	0/22	2/15	2/9	ND	75
CBDCA	50	0/33	0/9	0/33	1/15	3/12	ND	75
DOX	1	1/15	0/10	0/15	3/15	4/10	ND	75

[a] ZD1839 given (qd × 5) × 2, and cytotoxic agents given q every 3–4 days × 4.
[b] ND, not determined.

Table 3 Antitumor activity of ZD1839 and PTXL given alone or in combination against the human A431 vulvar tumor xenografted to nude mice

Two to three experiments of three to four mice/group.

Drug		Average weight change (%)	Average tumor diameter[b,c] (mm ± SE)	Change in tumor volume[b,c] (mm^3)	Tumor-free mice (no./total)
Compound[a]	Dose (mg/kg)				
		+2	8.8 ± 2	+349	0/9
ZD1839	50	−4	5.6 ± 1	+18	0/9
ZD1839	150	−6	4.2 ± 1	−15	0/9
PTXL	25	−3	4.1 ± 1	−16	0/9
ZD1839 + PTXL	50 + 25	−6	2.4 ± 1	−48	3/9[d]
ZD1839 + PTXL	150 + 18	−7	3.4 ± 1	−33	1/9[d]

[a] ZD1839 given (qd × 5) × 2 and platinums, PTXL, and GEM given every 3–4 days × 4 at 3–4 days after transplant.
[b] Initial tumor mass was 54 ± 8 mg (4.6 ± 0.6 diameter).
[c] Measurements made 2–3 days after dose or at the nadir for regressing tumors.
[d] Tumor regrowth in two of three and one of one mice 21 days after treatment.

LX-1 tumor. The doses of ZD1839 used in these studies were all below the dose that would cause lethal toxicity on the schedule of administration used. However, data shown in Fig. 4 record substantial (≥10%) weight loss in mice after a full course of treatment at doses >150 mg/kg. With lower doses of ZD1839, weight loss was significantly less, and dose dependence varied from as little as 2% (50 mg/kg) to 5% (100 mg/kg) at the end of treatment.

Tolerance Studies Combining ZD1839 with Cytotoxic Agents. Before evaluating the antitumor activity of ZD1839 in combination with cytotoxic agents, we determined tolerances of mice to these various combinations. Coadministration of cytotoxic agents reduced the tolerability of ZD1839 by varying extents that depended on the cytotoxic agent used (Table 2). The maximum nonlethal dose of ZD1839 was 75 mg/kg in combination with DOX, EDX, and both platinum compounds (CDDP and CBDCA) and 50 mg/kg in combination with the taxanes (PTXL and DTXL) and GEM. Tolerance of ZD1839 with VLB was extremely low (<25 mg/kg), and this combination was not used extensively in these studies because of lack of efficacy (data not shown). An examination of mice that did not survive treatment with these various combinations when administered ZD1839 above the MTD showed that pathological changes were limited to the small intestine (data not shown). This was in the form of multifocal ulcerative enteritis

Combination Treatment against the A431 Tumor. PTXL markedly inhibited the growth of the A431 tumor, which resulted in a similar level of regression to that observed with ZD1839 at 150 mg/kg (Table 3). When PTXL was coadministered with ZD1839, with the dose of either ZD1839 (50 mg/kg ZD1839 plus 25 mg/kg PTXL) or PTXL (150 mg/kg ZD1839 plus 18 mg/kg PTXL) attenuated, nearly complete regression was obtained ($P < 0.05$), and some tumor-free mice were observed.

Combination Treatment against Lung Tumors. The growth inhibitory action of CDDP and CBDCA against the A549 tumor was increased 4-fold in combination with ZD1839 ($P < 0.01$; Table 4). In addition, coadministration of ZD1839 increased the activity of CBDCA 3-fold against the LX-1 tumor ($P < 0.01$). Both taxanes markedly inhibited the growth of the LX-1 tumor, although PTXL was more effective than DTXL (Table 5). When combined with ZD1839, the activity of DTXL against LX-1 was increased 4-fold ($P < 0.01$), whereas PTXL resulted in pronounced tumor regression with three of nine mice having no detectable tumor at the end of treatment. In mice treated with a combination of PTXL and ZD1839, regression of

Table 4 Antitumor activity of ZD1839 and platinum compounds given alone or in combination against human lung tumors xenografted to nude mice

Two to three experiments of three to four mice/group.

Tumor	Drug compound[a]	Average weight change (%)	Average tumor diameter[b,c] (mm ± SE)	Change in tumor volume[b,c] (mm^3)	Tumor-free mice (no./total)
A549		+1	13.1 ± 2	+1185	0/8
	ZD1839 (75)	−6	9.9 ± 1	+527	0/8
	CDDP (1.5)	−2	7.7 ± 2	+189	0/8
	CBCDA (50)	−1	7.4 ± 2	+168	0/8
	ZD1839 + CDDP	−7	5.6 ± 1	+41	0/8
	ZD1839 + CBCDA	−8	5.9 ± 1	+38	0/8
LX-1		−4	15.2 ± 1	+1801	0/9
	ZD1839 (75)	−7	12.2 ± 2	+956	0/9
	CBCDA (50)	+1	10.2 ± 1	+545	0/9
	ZD1839 + CBCDA	−7	7.4 ± 2	+188	0/9

[a] ZD1839 given (qd × 5) × 2 and platinums given every 3–4 days × 4 at 3–4 days after transplant.
[b] Initial tumor mass was 50–65 mg (4.8 ± 0.2 mm diameter).
[c] Measurements made 2–3 days after dose.

Table 5 Antitumor activity of ZD1839, taxanes, and GEM given alone or in combination against human lung tumors xenografted to nude mice

Two to three experiments of three to four mice/group.

Tumor	Drug compound[a]	Average weight change (%)	Average tumor diameter[b,c] (mm ± SE)	Change in tumor volume[b,c] (mm^3)	Tumor-free mice (no./total)
LX-1		−4	15.2 ± 2	+1805	0/9
	ZD1839 (50)	−6	12.5 ± 3	+981	0/9
	PTXL (25)	−3	6.3 ± 1	+89	0/9
	DTXT (20)	−4	7.4 ± 2	+162	0/9
	ZD1839 + PTXL	−5	2.8 ± 1	−51	3/9[d]
	ZD1839 + DTXT	−6	5.6 ± 1	+42	0/9
A549		+1	12.2 ± 2	+907	0/8
	ZD1839 (50)	−3	9.6 ± 1	+809	0/8
	PTXL (25)	−2	4.4 ± 1	−9	0/8
	DTXT (20)	−1	4.3 ± 1	−8	0/8
	GEM (350)	−3	5.2 ± 2	+31	0/8
	ZD1839 + PTXL	−4	2.4 ± 1	−52	2/8[d]
	ZD1839 + DTXT	−3	2.5 ± 1	−55	1/8[d]
	ZD1839 + GEM	−5	5.3 ± 1	+35	0/8

[a] ZD1839 given (qd × 5) × 2 and cytotoxics given every 3–4 days × 4 at 3–4 day after transplant.
[b] Initial tumor mass was 55–63 mg (4.9 ± 0.1 mm diameter).
[c] Measurements made 2–3 days after dose or at the nadir for a regressing tumor.
[d] Tumor regrowth in two of three (LX-1), one of two (A549) and one of one mice 21 days after treatment.

the LX-1 tumor was evident within the first week of treatment and continued for ~10 days after treatment was stopped (Fig. 5). In other experiments, both PTXL and DTXL resulted in complete growth inhibition of the A549 tumor with some indication of slight regression (Table 5). By comparison, when given with ZD1839, both agents induced pronounced regression ($P < 0.01$) with some tumor-free mice found after treatment.

ZD1839 also markedly enhanced the highly growth inhibitory action of EDX against both LX-1 and SK-LC-16 tumors (Table 6). This combination resulted in nearly complete regression of LX-1 in all mice, with some tumor-free mice found after the completion of therapy. A similar but somewhat more modest result was obtained with this combination against SK-LC-16. In addition, ZD1839 substantially potentiated the activity of DOX against the A549 tumor. Whereas DOX alone inhibited tumor growth by nearly 90%, coadministration of ZD1839 resulted in almost complete growth inhibition ($P < 0.005$). In contrast, ZD1839 did not improve the activity of GEM against A549 or SK-LC-16 tumors, although nearly complete growth inhibition was observed with GEM alone (Tables 5 and 6).

Combination Treatment against Prostate Tumors. Coadministration of ZD1839 increased antitumor activity of CBDCA against PC-3 tumors by 5-fold ($P < 0.01$), resulting in tumor regression and two of eight tumor-free mice (Table 7). Whereas PTXL alone had pronounced growth inhibitory activity against both PC-3 and TSU-PR1 tumors, combination with ZD1839 resulted in tumor regression in each case, with five of nine and two of eight complete regressions of PC-3 and TSU-PR1, respectively (Table 8 and Fig. 6). The combination of PTXL and ZD1839 against PC-3 induced onset of regression after the first week of therapy, with regression continuing for 8–10 days after the cessation of therapy. Finally, although EDX

Table 6 Antitumor activity of ZD1839, adriamycin, GEM, and EDX given alone or in combination against human lung tumors xenografted to nude mice

Two experiments of four mice/group.

Tumor	Drug compound[a]	Average weight change (%)	Average tumor diameter[b,c] (mm ± SE)	Change in tumor volume[b,c] (mm^3)	Tumor-free mice (no./total)
A549		2	13.2 ± 2	+1145	0/8
	ZD1839 (75)	−2	10.1 ± 2	+485	0/8
	DOX (1)	1	7.0 ± 1	+139	0/8
	ZD1839 + DOX	−2	4.8 ± 1	+12	0/8
LX-1		−3	14.3 ± 2	+1575	0/8
	ZD1839 (75)	−6	11.1 ± 2	+638	0/8
	EDX(45)	−4	6.6 ± 1	+91	0/8
	ZD1839 + EDX	−7	2.6 ± 1	−51	3/8[d]
SK-LC-16		1	11.1 ± 3	+652	0/8
	ZD1839 (50)	−3	8.4 ± 2	+309	0/8
	ZD1839 (75)	−4	8.0 ± 2	+229	0/8
	GEM (350)	−2	4.8 ± 1	+16	0/8
	EDX (45)	−1	5.4 ± 1	+39	0/8
	ZD1839(50) + GEM	−5	4.9 ± 1	+18	0/8
	ZD1839(75) + EDX	−4	3.8 ± 2	−23	1/8[d]

[a] ZD1839 given (qd × 5) × 2 and cytotoxics given every 3–4 days × 4 at 3–4 day after implant.
[b] Initial tumor mass was 48–64 mg (4.7 ± 2 mm diameter).
[c] Measurements made 2–3 days after dose or at the nadir for a regressing tumor.
[d] Tumor regrowth in one of three (LX-1) and one of one (SK-LC-16) mice 21 days after treatment.

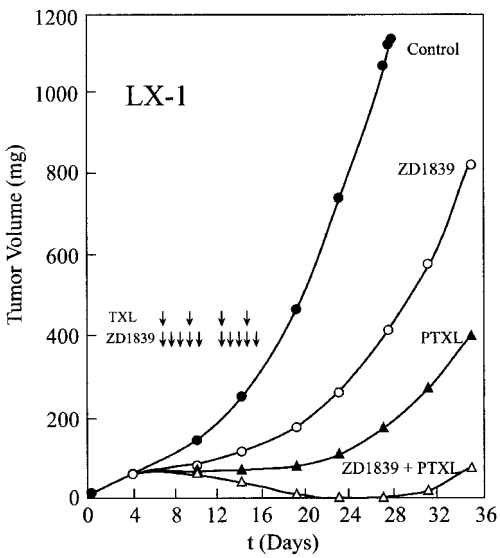

Fig. 5 The effect of ZD1839 alone and in combination with PTXL against the LX-1 tumor. Average of three experiments at three to four mice/group with SE of <13%. Animals treated with ZD1839 (qd × 5) × 2 and PTXL (every 3–4 days × 4). Additional details are given in the text.

was less growth inhibitory than PTXL against TSU-PR1, coadministration of ZD1839 with EDX resulted in partial tumor regression (Table 8).

DISCUSSION

As a single agent, ZD1839 resulted in regression of the vulvar A431 tumor, which markedly overexpresses EGFR. Against the tumors (A549, SK-LC-16, PC-3, and TSU-PR1 LX-1) with much lower levels of EGFR expression, ZD1839 was only growth inhibitory. Growth inhibition by ZD1839 was lowest against the lung LX-1 tumor, which had the lowest EGFR expression. These results are consistent with an earlier study (17) that demonstrated antitumor activity for ZD1839 in human vulvar, lung, prostate, and ovarian tumor xenografts. ZD1839 monotherapy was well tolerated in mice, and the maximum nonlethal dose was 150 mg/kg.

The combination of ZD1839 with all cytotoxic agents in this study required a 2-fold or greater attenuation of the ZD1839 dose below its single-agent maximum nonlethal dose of 150 mg/kg for optimum tolerance. Interestingly, limiting toxicity for ZD1839 in combination was associated only with the small intestine and appears to reflect the extraordinarily rapid renewal of the mouse intestinal epithelium, compared with proliferative compartments elsewhere. As such unique sensitivity at this organ site is not usually a characteristic of humans exposed to these cytotoxic agents, similar dose attenuation may not be necessary in clinical trials.

Coadministration of ZD1839 markedly enhanced the antitumor activity of a number of cytotoxic agents with highly diverse mechanisms of action. The effects of ZD1839 were most pronounced in combination with taxanes (PTXL, DTXL), platinums (CDDP, CBDCA), and the folate antagonist, EDX. Similar potentiation occurred with DOX, although the overall level of efficacy observed was much lower than that obtained with taxanes, platinums, and EDX. Combination with GEM neither added nor detracted from baseline cytotoxic efficacy, whereas toxicity precluded assessment of efficacy in combination with VNR. Although the platinums, DOX, and EDX ultimately induce damage of DNA, they achieve this by very different mechanisms. The taxanes, on the other hand, effectively target microtubules. Thus, the interaction between ZD1839 and cytotoxic agents underlying the observed potentiation is most likely

Table 7 Antitumor activity of ZD1839 and platinum compounds given alone or in combination against human prostate tumors xenografted to nude mice

Two experiments of four mice/group.

Tumor	Drug compound[a]	Average weight change (%)	Average tumor diameter[b,c] (mm ± SE)	Change in tumor volume[b,c] (mm^3)	Tumor-free mice (no./total)
TSU-PR1		1	12.1 ± 2	+886	0/8
	ZD1839 (75)	−2	9.7 ± 1	+495	0/8
	CBCDA (50)	−1	8.2 ± 1	+247	0/8
	ZD1839 + CBCDA	−2	5.7 ± 1	+55	0/8
PC-3		1	9.5 ± 2	+449	0/8
	ZD1839 (75)	−1	7.4 ± 1	+208	0/8
	CBCDA (50)	−1	5.8 ± 1	+62	0/8
	ZD1839 + CBCDA	−3	3.7 ± 1	−21	2/8[d]

[a] ZD1839 given (qd × 5) × 2 and platinums given every 3–4 days × 4.
[b] Initial tumor mass was 50–65 mg (4.8 ± 0.2 mm diameter).
[c] Measurements made 2–3 days after dose.
[d] Tumor regrowth in one of two mice (PC-3) 21 days after treatment.

Table 8 Antitumor activity of ZD1839, taxol, and EDX given alone or in combination against human prostate tumors xenografted to nude mice

Two to three experiments of three to four mice/group.

Tumor	Drug compound[a]	Average weight change (%)	Average tumor diameter[b,c] (mm ± SE)	Change in tumor volume[b,c] (mm^3)	Tumor-free mice (no./total)
PC-3		−3	14.2 ± 2	+1449	0/9
	ZD1839 (50)	−4	11.8 ± 2	+823	0/9
	PTXL (25)	−4	4.9 ± 2	+14	1/9[d]
	ZD1839 + PTXL	−6	1.6 ± 1	−48	5/9[d]
TSU-PR1		−2	13.8 ± 2	+1336	0/8
	ZD1839 (50)	−3	11.4 ± 3	+708	0/8
	PTXL (25)	1	5.2 ± 1	+25	0/8
	ZD1839 + PTXL	−4	2.8 ± 1	−31	2/8[d]
TSU-PR1		2	13.3 ± 2	+1147	0/8
	ZD1839 (75)	−2	9.7 ± 2	+405	0/8
	EDX (45)	1	7.3 ± 1	+144	0/8
	ZD1839 + EDX	−3	4.2 ± 1	−23	1/8[d]

[a] ZD1839 given (qd × 5) × 2 and cytotoxics given every 3–4 days × 4 at 3–4 days after implant.
[b] Initial tumor mass was 48–59 mg (4.8 ± 0.1 mm diameter).
[c] Measurements made 2–3 days after dose or at the nadir for a regressing tumor.
[d] Tumor regrowth in one of one and three of five (PC-3), one of two and one of one (TSU-PR1) mice 21 days after treatment.

downstream of the sites of specific pharmacological effects of these agents and more globally determined at the level of growth control signaling. In this respect, ZD1839 is similar to anti-EGFR Mabs (10, 11) in their ability to potentiate the action of various cytotoxic agents with different mechanisms of action. In view of the above, it was of interest to note that ZD1839 did not potentiate the action of the pyrimidine nucleoside analogue, GEM. The underlying basis for the lack of efficacy of this combination, as well as the exceedingly high toxicity of VNR with ZD1839, is unknown.

Strikingly, the degree of potentiation of cytotoxic action in a variety of tumor types was not dependent upon high levels of expression of EGFR. The basis for this result is not known. However, these results may indicate that a lower threshold of antitumor response to ZD1839 at the level of EGFR tyrosine kinase was necessary for potentiation of cytotoxic agents. Alternatively, EGFR expression may be up-regulated by the cytotoxic agent (20), possibly to counteract the induction of apoptosis by the cytotoxic drug. Indeed, high levels of EGFR expression have been found in drug-resistant cell lines (21). Finally, it should be mentioned that significant inhibition by ZD1839 of kinases other than EGFR tyrosine kinase, although not completely ruled out, would appear unlikely. Earlier *in vitro* studies (22) have shown that in contrast to its potent inhibition of EGFR tyrosine kinase, inhibition by ZD1839 was 10^2-10^4-fold less against kinases associated with erbB2, vascular EGFRs, KDR, and eflt or against a large group of serine/threonine kinases, including protein kinase C, mitogen-activated protein/extracellular signal-regulated kinase-1, and extracellular signal-regulated kinase-2.

Given that the combinations of ZD1839 with all of these cytotoxic agents required substantial attenuation of the dose of ZD1839 for optimum tolerance and the observation that the degree of potentiation was not dependent upon the level of EGFR expression, the results become even more impressive and suggest promising clinical potential for the use of these combined agents in the treatment of at least two major neoplastic disorders (lung and prostate cancer) and possibly others.

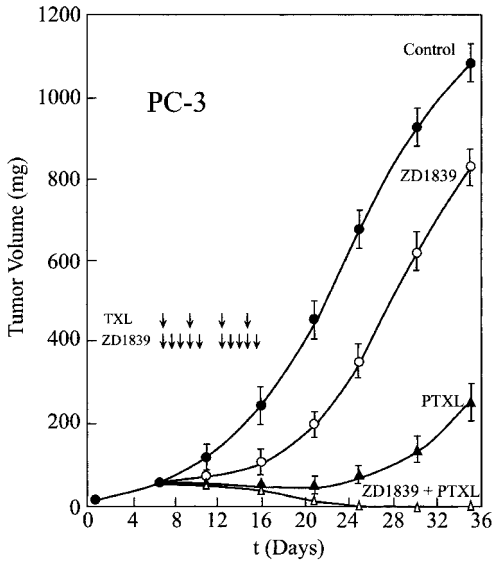

Fig. 6 The effect of ZD1839 alone and in combination with PTXL against the PC-3 tumor. Average of three experiments at three to four mice/group with a SE of $<\pm 14\%$. Animals treated with ZD1839 (qd $\times$ 5) $\times$ 2 and PTXL (every 3–4 days $\times$ 4). See text for additional details.

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