

Erlotinib (OSI-774)-induced inhibition of transitional cell carcinoma of bladder cell line growth is enhanced by interferon- α

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OBJECTIVE

To examine whether erlotinib gives similar results to gefitinib, a small molecule epidermal growth factor receptor (HER1/EGFR) tyrosine kinase (TK) inhibitor that inhibits the growth of human bladder cancer cell lines *in vitro*, and given that interferon- α (IFN α) promotes an antiproliferative effect of HER1/EGFR inhibitors on colon cancer cell lines, to also determine the effects of erlotinib alone or together with IFN α on bladder cancer cell lines, and whether sensitivity is influenced by HER1/EGFR mutation status.

MATERIALS AND METHODS

Seven bladder cancer cell lines were characterized for HER1/EGFR expression, then

treated with erlotinib alone, IFN α alone, or IFN α plus erlotinib. Cell growth inhibition was assessed by crystal-violet staining and HER1/EGFR expression by flow cytometry. Synergy was evaluated using the combination index of Chou and Talalay. DNA from these cell lines in the linear growth phase and from 14 bladder cancer tissue samples were tested for HER1/EGFR mutations.

RESULTS

Cell-surface HER1/EGFR expression was present in all seven bladder cancer cell lines. Both erlotinib and IFN α independently were significantly antiproliferative, and combined treatment synergistically enhanced the sensitivity in six of the seven cell lines. No

bladder cancer cell lines or tissues tested expressed HER1/EGFR mutations.

CONCLUSION

Erlotinib inhibits the growth of human bladder cancer cell lines. Enhanced inhibition in the presence of IFN α is not determined by the presence of HER1/EGFR mutations. This study might have clinical implications for improving the treatment of bladder cancer.

KEYWORDS

bladder cancer cell line, combined therapy, erlotinib, HER1/EGFR mutation, interferon- α , isobologram, synergism

INTRODUCTION

Bladder cancer is the fourth most common type of cancer in men and the eighth in women [1]. In the USA, bladder cancer occurs more in Caucasians than in African-Americans and Hispanics. With \approx 132 000 directly related deaths in 2000 worldwide [2], there are currently no cures for relapsed or inoperable patients with invasive tumours [3]. New therapies are needed for progressive tumours.

Targeted therapies have been used successfully for treating cancers, e.g. erlotinib (an epidermal growth factor receptor, HER1/EGFR, tyrosine kinase, TK, inhibitor) for lung

cancer [4] gives a survival benefit with minimal side-effects to patients with sensitive tumours. HER1/EGFR and its signals contribute to cell-cycle progression, angiogenesis, metastatic cancer spread, and inhibition of apoptosis. In bladder cancer, HER1/EGFR expression is often dysregulated, possibly contributing to the growth and progression of the tumours [5]. Alterations occur in the expression of the genes of both the receptors and ligands of the four members of the HER (ERBB) gene family [6,7]. Prognosis appears to be related to the relative expression of each of the different receptor and ligand genes in combination. Increased cell replication can occur through enhanced stimulation by external ligand or by mutation

of HER/EGFR in the extracellular domain and autocrine growth enhancement in the absence of external stimulation [5]. High levels or over-expression of HER1/EGFR are associated with aggressive disease and a poor clinical outcome [8,9]. However, there are no reports on the clinical treatment of bladder cancers using HER1/EGFR inhibitors. One of these, gefitinib, inhibits the growth of human bladder cancer cell lines *in vitro* [10] and *in vivo* [11]. We hypothesized that erlotinib would have similar activity.

There have been several clinical trials, particularly in lung cancer, of EGFR-targeted therapy [4]. As only a minority of patients in these trials benefited from HER1/EGFR-

targeted therapy (given singly or together with chemotherapy [4,5]), enhancing inhibition or the proportion of susceptible tumours might be clinically relevant. We showed in colon cancer cell lines that combination with interferon- α (IFN α) increased the efficacy of gefitinib through synergy, such that reduced doses of gefitinib in the presence of a low dose of IFN α achieved higher growth inhibition than a full dose of gefitinib alone [12]. Moreover, IFN α -induced growth inhibition of some human bladder cancer lines was not paralleled by complete inhibition of the EGF-HER1/EGFR pathway, as the antiproliferative effect of IFN α was reduced by adding EGF [13]. We therefore hypothesized that in bladder cancer cells in which the HER1/EGFR pathway is present/functional, IFN α might enhance growth inhibition by HER1/EGFR blockers. In the present study we aimed to determine in bladder cancer cell lines whether erlotinib is antiproliferative and whether combining erlotinib with IFN α increased the antitumour effect over either alone. Given recent clinical research that suggests that effective inhibition might be related to the activation status of HER1/EGFR, or to the presence of mutations in the TK domain of the receptor [14], we also determined the mutation status of the cell lines tested and of 14 bladder cancer tissue samples.

MATERIALS AND METHODS

Seven human bladder cancer cell lines of different histological grades were used (Table 1) [15–21]; BL-28/0, BL-17/2 and BL17/0/X1 were established by us. Other cell lines were gifts (Table 1) including HT1376, MGHU1R, MGHU3 and T24. Two colon cancer cell lines, SW620 (HER1/EGFR-negative) and HCT116 (HER1/EGFR-positive) used as controls, were from the American Type Culture Collection (Manassas, VA, USA).

Bladder cancer tissues were from 14 consecutive patients undergoing cystoscopy at the Department of Urology, St Vincent's Hospital, in 2003–2004, with informed consent (Approval by the Human Research Ethics Committee of the Hospital, H03/044). Tumours were snap-frozen in liquid nitrogen within minutes of excision. Twelve were noninvasive/superficial (Ta or T1 tumours) and two were muscle-invasive TCCs.

Cells were grown in RPMI-1640 with 10% fetal bovine serum, 2 mM L-glutamine,

Cell line	Tumour stage	Tumour grade	Reference
BL-17/0/X1	T4b	III	Russell <i>et al.</i> [15]
BL-17/2	T4b	III	Brown <i>et al.</i> [16]
BL-28/0	T4b	II	Russell <i>et al.</i> [17]
HT1376	T2	III	Rasheed <i>et al.</i> [18]
MGH-U1R	NR	III	Lin <i>et al.</i> [19]
MGH-U3	NR	I	Zhang <i>et al.</i> [20]
T24	NR	III	Bubenik <i>et al.</i> [21]

TABLE 1

The origins of the bladder cancer cell lines

NR, not recorded.

penicillin and streptomycin at 37 °C in humidified 5% CO₂/air. Cells were fed every 3–4 days, and harvested using 0.02% EDTA-PBS (ICN, Aurora, OH, USA).

For the crystal violet colorimetric assay, cells were cultured in 24-well plates at 5×10^4 /well for 72 h, then treated with different agents (see Results), washed with PBS with divalent cations (Sigma, St. Louis, MO, USA), stained with 0.5% crystal violet, rinsed in distilled water and air-dried, then lysed with 100 μ L 2% sodium deoxycholate per well. Absorbance was measured at 540 nm using a plate reader (Tecan, Grodig, Salzburg, Austria).

Cell-surface HER1/EGFR levels at 72 h of culture were measured by flow cytometry. Cells seeded in 60 \times 15 mm dishes were harvested, washed twice with ice-cold PBS and counted. Mouse monoclonal antibody against the extracellular domain of human HER1/EGFR (MO886, Dako, Carpinteria, CA, USA; 100 μ L, 1/100 in PBS) or an irrelevant IgG2b isotype-matched control in the same volume and dilution, was added to the cells (1×10^6 cells) for 60 min on ice. Cells were then washed, incubated with fluorescein-isothiocyanate-conjugated goat-anti-mouse IgG (81–6511, Zymed, Invitrogen, Carlsbad, CA, USA; 100 μ L, diluted 1/200 in PBS) on ice for 30 min in the dark, washed twice and resuspended in 1% formaldehyde in PBS. Cell surface immunofluorescence was analysed on a FACSCalibur (Beckton-Dickinson, Franklin Lakes, NJ, USA) using CELLQuest software.

For HER1/EGFR mutation analysis, DNA was extracted from cell lines and bladder cancer tissues using a DNA extraction kit (Qiagen, Hilden, Germany). The entire TK domain of the *HER1/EGFR* gene, including exons 18–24 and all relevant donor/acceptor splice sites, was amplified and pre-screened for sequence variation using denaturing gradient gel electrophoresis (DGGE). DGGE primers were designed using the 'melt87'

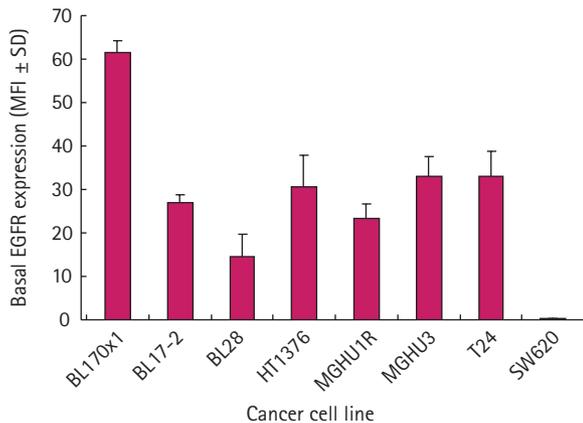
computer program. Amplicons were electrophoresed in 9% polyacrylamide gel (acrylamide : bisacrylamide; 37.5 : 1) containing a 40% differential urea and formamide (100% is 7 M urea/40% deionized formamide) denaturing gradient in a tris-borate-EDTA buffer, at 60 °C and 110 V overnight. DGGE conditions were optimized as previously described [22]. Heterozygous sequence variation was identified after ethidium bromide staining as four DGGE bands (two homoduplex and two heteroduplex); a lack of sequence variation was depicted as a single unshifted DGGE band. Amplicons showing aberrant DGGE banding patterns were sequenced (ABI 3100 sequencer, using dye terminator sequencing kit, Applied Biosystems, Foster City, USA).

IFN α (recombinant IFN α -2a) was provided by Roche Pharmaceuticals, Dee Why, Sydney, Australia with specific activity of 2.7×10^6 IU/mg, and erlotinib, by OSI Pharmaceuticals, Uniondale, NY, USA. Antiproliferative effects obtained with IFN α plus erlotinib were analysed using the Chou and Talalay method [23] on CalcuSyn software (Biosoft, Cambridge, UK). Interaction between drugs A and B was assessed using an automatically computed combination index (Col), determined at 50%, 75% and 90% inhibition of cell growth. The Col was defined as:

$$Col_{A+B} = (D_{A/A+B})/D_A + (D_{B/A+B})/D_B + \alpha(D_{A/A+B})(D_{B/A+B})/D_A D_B$$

where Col_{A+B} is the Col for a fixed effect (F) for the combination of drug A and a drug B; D_{A/A+B} is the concentration of drug A in the combination A + B giving an effect F; D_{B/A+B} is the concentration of drug B in the combination A + B giving an effect F; D_A is the concentration of drug A alone giving an effect F; D_B is the concentration of drug B alone giving an effect F; and α is a parameter with value 0 when A and B are mutually exclusive

FIG. 1. Basal cell-surface HER1/EGFR expression measured by flow cytometry in triplicate samples of individual cell lines at 72 h of culture. Protein expression was estimated as the mean fluorescence intensity of anti-HER1/EGFR minus that of the isotype-matched antibody control, counting 10 000 singlet and viable cells/treatment. The receptor expression is shown for each bladder cancer cell line and control colon cancer cell line (SW620) as mean (SD) fluorescence intensity. Experiments were done in duplicate.



Cell line	Number of experiments	Mean (SD) EC ₅₀ at [erlotinib] $\mu\text{g}/\text{mL}$	Mean (SD) EC ₅₀ at [erlotinib] $\mu\text{mol}/\text{L}$
BL17/0/X1	5	2.2 (1.4)	5.1 (3.2)
BL17/2	5	3.8 (2.7)	8.8 (6.2)
BL28/0	4	6.4 (2.9)	14.8 (6.7)
HT1376	5	4.6 (1.2)	10.7 (2.7)
MGHU1R	5	6.9 (1.9)	16.0 (4.4)
MGHU3	5	3.1 (0.7)	7.2 (1.6)
T24	5	6.1 (2.3)	14.1 (5.3)

TABLE 2
The EC₅₀ of erlotinib for treating bladder cancer cell lines

and 1 for mutually nonexclusive drugs that have independent modes of action. The Col indicates synergism when <0.90 , antagonism when >1.10 , and additivity when $0.90-1.10$.

ANOVA was used to evaluate quantitative HER1/EGFR expression across variables. The nonparametric Kruskal-Wallis or Mann-Whitney *U*-test was used to evaluate differences in percentage cell growth inhibition. A *post hoc* Bonferroni test was then used to detect differences between individual groups. Statistical values (two-tailed) of $P < 0.05$ or 95% CIs excluding zero were considered to indicate significance.

RESULTS

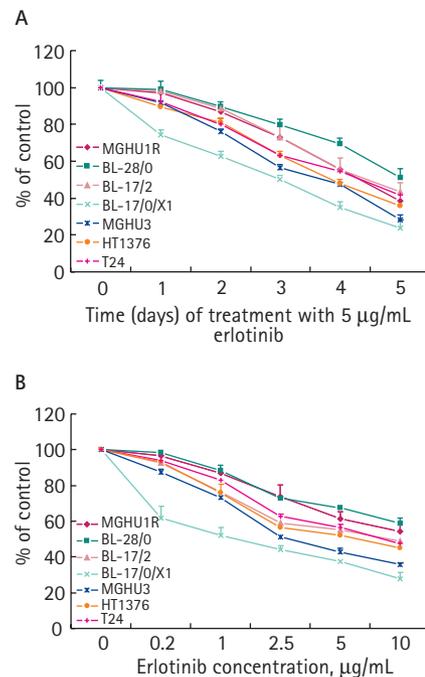
HER1/EGFR expression was assessed on each bladder cancer cell line to identify the feasibility of using individual cell lines in HER1/EGFR-targeted therapy experiments. Cells were harvested in the linear growth phase (72 h of culture) and measured by flow cytometry. MGHU1R, MGHU3, BL-17/2, BL-

17/0/X1, BL-28/0, HT1376 and T24 all expressed HER1/EGFR (Fig. 1). The control colon cancer cell line, HCT116, was HER1/EGFR-positive (data not shown) and SW620, HER1/EGFR-negative.

To select optimum times for the analysis of drug effects, the time course of growth of untreated cells was tested over 5 days. The bladder cancer lines differed in growth rates, with a more significant difference at 4 days (ANOVA, $P < 0.010$). No untreated lines reached confluence by 5 days (data not shown). We selected a linear growth phase period including day 4 for studying drug effects on treated cells for analysis of the degree of cooperativity at different times and drug concentrations.

Antiproliferative effects of erlotinib on bladder cancer cells were dose- and time-dependent. First, treatment of cells with $5 \mu\text{g}/\text{mL}$ ($11.6 \mu\text{mol}/\text{L}$, molecular weight 429.9) erlotinib with harvest at 1, 2, 3, 4, and 5 days, showed that the growth inhibitory effects of

FIG. 2. (A) Time-dependent growth inhibitory effect of erlotinib vs vehicle control on bladder cancer cell lines, and (B) dose-dependent growth inhibitory effects at 4 days of erlotinib on bladder cancer cell lines: the error bars show the variation of all replicates. Experiments were duplicated, with samples in triplicate.



the drug vs vehicle control (0.1% DMSO) were increased with increasing duration of culture (Fig. 2A). This trend was statistically significant for all bladder cancer cell lines (Kruskal-Wallis, all $P \leq 0.010$).

Second, the effects were dose-dependent. Using erlotinib at $0.2-10 \mu\text{g}/\text{mL}$ there was significantly greater growth inhibition at higher concentrations of erlotinib for all cell lines (Kruskal-Wallis, all $P \leq 0.015$; Fig. 2B). The mean 50% effective doses (ED₅₀) from quadruplicate or quintuplicate experiments were calculated using the CalcuSyn software (Table 2).

To improve the efficacy of erlotinib we added IFN α ; different doses of erlotinib ($0.1-2 \mu\text{g}/\text{mL}$) were used combined with $100 \text{ IU}/\text{mL}$ of IFN α , with significantly greater growth inhibition in the presence of higher concentrations of erlotinib for all cell lines (Fig. 3A, Kruskal-Wallis, all $P \leq 0.036$). The T24 cell line was least sensitive of all to the increasing doses of erlotinib in the experiment.

FIG. 3. Dose-dependent growth inhibitory effect at 4 days of the combined therapy: (A) different doses of erlotinib combined with 100 IU/mL of IFN α and (B) 1 μ g/mL erlotinib together with 50 or 100 IU/mL of IFN α . Cell growth inhibition is shown as the mean percentage of growth of combination therapy against vehicle control. The error bars show the variation of all replicates. Experiments were duplicated or triplicated, with duplicate samples within each experiment. For the single-drug control, 100 IU/mL of IFN α inhibited T24 cell growth at the same time by 9.8%, HT1376 by 27.2%, MGHU3 by 40.7%, BL28/0 by 30.9%, BL17/0/X1 by 49.8%, BL17/2 by 43.4% and MGHU1R by 47.1%, whilst 1 μ g/mL erlotinib delayed T24 cell growth by 12.9%, HT1376 by 24.2%, MGHU3 by 26.7%, BL28/0 by 11.5%, BL17/0/X1 by 37.9%, BL17/2 by 23.7% and MGHU1R by 13.2%.

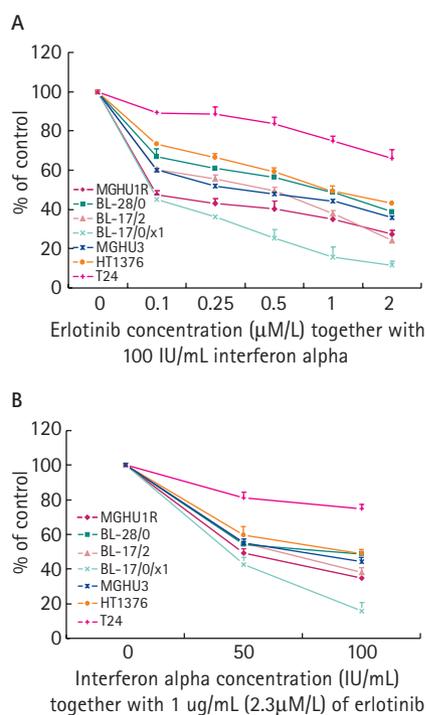


Figure 3B shows the effect of 1 μ g/mL erlotinib together with 50 or 100 IU/mL of IFN α , with significantly less cell growth in the presence of higher concentrations of IFN α for all bladder cancer lines (Mann–Whitney, all $P \leq 0.050$). T24 was again the least sensitive cell line of all to the increasing doses of IFN α in the experiment.

We used the isobologram method [14] to determine whether IFN α and erlotinib were synergistic. We extracted data from experiments performed in duplicate (MGHU3, HT1376) or triplicate (MGHU1R, BL-28/0, BL-

TABLE 3 The Col for treating bladder cancer cell lines with IFN α and erlotinib

Cell line (n)*	Col			Range	Significance
	ED ₅₀	ED ₇₅	ED ₉₀		
BL17/0/X1 (3)	0.2642	0.0195	0.0014	0.1–0.3	strong synergism
BL17/2 (3)	0.4469	0.1616	0.1415	0.3–0.7	synergism
BL28/0 (3)	0.2726	0.1128	0.0987	0.1–0.3	strong synergism
HT1376 (2)	0.1125	0.0483	0.0251	<0.1	very strong synergism
MGHU1R (3)	0.2007	0.0422	0.0195	<0.1	very strong synergism
MGHU3 (2)	0.1764	0.1276	0.1224	0.1–0.3	strong synergism
T24 (4)	1.7701	3.1790	5.7095	>1.10	antagonism

*number of experiments.

17/2, BL17/0/X1 and T24) including data from dose–response experiments (not shown) using single drugs (IFN α : 1, 10, 100, 1000, and 10 000 IU/mL for 1, 2, 3, 4 and 5 days; and erlotinib: 0.1, 0.25, 0.5, 1, 2, 5, 10 μ g/mL for 1, 2, 3, 4 and 5 days) or combined 100 IU/mL IFN α , and 50 IU/mL IFN α with 0.1, 0.25, 0.5, 1 and 2 μ g/mL erlotinib, respectively, for 4 days. When concomitant treatment was used, control cells were exposed to each drug alone at each dose.

We chose day 4 data from single drug dose–response experiments to establish the median–effect and dose–response plots using the CalcuSyn software. We entered concomitant treatment at two doses of IFN α (IU/mL) combined with selected erlotinib (μ g/mL) doses in a fixed ratio (IFN α /erlotinib 100/1.0 and 50/0.5) for establishing the plots for combined drugs. We checked effects of drugs used alone; they matched well with the single-drug plots. The software generated a report for each cell line and provided representative figures.

Results of these analyses are summarized in Table 3 and representative figures (Fig. 4Aa–e for median–effect plots and Fig. 4Ba–e for dose–response plots). The combination of IFN α and erlotinib resulted in heterogeneous growth inhibition among the bladder cancer cell lines, with two lines showing very strong synergism (HT1376 and MGHU1R), three a strong synergism (BL17/0/X1, BL28–0 and MGHU3), one synergism (BL17/2) and one antagonism (T24).

Seven bladder cancer cell lines and 14 bladder cancer tissues were screened for mutations in the TK domain (exons 18–24) of the *HER1/EGFR* gene; no rare sequence variants were detected. The previously reported single

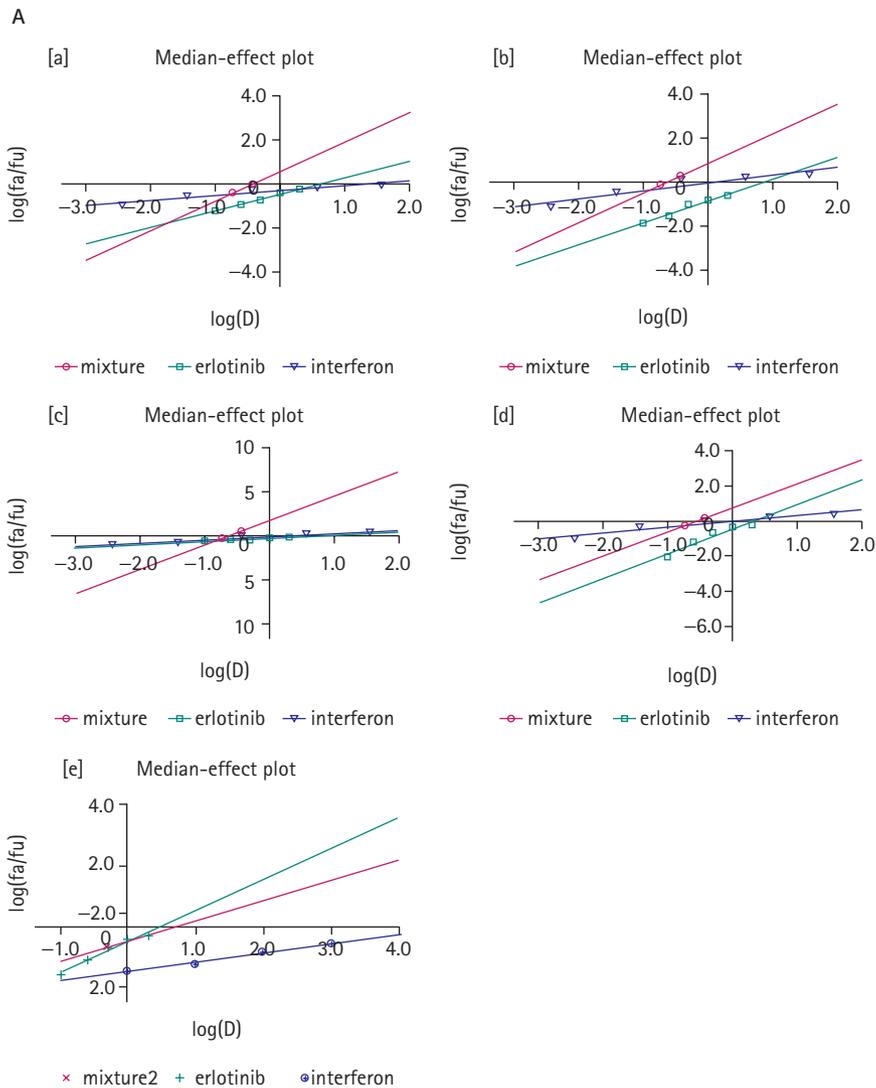
nucleotide polymorphisms in exon 20 (rs10251977, Gln787Gln G >A) and exon 23 (rs1140475, Thr903Thr C >T) occurred at an allele frequency of 0.73 and 0.09, respectively.

DISCUSSION

This study is the first to show that erlotinib, an HER1/EGFR TK inhibitor, inhibits the growth *in vitro* of several bladder cancer cell lines, similar to gefitinib [10]. However, this effect could be considered suboptimal according to others [24], who defined the sensitivity of lung cancer cell lines as >50% *in vitro* growth inhibition with erlotinib at <5 μ mol/L (2.1 μ g/mL). The only clinical reports on the use of IFN in bladder cancer relate to the finding that IFN α can inhibit superficial bladder cancer growth and reduce recurrence after intravesical administration [25]. We were encouraged by this experience to examine its efficacy when combined with erlotinib on invasive bladder cancer cell lines, as erlotinib can block EGF downstream signals that play a negative regulatory role on IFN α . We showed that combining IFN α treatment with erlotinib has a synergistic inhibitory effect, as evaluated by the isobologram method [23], suggesting that using the two drugs together can overcome the insensitivity of selected bladder cancer cell lines. This study is supported by our earlier report on colon cancer [12] and by others in head and neck cancer cells [26] using gefitinib and IFN α . These findings might have implications for identifying patients suitable for treatment, by improving HER1/EGFR-targeted therapy.

IFN α binds to cell membrane receptors, IFN α R1 and IFN α R2 [27], leading to activation of IFN α -associated Janus kinases, JAK1 and TYK2, that mediate cytokine-induced signal transduction [28]. Their

FIG. 4. (A) The median-effect plots and (B) the dose-effect plots of IFN α and/or erlotinib for representative bladder cancer cell lines: (a) HT1376 and (b) MGHU1R showed very strong synergism; (c) BL17/0/X1 showed strong synergism, with (d) BL17/2 showing synergism, and (e) T24 showing antagonism.



phosphorylation activates cytoplasmic signalling proteins including STAT (signalling transducers and activators of transcription) transcription factors, that regulate target gene expression, leading to cell growth inhibition [28]. This pathway co-operates in mediating downstream signalling by EGF and is enhanced in cells over-expressing EGF [29], providing a possible escape mechanism to overcome the antiproliferative effects of IFN α [30]. The resistance of T24 cells in this study to one IFN α treatment might reflect this mechanism. Downstream pathways of EGF-stimulated proliferation are associated with decreased antiproliferative effects of IFN α [31] and the chemical inhibition of these

pathways at different levels reverses that effect [28]. A recent study showed that inhibition of an alternative growth signalling pathway (platelet-derived growth factor, PDGF) can overcome resistance to HER1/EGFR TK inhibitors [32]. Such dual blockade might also account for the synergistic growth inhibition in the present study.

Recent data indicate no clear relationship between sensitivity to HER1/EGFR inhibition and the degree of HER1/EGFR expression [33]. By contrast, the presence of gain-of-function mutations in the TK domain of the *HER1/EGFR* gene has been associated with sensitivity to growth inhibition by

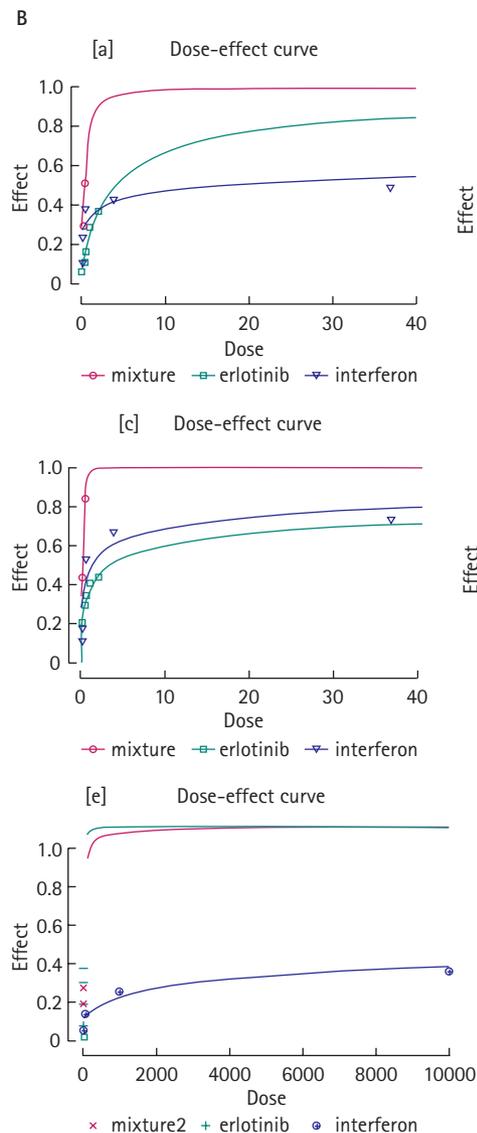
HER1/EGFR inhibitors in lung cancer [14]. Such gain of function might increase dependency on this pathway and so increase sensitivity to its inhibition [34,35]. In screening for similar mutations in colon cancer cell lines in our laboratories, we detected only one of 10 colon cancer cell lines with an *HER1/EGFR* mutation (unpublished data). Others have detected a single mutation (G-to-S substitution at amino acid 719) in only 1 of 293 colon cancer tissues [36]. Our results in the bladder cancer cell lines and tissues where no *HER1/EGFR* mutations were detected suggest that gain-of-function mutations [14] might be uncommon in cancers other than lung cancer, although more samples should be tested. Our data suggest that the successful use of TK inhibitors need not be confined to tumours that overexpress the *HER1/EGFR* gene or carry an enhancing mutation. It is possible that IFN α might induce a 'gain of function' phenotype that enhances EGF dependence, thus increasing susceptibility to HER1/EGFR inhibition.

Erlotinib inhibits activation of the HER1/EGFR intracellular TK and downstream signalling pathways such as PI3K/Akt and Ras/Raf/MAPK [5]. Recent data show that lung tumours with higher baseline phosphorylation of downstream signals, such as PI3K/Akt, are more likely to respond to gefitinib [36]. Thus an alternative explanation for our results might be that IFN α , by enhancing signalling of the JAK/STAT and PI3K/Akt pathways, sensitises the cells to the HER1/EGFR inhibitor, reducing the dose required to block relevant shared pathways of growth stimulation in the absence of mutations.

The synergy might also occur through a direct effect of IFN α on HER1/EGFR synthesis and function; IFN α induces expression of IFN regulatory factor-1, a transcription factor that modulates the expression of many genes including *HER1/EGFR* [37]. Alternatively, down-regulation of phosphorylation and/or alterations in affinity or bound complex internalization might enhance inhibition of HER1/EGFR activity [28].

The concentrations of 100 IU/mL for IFN α and 5 $\mu\text{mol/L}$ (2.1 $\mu\text{g/mL}$) for erlotinib used here are comparable to serum levels achieved in humans treated with current dose schedules [38,39]. Combined therapy at half of the concentration of both drugs achieved a

FIG. 4. Continued



synergistic antiproliferative effect. The clinical implication is one of enhanced anticancer efficacy with no greater toxicity. Whether alternative routes of administration, e.g. intravesical, would enhance this effect requires additional modelling.

In the present study we examined the broadest range of bladder cancer cell lines yet reported, and this encourages further study of this drug combination approach to therapy. Future studies will focus on the mechanism of synergy induced by combined treatment, by examining the activity of target receptors including HER1/EGFR and IFN α R1/2, key downstream signalling factors, e.g. STATs, Akt, ERK1/2, and PDGF signalling markers, and

how cell growth is inhibited (cell cycle and apoptosis analysis). Our focus will be to identify reliable predictors of which patients are most likely to benefit from this combined approach to targeted therapy.

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CONFLICT OF INTEREST

Jia-Lin Yang, Xian-Jun Qu, Pamela J. Russell and David Goldstein are all patent inventors with OSI (no financial gain).

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Abbreviations: EGF(R), epidermal growth factor (receptor); TK, tyrosine kinase; ED_x, the effective dose for x% inhibition; IFN, interferon; DGGE, denaturing gradient gel electrophoresis; ColI, combination index; PDGF, platelet-derived growth factor.