Cytotoxic Effects of Gemcitabine-containing Regimens against Human Non-Small Cell Lung Cancer Cell Lines Which Express Different Levels of p185<sup>neu</sup>

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ABSTRACT

A novel pyrimidine analogue, gemcitabine, has been found to inhibit DNA replication and repair. We speculated that gemcitabine in combination with DNA-damaging agents might be more active against high- than low-p185<sup>neu</sup>-expressing non-small lung cancer (NSCLC) cells because the high-p185<sup>neu</sup> expressors were proposed to possess a more effective DNA repair ability. We therefore compared the combination effects of gemcitabine plus cisplatin, gemcitabine plus etoposide, and cisplatin plus etoposide in a panel of 12 NSCLC cell lines. We also investigated the correlations between the level of p185<sup>neu</sup> and the cytotoxicities of each single agent and the three combinations. We found that as single agents the cytotoxicities of cisplatin and etoposide but not gemcitabine were significantly correlated with the level of p185<sup>neu</sup>. In contrast to the tight cross-resistance between cisplatin and etoposide, gemcitabine demonstrated little cross-resistance to either etoposide or cisplatin. Both gemcitabine-containing combinations demonstrated equivalent or more active cytotoxicities compared to cisplatin plus etoposide, with gemcitabine plus cisplatin showing a greater synergistic activity which was effect (dose) dependent. The effect of cisplatin plus etoposide was not p185<sup>neu</sup>-related, whereas gemcitabine-containing regimens, especially gemcitabine plus cisplatin, had a greater cytotoxicity against the high- than the low-p185<sup>neu</sup> expressors. Our findings indicate that gemcitabine in combination with cisplatin is active against NSCLC cells in vitro. The gemcitabine-cisplatin interaction is more active than the etoposide-cisplatin interaction in cells with high-p185<sup>neu</sup> expression.

INTRODUCTION

NSCLC<sup>3</sup> tumors are notorious for their resistance to chemotherapeutic agents. Most chemotherapeutic drugs, both alone and in combination, are not particularly active against this type of tumor and are at best strictly palliative in the clinical setting. Although current cisplatin-based combination chemotherapeutic regimens produce objective tumor responses in up to 40% of patients with advanced NSCLC, the routine use of chemotherapy in these patients remains controversial (1, 2). To improve the likelihood of response, response duration, and survival, it is clear that more active drugs or drug combinations are required.

Using large panels of lung cancer cell lines and determining their chemosensitivity profiles for several commonly used cytotoxic drugs, we and others have demonstrated that in vitro activity of most, if not all, of the drugs were highly correlated with each other (3–5). In NSCLC, the spectrum of cross-resistance/sensitivity observed is closely associated with the expression of the HER-2/neu gene (6–8). The HER-2/neu gene encodes a transmembrane glycoprotein receptor (p185<sup>neu</sup>) with tyrosine kinase activity (9). Overexpression of the HER-2/neu gene is encountered in >30% of NSCLC tumors but not in small cell lung cancer tumors (10–12). Enhanced chemoresistance associated with the elevated levels of p185<sup>neu</sup> in HER-2/neu-transfected NSCLC cells suggested the possibility that a high level of p185<sup>neu</sup> confers multiple-drug resistance to certain human cancers (13).

Previously, we have demonstrated that caffeine enhances chemosensitivity to a greater degree in high- than in low-p185<sup>neu</sup>-expressing NSCLC cell lines (7). Caffeine is known to enhance cell killing by overriding the cell cycle arrest triggered by DNA-damaging agents (14, 15). It is presumed that cell cycle arrest induced by such agents would allow cells to repair DNA lesions (16, 17). Our findings (7), therefore, suggested that the chemoresistant high-p185<sup>neu</sup>-expressing cells might utilize DNA repair machinery more effectively, attenuating the lethal effects of chemotherapeutic agents, and that this survival advantage would be diminished by caffeine.

Gemcitabine (2′,2′-difluorodeoxycytidine) is a novel pyrimidine analogue with structural and metabolic similarities to cytarabine (arabino-uracil). Compared to cytarabine, gemcitabine is, however, markedly distinctive in its cellular pharmacology and biological action and exhibits a much wider spectrum of activity against murine solid tumors and human tumor xenografts (18, 19). Gemcitabine is a prodrug that requires the intracellular conversion by deoxycytidine kinase to its active forms, gemcitabine diphosphate and gemcitabine triphosphate. Gemcitabine triphosphate competes directly with dCTP as a substrate for incorporation into DNA by DNA polymerases (20). The gemcitabine metabolic pathway exhibits an interesting and unique combination of self-potentiating mechanisms whereby gemcitabine diphosphate contributes to a reduction of cellular dCTP levels (21), facilitating both increased gemcitabine phosphorylation by reducing the inhibition of deoxycytidine kinase and decreased elimination by inactivating dCMP deaminase (22). This results in the accumulation of the active metabolites within the cell, and hence continuing inhibition of cellular DNA synthesis and repair. The inherent ability of gemcitabine to inhibit DNA synthesis and repair led us to propose the following hypotheses: (a) the intrinsic chemoresistance for gemcitabine of NSCLC cells might not be tightly correlated with the level of p185<sup>neu</sup>; and (b) DNA-damaging agents when combined with gemcitabine might be more active against the high-p185<sup>neu</sup>-expressing NSCLC cell lines, which are presumably able to repair damaged DNA more effectively as compared to the low-p185<sup>neu</sup>-expressing cell lines.

Currently, cisplatin in combination with etoposide is one of the most commonly used chemotherapeutic regimens in the treatment of NSCLC. This combination has been shown to have synergistic or additive antitumor activity in NSCLC cells in a variety of experimental models (23–25). The combination of cisplatin and etoposide produces an objective response in 30–35% of patients with advanced NSCLC (1, 2). In the present study, we have evaluated and compared the combination effects of gemcitabine plus cisplatin, gemcitabine plus etoposide, and cisplatin plus etoposide at the cellular level using a panel of NSCLC cell lines. We have also investigated the correlations between the level of p185<sup>neu</sup> and the cytotoxicities of each single agent and the three drug combinations.
MATERIALS AND METHODS

Cell Lines. Twelve NSCLC cell lines were studied. There were eight adenocarcinomas (NCI-H23, H322, H358, H820, H1355, H1435, and H1437); one adenosquamous carcinoma (H125); and three large cell carcinomas (H460, H1155, and H1299). These cell lines expressed various levels of p185e5 (6—8). All lines were derived from previously untreated patients and were grown in RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum and adapted in RPMI 1640 medium supplemented with 5% fetal bovine serum for >6 months before this study. All cell lines were in the logarithmic phase of growth at the time of the drug sensitivity assays.

Quantitative Measurement of the P185e5 Protein. Nearly confluent cells were harvested by scraping, followed by centrifugation to form a cell pellet. Cells were incubated in lysis buffer [10 mM Tris-HCl (pH 7.6), 1.5 mM EDTA, 10% glycerol, and 0.1% sodium azide] and Dounce homogenized. After homogenization the proteins were detergent extracted. After centrifugation, supernatants were collected and protein concentrations determined. The protein samples were diluted with lysis buffer, mixed with 2x loading buffer, and boiled for 5 min. The samples were resolved on a 10% polyacrylamide gel. After electrophoresis, the proteins were transferred to a nitrocellulose membrane. The blots were blocked with 5% non-fat milk in TBST (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween 20) and incubated with the following antibodies: monoclonal antibodies NB-3 (coated onto microtiter wells) and TA-1 (biotin labeled) (human neu assay kits: Oncogene Science, Inc., Uniondale, NY; Ref. 7). The biotin-labeled TA-1 was detected using a streptavidin immunoperoxidase conjugate and of each two-drug combination. In vitro drug combination testing was designed and performed using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide colorimetric assay as described in detail previously (26, 27). The single drugs were tested over seven concentrations (in 0.5- or 1-log increments) to cover the entire dose-response curves whenever possible. Sixteen survival curves were generated from every set of experiments, one from each of the single drugs and 14 from the 49 pairs of drug combinations (27). The drugs were dissolved in PBS to 1 mM, and then all were subsequently diluted in culture medium. For gemcitabine-containing regimens, gemcitabine was added 20 min before the addition of the second drug. For cisplatin plus etoposide, etoposide was added 20 min after the addition of cisplatin. The percentage of control absorbance was considered to be the surviving fraction of cells, and the IC50, IC50, and IC50 values were defined as the concentrations of drug that produced 50, 50, and 70% reduction in control absorbance, respectively. The results were the means of three independently performed assays. Each assay was performed in four wells at the same time.

Identification of the Combination Effects. We used the classical isobole method (26—28) to determine the in vitro effects of drug combinations. The CI
was used to express the cytotoxic effects of drug combinations. For detecting true in vitro synergy between drug combinations, there is still controversy over which method is best (29). The classical isobole method with application of the CI is one of the most commonly used methods. By using this method to test a variety of drug combinations, our previous findings were in accordance with the findings obtained from other experimental model systems as well as with the clinical observations (7, 26, 27). The CI was defined as the sum of the relative doses of each drug which yields an isoeffect cell kill when added together. For example, for the combination of drug A + drug B, CI = (dose of drug A/ICr of drug A) + (dose of drug B/ICX of drug B). Here, the dose of drug A and dose of drug B when added together yielded an isoeffect (% cell kill). The CI at the 30%, 50%, and 70% effect levels were determined and designated as CI30, CI50, and CI70. Within the designed assay range, a set of CI values or data points was generated because there were multiple drug concentrations that achieved the same isoeffect. In the present study, the mean values of the survival fractions of the three replicate tests were used to generate the set of CI values and construct the isobole for a particular cell line and drug combination. The mean CI value for this set was reported as the summary measure. Upper and lower bounds of 1.05 and 0.95 were selected as being of interest (based on a preset “null” interval of 0.95–1.05), so that mean CI values >1.05 or <0.95 are interpreted as being suggestive of antagonism and synergism, respectively.

**Data Analysis.** Sign tests were performed on each set of CI values to formally evaluate whether antagonism or synergism was evident for a particular cell line, drug combination, and effect levels. In addition, Wilcoxon signed rank tests were computed to evaluate whether significant differences in the cell line means occurred as compared to a null hypothesized CI of 1 and to analyze the differences of drug interactions of the tested regimens. The Spearman rank correlation was used to analyze the correlations between p185 expression and the IC values of the drug. The Mann-Whitney U rank sum test was used to test for differences in the IC values of drugs and the CI values of drug combinations of the six cell lines with low and the other six cell lines with high p185 expression. All tests were two sided, and significance was assumed if \( P < 0.05 \).

### RESULTS

**Little Cross-resistance between Gemcitabine and Either Cisplatin or Etoposide.** In this panel of cell lines, we found that the inhibitory concentrations of cisplatin versus etoposide were closely correlated through the effect levels studied (all \( P \) values ≤ 0.009, Fig. 1, left set of panels). Gemcitabine toxicity correlated with etoposide toxicity (\( P = 0.025 \) and 0.0413 for IC50 and IC70, respectively, Fig. 1, middle center and middle bottom panels) but correlated weakly if at all with cisplatin toxicity (all \( P \) values ≥ 0.067, Fig. 1, right set of panels). As compared to the tight cross-resistance between cisplatin and etoposide, gemcitabine showed little cross-resistance to either cisplatin or etoposide.

**Correlation of the Level of p185**\(^\text{new}^{*}\) **with the Cytotoxic Effects of Cisplatin and Etoposide, but not with the Effect of Gemcitabine.** The 12 cell lines utilized were divided into two equal groups according to the level of p185\(^\text{new}^{*}\) expressed, high and low expressors (Table 1). The values of p185\(^\text{new}^{*}\), IC30, IC50, and IC70 of gemcitabine, cisplatin, and etoposide of individual cell lines as well as their mean values of the total and the two subgroups are shown in Table 1. In the entire panel of 12 cell lines, the cytotoxicities of cisplatin and etoposide but not of gemcitabine showed a statistically significant correlation with the level of p185\(^\text{new}^{*}\) at all of the effect levels studied (Fig. 2).

### Table 1 Chemosensitivities for single agents of NSCLC cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>p185(^\text{new}^{*}) (HNU/µg)</th>
<th>Cisplatin (µM)</th>
<th>Etoposide (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1155</td>
<td>0.62 (0.05)</td>
<td>1.07 (0.055)</td>
<td>0.337 (0.027)</td>
</tr>
<tr>
<td>H23</td>
<td>2.95 (0.12)</td>
<td>0.323 (0.033)</td>
<td>0.029 (0.008)</td>
</tr>
<tr>
<td>H1299</td>
<td>3.57 (0.29)</td>
<td>1.472 (0.033)</td>
<td>0.021 (0.002)</td>
</tr>
<tr>
<td>H460</td>
<td>4.81 (1.08)</td>
<td>0.208 (0.021)</td>
<td>0.021 (0.007)</td>
</tr>
<tr>
<td>H358</td>
<td>11.62 (1.65)</td>
<td>0.513 (0.016)</td>
<td>0.007 (0.001)</td>
</tr>
<tr>
<td>H125</td>
<td>12.65 (0.74)</td>
<td>1.060 (0.015)</td>
<td>0.020 (0.003)</td>
</tr>
<tr>
<td>Mean</td>
<td>6.04 (2.01)</td>
<td>0.776 (0.194)</td>
<td>0.227 (0.068)</td>
</tr>
<tr>
<td>H820</td>
<td>26.34 (1.76)</td>
<td>0.751 (0.137)</td>
<td>0.876 (0.077)</td>
</tr>
<tr>
<td>H1347</td>
<td>29.16 (5.04)</td>
<td>1.709 (0.190)</td>
<td>0.633 (0.064)</td>
</tr>
<tr>
<td>H322</td>
<td>35.75 (3.85)</td>
<td>0.960 (0.229)</td>
<td>0.446 (0.044)</td>
</tr>
<tr>
<td>H1355</td>
<td>42.06 (3.21)</td>
<td>1.473 (0.018)</td>
<td>0.048 (0.008)</td>
</tr>
<tr>
<td>H1435</td>
<td>51.70 (3.82)</td>
<td>2.645 (0.008)</td>
<td>0.087 (0.001)</td>
</tr>
<tr>
<td>H522</td>
<td>73.94 (4.00)</td>
<td>2.279 (0.089)</td>
<td>0.197 (0.038)</td>
</tr>
<tr>
<td>Mean</td>
<td>43.50 (7.29)</td>
<td>2.643 (1.049)</td>
<td>0.232 (0.109)</td>
</tr>
</tbody>
</table>

### Notes

\* IC30, IC50, and IC70 designated for the inhibitory concentrations of drugs required to inhibit cell growth by 30, 50, and 70%, respectively. Numbers in parentheses. SE.

\^ Statistical evaluation using the Mann-Whitney U rank sum test for the differences between the low- and the high-p185\(^\text{new}^{*}\) groups. All statistical tests were two-sided, and statistical significance was assumed if \( P < 0.05 \).
GEMCITABINE-CONTAINING REGIMENS AGAINST NSCLC CELLS

30% Effect Level  50% Effect Level  70% Effect Level

(A) Cisplatin

(B) Etoposide

(C) Gemcitabine

Fig. 2. Correlations between the level of p185^neu^ and chemosensitivities for cisplatin, etoposide, and gemcitabine of the 12 tested cell lines at the 30, 50, and 70% effect levels. The immunor assay for detection and quantitation of p185^neu^ was a sandwich assay utilizing monoclonal antibodies NB-3 and TA-1. Results expressed as HNU/µg protein were the mean values of three separate assays (each assay was performed in duplicate wells). (O), high-p185^neu^ expressors; (□), low-p185^neu^ expressors. The R and P values calculated using the Spearman rank correlation test are labeled.

The high-p185^neu^ group was more chemoresistant when treated with cisplatin and etoposide but not with gemcitabine as compared to the low-p185^neu^ group (Table I). The differences of the mean IC values of cisplatin and etoposide but not of gemcitabine between the two cell line groups were statistically significant for all three effect levels studied, except that of cisplatin at the 30% effect level where the difference was marginally significant (P = 0.055; Table I).

More Active Cytotoxicities of Gemcitabine-containing Regimens, Especially Gemcitabine Plus Cisplatin. In the entire panel of cell lines, gemcitabine-containing regimens showed an additive effect, and cisplatin plus etoposide demonstrated a synergistic effect at the 30% effect level (Table 2); however, the difference between the mean CI values of any two combinations was minimal and not statistically significant (Fig. 3A). At higher (50 and 70%) effect levels, all of the combinations demonstrated synergistic interactions, but the mean CI values of the gemcitabine-containing regimens were smaller than those of cisplatin plus etoposide (Fig. 3A), indicating a greater degree of synergy. At the 70% effect level, gemcitabine plus cisplatin was statistically more active than cisplatin plus etoposide (P = 0.015, Fig. 3A). We also found that the synergistic combination effects of gemcitabine plus cisplatin was effect dependent (Fig. 3A; r = -0.998, P = 0.037 by simple correlation).

Greater Enhancement of the Cytotoxicities of DNA-damaging Agents by Gemcitabine in the High-p185^neu^-expressing Cell Lines. In the low-p185^neu^ group, the three combination regimens, in general, demonstrated additive or marginally synergistic effects at the 30 and 50% effect levels and synergistic effects at the 70% effect level (Table 2 and Fig. 3B), but the differences between the mean CI values of

797
### Table 2 Mean values of CI of two-drug combinations

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CI&lt;sub&gt;10&lt;/sub&gt;</th>
<th>CI&lt;sub&gt;50&lt;/sub&gt;</th>
<th>CI&lt;sub&gt;70&lt;/sub&gt;</th>
<th>CI&lt;sub&gt;10&lt;/sub&gt;</th>
<th>CI&lt;sub&gt;50&lt;/sub&gt;</th>
<th>CI&lt;sub&gt;70&lt;/sub&gt;</th>
<th>CI&lt;sub&gt;10&lt;/sub&gt;</th>
<th>CI&lt;sub&gt;50&lt;/sub&gt;</th>
<th>CI&lt;sub&gt;70&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>H115</td>
<td>1.035</td>
<td>0.995</td>
<td>0.902</td>
<td>0.987</td>
<td>0.927</td>
<td>0.857</td>
<td>0.103</td>
<td>1.003</td>
<td>1.033</td>
</tr>
<tr>
<td>H23</td>
<td>(0.02)</td>
<td>(0.018)</td>
<td>(0.019)</td>
<td>(0.026)</td>
<td>(0.011)</td>
<td>(0.017)</td>
<td>(0.031)</td>
<td>(0.022)</td>
<td>(0.014)</td>
</tr>
<tr>
<td>H1299</td>
<td>0.825</td>
<td>0.813</td>
<td>0.743</td>
<td>0.867</td>
<td>0.855</td>
<td>0.625</td>
<td>0.968</td>
<td>0.977</td>
<td>0.933</td>
</tr>
<tr>
<td>H460</td>
<td>1.100</td>
<td>1.005</td>
<td>0.969</td>
<td>1.151</td>
<td>1.081</td>
<td>1.054</td>
<td>0.889</td>
<td>0.887</td>
<td>0.867</td>
</tr>
<tr>
<td>H358</td>
<td>(0.056)</td>
<td>(0.037)</td>
<td>(0.035)</td>
<td>(0.071)</td>
<td>(0.041)</td>
<td>(0.024)</td>
<td>(0.033)</td>
<td>(0.019)</td>
<td>(0.021)</td>
</tr>
<tr>
<td>H125</td>
<td>0.982</td>
<td>0.935</td>
<td>0.889</td>
<td>0.930</td>
<td>0.912</td>
<td>0.881</td>
<td>0.897</td>
<td>0.903</td>
<td>0.882</td>
</tr>
<tr>
<td>Mean&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.981</td>
<td>0.962</td>
<td>0.874</td>
<td>0.972</td>
<td>0.942</td>
<td>0.859</td>
<td>0.938</td>
<td>0.942</td>
<td>0.910</td>
</tr>
<tr>
<td>Group results&lt;sup&gt;d&lt;/sup&gt;</td>
<td>A</td>
<td>A</td>
<td>S</td>
<td>A</td>
<td>A</td>
<td>S</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Total, 12 cell lines</td>
<td>A</td>
<td>S</td>
<td>A</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

<sup>a</sup> See text.
<sup>b</sup> CI<sub>10</sub>, CI<sub>50</sub>, and CI<sub>70</sub> were designated for the group mean values of CI at the 30, 50, and 70% effect levels, respectively. Mean CI values > 1.05 or < 0.95 are interpreted as being suggestive of antagonism and synergism, respectively. Numbers in parentheses, SE.
<sup>c</sup> Wilcoxon signed rank tests were computed to evaluate whether significant differences in the group means occurred as compared to a null hypothesized CI of 1.
<sup>d</sup> Group results (analyzed using the Wilcoxon signed rank test). A, additive; Ant, antagonistic; S, synergistic.
<sup>e</sup> Statistical evaluations using the Mann-Whitney U rank sum test for the differences between the low- and the high-pl85<sup>eu</sup> groups. All statistical tests were two-sided, and significance was assumed if P < 0.05.

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**Fig. 3. The mean values of the CI (see text) of gemcitabine plus cisplatin (G + C), gemcitabine plus etoposide (G + V), and cisplatin plus etoposide (C + V) at the 30, 50, and 70% effect levels are plotted to demonstrate the differences of drug interactions of the tested regimens which were statistically analyzed using the Wilcoxon signed rank test.**

**Shaded area,** additivity based on null interval of 0.95–1.05. 
A, the entire panel of cell lines; B, the low-pl85<sup>eu</sup> expressors; and C, the high-pl85<sup>eu</sup> expressors. * G + C versus C + V, P = 0.015; ** G + C versus G + V and G + C versus C + V, P = 0.028. In the entire panel of cell lines, gemcitabine-containing regimens (particularly, gemcitabine plus cisplatin) demonstrated greater synergistic effects than the combination of cisplatin plus etoposide at middle and higher effect levels, presumably primarily related to their more active effects against the high-pl85<sup>eu</sup>-expressing cells.**
Low-p185<sub>neu</sub> expressing cell line NCI-H460  
High-p185<sub>neu</sub> expressing cell line NCI-H1355

(A) Gemcitabine + Cisplatin

\[
\begin{align*}
\text{mCl}_{30} &= 1.100 \pm 0.056 (7) \\
\text{mCl}_{50} &= 1.005 \pm 0.037 (7) \\
\text{mCl}_{70} &= 0.969 \pm 0.035 (9) \\
mCl_{30} &= 0.830 \pm 0.045 (7) \ast \\
mCl_{50} &= 0.812 \pm 0.031 (8) \ast\ast \\
mCl_{70} &= 0.627 \pm 0.058 (9) \ast\ast\ast 
\end{align*}
\]

(B) Gemcitabine + Etoposide

\[
\begin{align*}
\text{mCl}_{30} &= 1.151 \pm 0.071 (6) \\
\text{mCl}_{50} &= 1.081 \pm 0.041 (7) \\
\text{mCl}_{70} &= 1.054 \pm 0.024 (9) \\
mCl_{30} &= 0.978 \pm 0.014 (6) \\
mCl_{50} &= 0.802 \pm 0.028 (8) \ast\ast \\
mCl_{70} &= 0.822 \pm 0.036 (10) \ast\ast\ast 
\end{align*}
\]

(C) Cisplatin + Etoposide

\[
\begin{align*}
\text{mCl}_{30} &= 0.889 \pm 0.033 (6) \\
\text{mCl}_{50} &= 0.887 \pm 0.019 (7) \ast \\
\text{mCl}_{70} &= 0.867 \pm 0.021 (8) \ast\ast \\
mCl_{30} &= 0.847 \pm 0.067 (6) \\
mCl_{50} &= 0.867 \pm 0.017 (8) \ast\ast \\
mCl_{70} &= 0.793 \pm 0.042 (9) \ast\ast\ast 
\end{align*}
\]

Fig. 4. The isobolograms of gemcitabine plus cisplatin (A), gemcitabine plus etoposide (B), and cisplatin plus etoposide (C) against the low-p185<sup>neu</sup>-expressing NCI-H460 (left panel) and the high-p185<sup>neu</sup>-expressing NCI-H1355 (right panel) cell lines at 30, 50, and 70% effect levels are shown. The values of the mean CI (see text) for a particular cell line, drug combination, and effect levels are also labeled. mCl<sub>30</sub>, mCl<sub>50</sub>, and mCl<sub>70</sub> were designated for the mean CI values at the 30, 50, and 70% effect levels, respectively. Numbers in parentheses, number of data points. Data points at 30, 50, and 70% effect levels (signified as x, □, and ●, respectively) were generated from the mean survival fractions of the three replicate experiments and were fitted to construct the isoboles (by 3rd order of polynomial, with all R values >0.964). Data points above the dashed diagonal line of the additive effect in the isobole suggest antagonism and those below the diagonal suggest synergism. Sign tests were applied on each set of data points to formally evaluate whether synergism or antagonism was evident for a particular cell line, drug combination, and effect levels. * P < 0.05; ** P < 0.01; *** P < 0.005. Although the effects of gemcitabine-containing regimens were additive against NCI-H460 and statistically synergistic against NCI-H1355, the effects of cisplatin plus etoposide were synergistic against both cell lines. In general, the cytotoxic effects of all combinations against these two cell lines tend to be more active at higher effect levels (isoboles shift leftward toward the axis and decrease in the mean CI values with the increase of the combination effects), with gemcitabine plus cisplatin against NCI-H1355 showing the greatest activity.
these regimens were not statistically significant throughout the studied effect levels (Fig. 3B). In contrast, in the high-p185 neu group (Fig. 3C), while all combinations showed synergistic interactions across the effect levels studied, the mean CI values of gemcitabine-containing regimens were smaller than those of cisplatin plus etoposide at the 50 and 70% effect levels. Gemcitabine plus cisplatin was statistically more active than the other two regimens at the 70% effect level (both P values = 0.028). Moreover, we found that cisplatin plus etoposide demonstrated no differential effects between the high- and the low-p185 neu groups (Table 2). In contrast, the combination effects of gemcitabine plus cisplatin at the 50 and 70% effect levels and that of gemcitabine plus etoposide at the 50% effect level were statistically more active in the high- than in the low-p185 neu group (all P values = 0.025; Table 2). These findings demonstrated that gemcitabine was able to enhance the cytotoxicities of cisplatin and etoposide to a greater degree in the high- than in the low-p185 neu-expressing cell lines. Fig. 4 demonstrates the representative examples of the drug combination isoboles of one low- and one high-p185 neu-expressing cell line, NCI-H460 and NCI-H1355, respectively.

**DISCUSSION**

Despite the fact that dozens of chemotherapeutic agents have been introduced over the past four decades, only a few of them, such as ifosfamide, mitomycin C, vinblastine, and vindesine, have consistently produced single-agent response rates greater than 15% in patients with NSCLC (1, 2). Nevertheless, several newly developed drugs, including navelbine, Taxol, and taxotere, the camptothecins CPT-11 and topotecan, and gemcitabine, have also been able to produce single-agent response rates above 20% (2, 30). Among these new drugs, gemcitabine attracted our attention because of its ability to inhibit DNA replication and repair. It is shown that many DNA replicative processes targeted by gemcitabine in growing cells are also required for the repair of DNA damage in growth-arrested cells (18–21). Therefore, it is plausible that DNA repair sites elicited by DNA-damaging agents could be the target sites for gemcitabine. Cells that are more actively undergoing DNA repair may be more susceptible to the blocking effect of gemcitabine. Gemcitabine, therefore, would be a good candidate to combine with DNA-damaging agents for treatment of human cancers, especially those cancers that are known to undergo DNA repair more effectively. NSCLC cells which express a high level of p185 neu have been proposed to be one of these kinds of cancer (7). A recent preliminary report has demonstrated that gemcitabine, through the inhibition of DNA repair, may enhance the cytotoxicity of cisplatin against human colon cancer cells (31).

In the present study, we have tested a panel of NSCLC cell lines and demonstrated that the intrinsic resistance against gemcitabine did not correlate with the level of p185 neu. As compared to the tight cross-resistance observed between cisplatin and etoposide, gemcitabine was shown to have little cross-resistance to etoposide and cisplatin. Gemcitabine-containing regimens, especially gemcitabine plus cisplatin, were shown to be more active than the combination of cisplatin and etoposide against NSCLC cell lines with high-p185 neu expression. This finding was unique in that the synergistic effect of gemcitabine plus cisplatin was effect (dose) dependent. Most important, gemcitabine was able to enhance the effects of DNA-damaging agents to a greater degree in the high- than in the low-p185 neu-expressing cell lines. The high-p185 neu expressions were proposed to have more efficient DNA repair and were hence more chemoresistant (7). A recent report (32), demonstrating that the cytotoxicity of cisplatin to the high-p185 neu-expressing human breast and ovarian cancer cells can be enhanced by a p185 neu-specific antibody through the inhibition of DNA repair, provides further evidence to support the view that high levels of p185 neu may promote DNA repair. Nevertheless, thus far the pathway mediating the relationship between p185 neu and DNA repair remains unclear. Whether the more active interactions between gemcitabine and either cisplatin or etoposide in high- than in low-p185 neu expressors is due to more inhibition on DNA repair must be verified in additional mechanistic studies.

Through the past decade, the increased knowledge of tumor biology of lung cancer has enabled us not only to develop new therapeutic modalities for clinical intervention (e.g., gene therapy), but also to maximize the therapeutic benefit of currently available combination chemotherapy. Overexpression of HER-2/neu has been detected in a subgroup of NSCLCs (10–12) and is associated with advanced disease stage (11), shortened survival (12), and multidrug resistance (6). Gemcitabine alone or in combination with other agents is currently being evaluated in Phase I and Phase II clinical trials in a variety of human cancers, including NSCLC (33–40). Several preliminary clinical studies have shown that the combination of gemcitabine and cisplatin may induce a considerable response rate (in the range of 36–58%) with modest side effects in the treatment of advanced NSCLC (36–40). Our results in the present study indicate that gemcitabine-containing regimens (especially gemcitabine plus cisplatin) are highly cytotoxic against NSCLC cell lines in vitro. The gemcitabine-cisplatin interaction is more active than the etoposide-cisplatin interaction in cells with high-p185 neu expression. This work clearly provides a rationale to test the gemcitabine-cisplatin combination in Phase II and Phase III clinical trials in a subgroup of NSCLC patients with tumors expressing high levels of p185 neu and which therefore may be more chemoresistant to commonly used combination therapy.

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**REFERENCES**


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