Synergistic cytotoxicity of bcl-2 antisense oligodeoxynucleotides and etoposide, doxorubicin and cisplatin on small-cell lung cancer cell lines

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Summary  Expression of Bcl-2 is life-sustaining for small-cell lung cancer cells and associated with drug resistance. In the present study, the interactions between the bcl-2 antisense oligodeoxynucleotide 2009 and the chemotherapeutic agents etoposide, doxorubicin and cisplatin were investigated on small-cell lung cancer cell lines to search for synergistic combinations. The cell lines NCI-H69, SW2 and NCI-H82 express high, intermediate—high and low basal levels of Bcl-2, respectively, which are inversely correlated with the sensitivities of the cell lines to treatment with oligodeoxynucleotide 2009 and the chemotherapeutic agents alone. Moreover, differences were found in the responsiveness of the cell lines to treatment with combinations of oligodeoxynucleotide 2009 and the chemotherapeutic agents. In the cell lines NCI-H69 and SW2, all combinations resulted in synergistic cytotoxicity. In NCI-H69 cells, maximum synergy with a combination index of 0.2 was achieved with the combination of oligodeoxynucleotide 2009 and etoposide. In SW2 cells, the combination of oligodeoxynucleotide 2009 and doxorubicin was the most effective (combination index = 0.5). In the cell line NCI-H82, which expresses a low basal level of Bcl-2, most of the combinations were slightly antagonistic. Our data suggest the use of oligodeoxynucleotide 2009 in combination with chemotherapy for the treatment of small-cell lung cancer that overexpresses Bcl-2.

Keywords: synergistic cytotoxicity; bcl-2 antisense oligodeoxynucleotide; chemotherapy; small-cell lung cancer

Lung cancer is the leading cause of cancer death, and its incidence continues to rise worldwide. The treatment of small-cell lung cancer (SCLC), which makes up about 25% of lung cancer cases, relies on different classes of chemotherapeutic agents, including epipodophyllotoxins, anthracyclines and platinum analogues. Although the introduction of combination chemotherapy as the principal form of treatment has led to an increase in median survival, only a small proportion of patients with SCLC are cured (Souhami and Law, 1990).

Mechanisms of drug resistance in solid tumours have been examined extensively over the last 10 years. In lung cancer cell lines selected in vitro genetic changes have been identified that alter drug transport and activity, such as overexpression of the P-glycoprotein or the multidrug resistance-associated protein (MRP) (Doyle, 1993; Gonzalez Manzano et al. 1996; Versantvoort et al. 1996). However, no correlation between overexpression of these drug transporters in lung cancer cells and response to therapy has been found in patients (Lai et al. 1989) suggesting that other mechanisms are more important for clinical drug resistance of lung cancer. It is now generally accepted that resistance to cytotoxic treatments relates to failure of cells to engage the process of apoptosis, and some of the genetic defects that antagonize apoptosis have already been unravelled. Most SCLC cell lines and tumour tissues overexpress the Bcl-2 oncprotein (Ben Ezra et al. 1994; Reeve et al. 1996), which has been associated with chemotherapy resistance of various tumour cells in vitro and in vivo (Campos et al. 1993; Kamesaki et al. 1993; Miyashita and Reed, 1993; Strasser et al. 1994). For example, ectopic expression of bcl-2 has been shown to confer resistance to etoposide and cisplatin in neuroblastoma and lymphoma cell lines (Miyashita and Reed, 1992; Dole et al. 1994; Strasser et al. 1994), and to doxorubicin in a SCLC cell line (Ohmori et al. 1993).

Modulation of gene expression by antisense oligodeoxynucleotides (ODNs) is a promising approach because of its target specificity and potential applicability to any sequenced gene. The mechanisms implicated in the action of antisense ODNs relate to RNase H-mediated hydrolysis of the target mRNA or to translational arrest arising from steric hindrance by the RNA–DNA heteroduplex (Stein and Cheng, 1993; Ho and Parkinson, 1997). These mechanisms differ greatly from those exerted by chemotherapeutic agents and thus might operate also in drug-resistant tumour cells. Antisense ODNs targeting the first six codons of the bcl-2 mRNA have been shown to increase the sensitivity of lymphoma cells to chemotherapeutic agents (Kitada et al. 1994). However, although the combination of bcl-2 antisense ODNs and chemotherapy seems to be appealing, the cytotoxic interaction of these treatments on tumour cells has not yet been addressed in detail. Recently, we have identified an antisense sequence (ODN 2009) targeting the bcl-2 coding region that effectively down-regulated bcl-2 expression and induced apoptosis in SCLC cells (Ziegler et al. 1997). In the present study, we examined the cytotoxic effects of ODN 2009 in combination with etoposide, doxorubicin and cisplatin on SCLC cell lines with different Bcl-2 levels. Analysis of potential synergy was performed by the median effect method described by Chou and Talalay (1984).
MATERIALS AND METHODS

Cell lines
The SCLC cell line SW2 was obtained from the Dana-Farber Cancer Institute, Boston, MA, USA. The SCLC cell lines NCI-N417, NCI-H82 and NCI-H69 were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Cells were cultured in RPMI-1640 (Hyclone Europe, Cramlington, UK) supplemented with 2 mM l-glutamine, 10% fetal calf serum (FCS) (Hyclone Europe), 50 IU ml⁻¹ penicillin and 50 μg ml⁻¹ streptomycin (cell culture medium) at 37°C in a humidified atmosphere with 5% carbon dioxide.

Bcl-2 antisense and control ODNs
The 20-mer phosphorothioate ODN 2009 with the sequence 5'-AATCTCTCCCCAGTTCACC-3' targets the coding region of the bcl-2 mRNA (Ziegler et al. 1997). The following ODNs were used as controls: sense 5'-GGGTGAAGGGGGAGATT-3', 5'–3' reversed 5'-CCCCATCTGCCCACTTCAA-3', the scrambled sc-21 5'-ACACCCCAATTCCTGGCCC-3'. and the four base mismatch 5'-AATCTCTGGATCTTACC-3'. A BLASTN search of a database containing all sequences of GenBank, EMBL, DDBJ and PDB revealed no homology of the control ODNs to human genes. All ODNs were provided by Genset (Paris, France) as ‘guaranteed oligos’. They had been purified by use of a Waters’ high-pressure liquid chromatography system equipped with a Nucleosil C18 column and a mobile phase consisting of a 15–25% gradient of acetonitrile. Purified ODNs were stored at −20°C in 10 mM Tris, pH 7.4, containing 1 mM EDTA.

Delivery of ODNs to SCLC cells
ODN 2009 and the control ODN sc-21 were delivered to cells in the form of complexes with the cationic lipid N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulphate (DOTAP; Boehringer Mannheim, Germany) as described previously (Ziegler et al. 1997). Briefly, equal volumes of ODNs (24 μM) and DOTAP (400 μM) in 20 mM HEPES-buffered saline were mixed and allowed to complex for 10 min at room temperature. The mixtures were diluted into nine volumes of cell culture medium to achieve solutions of 1.2 μM ODN. For use in experiments, ODN stock solutions were further diluted serially into cell suspensions containing medium alone or medium with various dilutions of the chemotherapeutic agents.

Western blot analysis
Western blotting was performed as described by Ziegler et al. (1997). Briefly, 1 ml of cells/ODN mixture was plated in a 24-well plate and incubated for 24 h at 37°C. ODN concentrations and cell densities were as above. Ten μg of soluble protein extract per sample was separated on a 12% polyacrylamide sodium dodecyl sulphate (SDS) gel at 150 V for about 3 h, and transferred to a polyvinylidenefluoride membrane (Immobilon-P, Millipore) was performed in a semi-dry blotting chamber (Schleicher and Schuell) at 1 mA cm⁻² for 1 h. The blots were blocked in Tris-buffered saline (TBS) containing 5% bovine serum and 5% non-fat dry milk, and then incubated overnight at 4°C with mouse anti-human Bcl-2 monoclonal antibody (Dako Diagnostics, Glostrup, Denmark). To detect the primary antibody, blots were incubated with a rabbit antimouse immunoglobulin peroxidase conjugate (Sigma Chemical, St. Louis, MO, USA) for 2 h at room temperature. Visualization of the immunocomplex was performed by enhanced chemiluminescence using the ECL kit (Amersham), followed by exposure to radiographic films (Fuji RX) for different time periods dependent on the cell line. Relative protein levels were quantified after scanning of the films using a flat bed scanner (Hewlett Packard ScanJet IIcx) and the ImageQuant software (Molecular Dynamics).

Chemotherapeutic agents
Doxorubicin (Adriblastine) was obtained from Farmitalia Carlo Erba (Zug, Switzerland). Etoposide (Vepesid) and cisplatin (Platinol) were obtained from Bristol-Myers Squibb (Baar, Switzerland). All agents were clinical grade and diluted with cell culture medium before use.

Measurement of cell viability
The cytotoxic effects of ODN 2009 and chemotherapeutic agents on SCLC cell lines were determined by use of the colorimetric WST-1 viability assay as described previously (Ziegler et al. 1997). Once the optimal growth conditions have been established for each cell line, the WST-1 viability assay provides reproducible results which correlate well with the actual number of viable cells determined by propidium iodide exclusion (Ziegler et al. 1997). Briefly, for each experiment, 100 μl each of cell suspensions containing ODN 2009 or control ODNs, the chemotherapeutic agents, or a combination of both types of agents were plated in triplicates in 96-well plates. Cell densities were 0.5 × 10⁴ cells ml⁻¹ (SW2, NCI-H82), or 10⁵ cells ml⁻¹ (NCI-H69). Cells were incubated for 4 days at 37°C, and then 10 μl of WST-1 reagent (Boehringer Mannheim, Germany) was added per well and allowed to react for 3–5 h at 37°C. Absorbance at 450 nm was measured by use of an enzyme-linked immunosorbent assay reader (2550 EIA reader, Bio Rad Laboratories, Hercules, CA, USA).

Determination of viable cell numbers based on propidium iodide exclusion
To determine the number of viable cells after cytotoxic treatment, 4 ml of cell suspensions containing ODN 2009, chemotherapeutic agents or a combination of both types of agents were plated in six-well plates. The same cell densities as for the WST-1 viability assay described above were used. At different time points of incubation, cells were harvested, briefly trypsinized and resuspended in phosphate-buffered saline (PBS). Immediately before measurement, propidium iodide was added to a final concentration of 1.25 μg ml⁻¹. The number of cells in the cultures was quantitated at a constant flow rate of 12 μl min⁻¹ by use of a FACS Calibur cytofluorometer (Becton Dickinson, Mountain View, CA, USA). Only cells that excluded propidium iodide were considered viable. Apoptotic cell death after cytotoxic treatment was confirmed by forward and side light scatter analysis as described previously (Cotter et al. 1992; Ziegler et al. 1997).

Analysis of combined ODN/drug effects
The median-effect method described by Chou and Talalay (1984) was used to determine the nature of interaction between ODN
Table 1 Cytotoxicity of ODN 2009 and chemotherapeutic agents on SCLC cell lines

| Cell line | Relative Bcl-2 level (%) | ODN 2009 | Etoposide | Doxorubicin | Cisplatin |
|-----------|--------------------------|----------|-----------|------------|-----------|
| SW2       | 100                      | 48 ± 8   | 12000 ± 900 | 37 ± 4     | 2100 ± 100 |
| NCI-H69   | 174 ± 21                 | 135 ± 20 | 16000 ± 2000 | 80 ± 8     | 5000 ± 400  |
| NCI-H82   | 23 ± 4                   | 32 ± 5   | 590 ± 60   | 11 ± 1     | 680 ± 40   |

IC_{50} ± s.d. (nm)

*Quantitated from Western blots.

2009 and the chemotherapeutic agents. This analysis is based on the median-effect principle of the mass action law and relies on linear regression as a well-accepted statistical approach. In each experiment, cells were treated with serial dilutions of ODN 2009 and drugs individually, and with fixed ratios of ODN 2009 and drugs simultaneously at doses in the range of the individual concentrations at which cell viability was inhibited by 50% (IC_{50}). The fraction affected (f_j) was calculated by dividing the per cent viability in ODN 2009 and drug-treated wells by the viability in untreated wells, and data were analysed by the median-effect method (Chou and Talalay, 1984). Briefly, log (1/f_j - 1) was plotted against log(drug dose). From the resulting median-effect lines, the x-axis intercept (log IC_{50}) and slope m were calculated for ODN 2009 and each drug and for the combinations by the least squares method. When ODN 2009 and drugs were administered at a fixed ratio, the dose of the combination required to produce f_j could be separated into the component doses (D1) and (D2) of ODN 2009 and drug respectively. For each level of cytotoxicity, a combination index (CI) was calculated at increasing cell kill and the combinations were compared with the cytotoxic effects of the respective single agent treatments in each experiment. Synergy is indicated by a CI less than 1, additivity by a CI equal to 1, and antagonism by a CI greater than 1. For each combination, CI-values were calculated based on the assumption that drug interaction was mutually exclusive and mutually non-exclusive (when drugs have different modes of action or act independently). Because we did not map the entire response surface (Greco et al. 1995), the data of interaction of ODN 2009 with the chemotherapeutic agents calculated for each cell line rely on the fixed ratios of the agents.

RESULTS

Bcl-2 levels in SCLC cell lines and effect of treatment with ODN 2009

To demonstrate the ability of ODN 2009 to down-regulate Bcl-2 expression in the SCLC cell lines. Western blot analysis was performed. Cells were incubated for 48 h with 75 nM or 150 nM ODN 2009. Untreated cells were used to determine the basal levels of Bcl-2 in the cell lines. The relative basal levels of Bcl-2 which were quantitated from the Western blots are shown in Figure 1. Bcl-2 was abundantly expressed in the cell lines NCI-H69 (174%), and SW2 (100%), but was barely detectable in the NCI-H82 cell line (23%). As shown in Figure 1, in all cell lines ODN 2009 caused a dose-dependent reduction in Bcl-2.

Cytotoxicity of single agents

The IC_{50} values of ODN 2009, etoposide, doxorubicin and cisplatin were determined for the SCLC cell lines SW2, NCI-H69 and NCI-H89 using the WST-1 assay. As shown in Table 1, there was an inverse correlation between the level of Bcl-2 and the sensitivity of the cell lines to all three chemotherapeutic agents tested (0.02 > P > 0.007).

Interaction between ODN 2009 and chemotherapeutic agents on SCLC cells expressing intermediate levels of Bcl-2

The dose–response curves and median-effect plots for the treatment of SW2 cells that express intermediate levels of Bcl-2 (Figure 1. Table 1) with the different agents are depicted in Figure 2. No effect on cell viability was seen with the ODNs sc-21 and sense, whereas the ODNs mismatch and 5′–3′ reversed slightly reduced the number of viable cells at concentrations higher than 80 nM. When cells were exposed to fixed ratios of ODN 2009 and etoposide (1:330), doxorubicin (1:1) or cisplatin (1:83) at doses that roughly corresponded to the IC_{50} values, the cytotoxic effects of the combinations were always greater than those observed with the single agents alone (0.03 > P > 0.01). When used in combination with etoposide, doxorubicin or cisplatin at concentrations equivalent to ODN 2009, none of the control ODNs could potentiate the cytotoxic effects of the chemotherapeutic agents. Median-effect analysis of the cytotoxicity data demonstrated linear curves with similar slopes for ODN 2009 and etoposide and different slopes for doxorubicin and cisplatin (Figure 2). This suggests the interaction of ODN 2009 to be mutually exclusive with etoposide and mutually non-exclusive with doxorubicin and cisplatin (Chou and Talalay, 1984). As calculated from the median-effect curves in Figure 2, the CI values of the treatment of SW2 cells with ODN 2009 and etoposide were about 0.6 ± 0.1 at all levels of toxicity (Figure 3). Likewise, treatment of SW2 cells with ODN 2009 in combination with doxorubicin and cisplatin also resulted in
Figure 2. Cytotoxicity of ODN 2009, etoposide, doxorubicin and cisplatin alone, or their combinations on SW2 cells. Cells were incubated with ODN 2009, control ODNs or chemotherapeutic agents alone, and with combinations of ODN 2009 or control ODNs and chemotherapeutic agents at the ratios indicated. The percentage of viable cells determined by using the WST-1 assay was plotted relative to untreated control cells. The median-effect plots were derived from the dose–response curves of the combinations and were used to calculate the combination index (CI) values shown in Figure 3. Dose–response data represent the means of three independent determinations from which the median-effect plots were calculated; line bars = s.d. The cytotoxic effects of ODN 2009 and control ODNs, and of the combinations of ODN 2009 and control ODNs with chemotherapeutic agents were significantly different (P < 0.01) as determined by two-sided t-tests.
Synergy between bcl-2 antisense and chemotherapy on SCLC

Synergistic activity with mean CI values of 0.6 ± 0.08 and 0.83 ± 0.07 respectively (Figure 3).

To support the observation of synergistic cytotoxicity between ODN 2009 and the chemotherapeutic agents by a different cell viability assay, flow cytometric analysis based on propidium iodide exclusion of cells was performed for the combination of ODN 2009 and doxorubicin on SW2 cells. As shown in Figure 4A, the combination of ODN 2009 (75 nM) and doxorubicin (6.3 nM) reduced the number of viable SW2 cells to 10% of the untreated control during a 96-h treatment. Although synergistic cytotoxicity cannot be directly deduced from the viability curves, it is obvious that this combination was significantly more cytotoxic to the cells than treatment with equivalent concentrations of ODN 2009 or doxorubicin alone (P < 0.003). In contrast, the effects of a combination of ODN mismatch and doxorubicin, and doxorubicin alone were not significantly different (P > 0.2). This indicates that potentiation of the cytotoxicity of doxorubicin was specific for ODN 2009 and its ability to down-regulate bcl-2 expression.

To demonstrate morphological changes of the cells typical for apoptosis, SW2 cells were subjected to forward and side light scatter analysis 72 h after treatment with 75 nM ODN 2009 in combination with 6.3 nM doxorubicin. As shown by the contour plots in Figure 4B, the treatment caused an obvious increase in side light scattering and a slight reduction in forward light scattering of the cells. Microscopic analysis of the treated cells revealed shrinkage, extensive plasma membrane blebbing and nuclear condensation (not shown).

Interaction between ODN 2009 and chemotherapeutic agents on SCLC cells expressing high levels of Bcl-2

Compared with the other cell lines used in this study, the NCI-H69 cell line has the highest level of Bcl-2 (Figure 1, Table 1). Treatment of these cells with ODN 2009 was synergistic in combination with etoposide, doxorubicin and cisplatin at all levels of toxicity. The slopes of the median-effect curves (data not shown)
suggest that on this cell line all combinations resulted in mutually non-exclusive interactions. As shown in Figure 3, the combination of either ODN 2009 and etoposide at a ratio of 1:43 or ODN 2009 and doxorubicin at a ratio of 4:1 was slightly less than additive and resulted in antagonistic effects (CI > 1) at all levels of toxicity. Synergistic cytotoxicity on NCI-H82 cells was obtained only if ODN 2009 was combined with cisplatin (1:43) at the IC_{50} and the IC_{75} of the combination with CI values of 0.72 ± 0.08 and 0.95 ± 0.1 respectively (Figure 3).

**DISCUSSION**

Etoposide, doxorubicin and cisplatin are routinely used for the treatment of SCLC. They work by damaging DNA which triggers a common death programme called apoptosis (Strasser et al. 1994). The Bcl-2 oncoprotein can counteract drug-induced apoptosis and its expression has been associated with multidrug resistance in a variety of tumour cells (Miyashita and Reed, 1993; Ohmori et al. 1993; Dole et al. 1994; Strasser et al. 1994). Here, we report on the cytotoxic effect of antisense-mediated down-regulation of bcl-2 expression in combination with etoposide, doxorubicin and cisplatin on three SCLC cell lines. Bcl-2 is abundantly expressed in the cell line NCI-H69, whereas intermediate–high and low levels are present in the cell lines SW2 and NCI-H82 respectively (Ziegler et al. 1997). We observed an inverse correlation of the Bcl-2 levels of these cell lines with the sensitivities to the chemotherapeutic agents tested. This suggests that Bcl-2 is critical for inhibiting drug-induced apoptosis in SCLC cells.

Antisense ODNs have been used to disrupt the expression of various cancer related genes and to inhibit tumour cell growth in preclinical studies (Dosaka Akita et al. 1995; Monia et al. 1996; Kitada et al. 1994; Szczylak et al. 1991), and first results of antitumour activity are also available from clinical studies (Webb et al. 1997). ODN 2009 is a 20-mer phosphorothioate that targets the coding region of the bcl-2 mRNA (Ziegler et al. 1997). In SCLC cells it effectively down-regulated bcl-2 expression and induced apoptosis to a degree inversely correlated with the level of expression. This suggests Bcl-2 to be a critical survival factor for SCLC cells.

Combination chemotherapy has become the standard treatment for SCLC (Souhami and Law, 1990). The cytotoxic interactions of various chemotherapeutic agents have been analysed in vitro by different calculation methods (Kaufmann et al. 1996; Photiou et al. 1997), and it has been shown that even clinically approved drug combinations may result in less than additive effects (Kaufmann et al. 1996). These findings imply that such in vitro studies might be useful for the selection and design of optimal drug combinations for clinical application. Combinations of antisense ODNs targeting specific oncogenes or genes involved in drug resistance, such as bcl-2, and less toxic doses of chemotherapeutic agents represent a rational therapeutic strategy to pursue. In models of human leukaemia and colon carcinoma xenografts in mice, it has been shown that antisense ODNs targeting the oncogenes bcr/abl (Skorski et al. 1997) or c-myc (Del Bufalo et al. 1996), or the multiple drug resistance gene mdr1 (Cucco and Calabretta, 1996) can indeed enhance the anti-tumour effect of chemotherapeutic agents. However, these studies did not directly investigate the interaction of the antisense ODNs with chemotherapeutic agents in vitro and did not search for synergistic combinations.

In the present study, we demonstrate for the first time that a combination of bcl-2 antisense ODN with etoposide, doxorubicin or cisplatin results in synergistic cytotoxicity on cell lines derived from SCLC.
from a solid tumour in which Bcl-2 is prevalent (Ben Ezra et al. 1994). Synergy was particularly pronounced on the cell lines NCI-H69 and SW2 that have high and intermediate–high Bcl-2 levels respectively. The cell line NCI-H82 which expresses barely detectable Bcl-2 levels was extremely sensitive to treatment with ODN 2009 and also to the chemotherapeutic agents alone. This might explain why, on this cell line, ODN 2009 and the chemotherapeutic agents did not interact synergistically.

Because the sulphur backbone of phosphorothioate ODNs non-specifically interacts with proteins and nucleic acid targets (Stein and Krieg. 1994; Stein. 1995), it is difficult to determine which of their biological effects are truly antisense in nature. Therefore, in the present study, a series of control phosphorothioate ODNs was used for comparison to ODN 2009. At concentrations less than 150 nM, only the ODNs mismatch and 5’–3’ reversed caused a slight reduction in cell viability. In combination experiments, none of the control ODNs potentiated the cytotoxic effects of the chemotherapeutic agents. This unequivocally excludes the possibility that the increase in cytotoxicity achieved with the combinations of ODN 2009 and chemotherapeutic agents was the result of enhanced cellular uptake of the latter in the presence of cationic lipids (Bennett et al. 1992).

Although the intracellular targets and the mechanisms of action of ODN 2009 and the chemotherapeutic agents are different, their effects eventually merge in a common final death pathway. In this case, one would expect the interactions between ODN 2009 and etoposide, doxorubicin or cisplatin to be mutually exclusive. For example, Bcl-2 has been shown to inhibit apoptosis induced by etoposide through effects on events early after topoisomerase II-induced DNA damage (Kamesaki et al. 1993). According to Chou and Talalay (1984), agents are claimed to be mutually exclusive if the slopes of the median-effect lines of the single-agents and their combination are identical. Based on this assumption, however, on SW2 cells a mutually exclusive interaction occurred between ODN 2009 and etoposide, but not between ODN 2009 and doxorubicin or cisplatin. On NCI-H69 cells, only the interaction of ODN 2009 and doxorubicin is suggested to be mutually exclusive. This indicates that: (1) cells can differ in the way they handle the apoptotic signals provided by ODN 2009 and the chemotherapeutic agents; (2) the assumption whether agents act independently or not cannot be made solely based on our current understanding of the initial steps in their mechanisms of action. Considering this uncertainty, in the present study all data were analysed assuming both mutually exclusive and non-exclusive interactions of the agents.

In considering the potential implication of this study, one limitation must be kept in mind. The data for each cell line were generated using fixed ratios of the agents. If a different ratio had been evaluated, a different CI plot would have resulted. Thus, because the entire response surfaces (Greco et al. 1995) were not mapped, the conclusions of the study are limited to the ratios of ODN 2009 and chemotherapeutic agents that were actually used. Nevertheless, our data provide clear evidence that synergistic interactions of ODN 2009 and the chemotherapeutic agents can be expected to be more pronounced on cells expressing higher levels of Bcl-2. It will be of interest to see to what extent the differences between synergistic and antagonistic effects observed in vitro translate to in vivo responses.

Our data suggest the use of ODN 2009 in combination with conventional chemotherapy as a novel approach to more effective treatment of SCLC and other types of tumours in which Bcl-2 is prevalent.

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