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COMBINED EFFECT OF DOCETAXEL AND CISPLATIN FOR NON-SMALL CELL LUNG CANCER CELL LINES *IN VITRO*

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ABSTRACT

Docetaxel (DOC) plus cisplatin (CDDP) is a novel combination chemotherapy for the treatment of advanced non-small-cell lung cancer (NSCLC). We investigated the combined effect of DOC with CDDP in sequence and the reverse schedule for NSCLC cell lines EBC-1 (squamous cell carcinoma) and RERF-LC-MS (adenocarcinoma) using an MTT assay and an improved isobologram method. The results showed that the combination of DOC and CDDP in human lung cancer cell lines was antagonistic. To investigate the possible mechanism of the antagonistic effect, we focused on the cell cycle perturbation and the inhibition of apoptosis fractions induced by chemotherapeutic agents. Pretreatment of CDDP significantly blocked the following DOC-induced apoptosis fraction. Therefore we consider that the suppression of apoptosis could be one of the mechanisms for antagonistic effects of combination chemotherapy of DOC and CDDP.

Key Words: docetaxel (DOC), cisplatin (CDDP), non-small-cell lung cancer (NSCLC), combination chemotherapy, isobologram, cell cycle

INTRODUCTION

Docetaxel (DOC) is a new hemisynthetic anticancer agent, isolated from the bark of the pacific yew, *Taxus brevifolia*.[1-3] Anticancer responses of DOC have been observed in a variety of solid tumors including non-small-cell lung cancer (NSCLC),[4,5] and several clinical trials for the docetaxel-based combination chemotherapy for the treatment of NSCLC have been performed.[6-8] DOC has been known to possess the unique property of inducing microtubule assembly *in vitro*. It induces the extensive formation of microtubule bundles, thereby blocking cell replication at a checkpoint between G_2 and M phase of the cell cycle, resulting in cell death.[9-12]

On the other hand, cisplatin (CDDP) remains a key drug in combination chemotherapy protocols for NSCLC, and it is the only drug for which significantly prolonged survival has been demonstrated for a cohort of stage IV NSCLC patients.[13,14] Therefore, combination chemotherapy of CDDP with DOC is one of the promising treatments for NSCLC.

However, a previous report demonstrated that some mitotic inhibitors including chemotherapeutic agents inhibited the binding of taxol to cells.[15] In addition, chemotherapeutic agents affect the cell cycle of tumor cells; some inhibit the cell cycle at G_1 or S phase, and others

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inhibit at G_2 or M phase. Therefore, combined chemotherapy with DOC may reduce cytotoxic activities because of the inhibition of DOC binding or the perturbation of cell cycle dependent killing. In this study, we investigated the effect of combination chemotherapy with DOC and CDDP for NSCLC cell lines *in vitro* assessed by the cytotoxic activity and cell cycle analysis.

MATERIALS AND METHODS

Drugs

DOC was kindly provided from Rhone-Poulenc Rorer (Vitry, France), and CDDP from Bristol-Myers-Squibb Co. (Tokyo, Japan). These drugs were adjusted at the designed concentrations by successive dilutions in growth medium.

Cell lines

The human NSCLC cell lines EBC-1 (squamous cell carcinoma) and RERF-LC-MS (MS) (adenocarcinoma) were originally obtained from the Japanese Cancer Resources Bank. They were maintained in Eagle's minimal essential medium supplemented with 1% nonessential amino acids, 1% L-glutamine, 1% sodium pyruvate, 1% penicillin-streptomycin and 10% fetal calf serum at 37°C in a humidified atmosphere with 5% CO_2.

Cell growth inhibition experiments and MTT assay

The DOC plus CDDP combination was scheduled in two ways. CDDP administration before DOC (CDDP→DOC), and CDDP administration after DOC (DOC→CDDP). Cells in the logarithmic phase were harvested and plated into 96 well microtiter plates in 100 μl medium with the appropriate seeding density (EBC-1; 10^4 cells / well, and MS; 7×10^3 cells / well). Twenty-four h after incubation, first drug was added into the medium at various doses, and then the second drug was added at various doses 24 h after the addition of the first drug. After 48-h exposure of chemotherapeutic agents, viable cell growth was determined using a tetrazolium-based colorimetric assay (MTT assay).[16] The assay is dependent on the cellular reduction of a tetrazolium salt, 3-(4,5-diethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT), by the mitochondrial dehydrogenase of viable cells to a blue formazan product, which can be measured spectrophotometrically. Five mg / ml (final concentration) of MTT was added to each well. After 3–4 h incubation at 37°C with MTT, the unreacted MTT and medium were removed, and 150 μl of dimethyl sulfoxide was added to solubilize the MTT formazan. After shaking the plates, the OD of each well was measured with a microplate spectrophotometer (SPECTRA 2, TECAN GmbH, Austria) equipped with a 540-nm filter. The spectrophotometer was calibrated at 0 absorbance using wells that only contained medium and MTT. The OD reading of control wells contained cells and MTT (maximum dye reduction). The % cytotoxicity was calculated by the following equation: % cytotoxicity = {1–(OD drug-treated) / (OD control)}×100. All tests were performed in 4–6 samples, and mean values were calculated. Each experiment was repeated at least three times.

Isobologram analysis

The effect of combination with two agents at the point of ID_{50} (drug concentration producing 50% inhibition of cell growth) was analyzed using the improved isobologram method described previously.[17-19] Based on the dose-response curves of two agents, three isoeffect lines of mode I, mode II_a and mode II_b were drawn (Fig. 1). Two isoeffect lines which surround the maximum area (for example, mode I and mode II_a in Figure 1) were selected. Then, we plotted

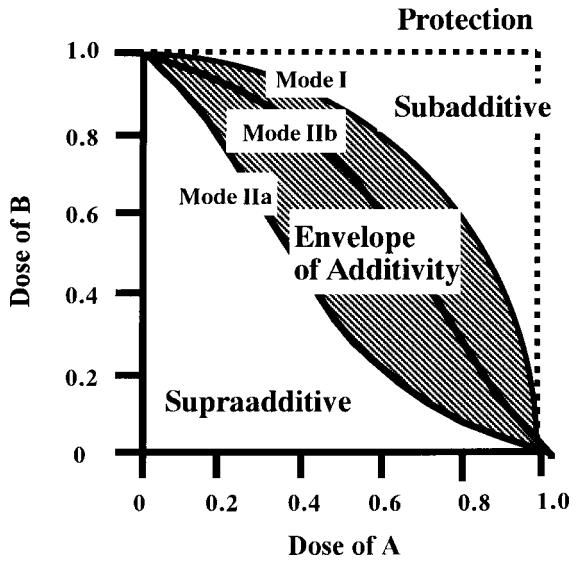

Fig. 1 Isobologram of the combination of drug A and drug B. Mode I, Mode II_a and Mode II_b lines are isoeffect lines drawn from the data for the dose response curves of drug A and drug B (see Materials and Methods).

each point which was given by the two-drug combination experiment on the isobologram. When each point of the drug combination falls within the area of the envelope of additivity, this combination is regarded as additive. When each point falls left of the envelope of additivity and the axis of coordinates, this combination is regarded as supraadditive. When each point falls right of the envelope and within or on the square dot line, this combination is regarded as subadditive. When each point is outside the square, the combination is considered to be mutually protective. Both subadditive and protective effects are considered to be antagonistic.

Apoptosis and cell cycle analysis

The cells were trypsinized into single cell preparations for all flow cytometric studies. Cells were washed once with phosphate buffered saline and resuspended in 1.0 ml of 0.1% sodium citrate buffer containing 50 μg / ml propidium iodide (PI) (Sigma Chemical Co.) and 0.2% nonionic detergent (Sigma Chemical Co.).[20] Samples were filtered using a nylon mesh before sample analysis. DNA ploidy analysis was performed using an Epics XL flow cytometer (Coulter Electronics Inc.). The PI was excited using the 488-nm line of an argon ion laser, and the PI fluorescence greater than 530 nm was collected for DNA detection. Both logarithmic and linear DNA histograms were collected and stored for analysis. The fractions of apoptosis and cell cycle phase were analysed using the Coulter system II™ software attached. The cell numbers were measured in units of ten thousands. The experiments were repeated over three times.

RESULTS

Cytotoxic effect of combination with DOC and CDDP *in vitro*

We first investigated the effect of cytotoxicity combined with DOC and CDDP against human lung cancer cell lines. Table 1-A showed that human lung cancer cell line EBC-1 was

treated with CDDP as a first drug and DOC secondly. Pretreatment with CDDP did not enhance the cytotoxic activity of DOC, but inhibited the cytotoxic activity of DOC. To determine if a different schedule of drug application resulted in a similar finding, we then treated EBC-1 cells with DOC firstly and CDDP secondly. As shown in Table 1-B, these two agents acted neither synergistically nor additively. In addition, when we used the cell line MS, we observed findings similar to those for EBC-1 cells (Table 2-A & 2-B). These findings suggested that the combination DOC and CDDP treatment for human lung cancer cells was antagonistic *in vitro*.

To confirm the above data and to assess the synergistic effect of the two-drug combination, we adopted isobologram analysis.[17-19] Figure 2 showed the representative data of isobologram analysis for EBC-1 cells with CDDP firstly and DOC secondly. After drawing the isoeffect lines, we plotted the data points which were given by ID_{50} of the two-drug combination experiments. Data points fell in the area from the protective to the subadditive. Figure 3 showed the isobologram analysis for MS cells first with DOC and then with CDDP, and the data points also fell in the area from the protective to the subadditive. These data obtained from the isobologram analysis supported the results obtained from the cell inhibition experiments by MTT assay, suggesting that the combination of DOC and CDDP produced the antagonistic effect.

Cell cycle analysis and fractions of apoptosis

To investigate the mechanism of antagonism for the combination of DOC and CDDP, we focused on the cell cycle perturbation and the inhibition of apoptosis fractions induced by chemotherapeutic agents. Short time exposure of EBC-1 cells to DOC caused the cells to accumu-

Table 1. Cell growth inhibition by chemotherapeutic agents in EBC-1 cell[a]

A) CDDP→DOC schedule[b]

CDDP	DOC (nM)				
(μM)	0	1	5	10	50
0	-	20.7 ± 13.6	54.6 ± 5.1	41.6 ± 4.6	46.8 ± 2.0
1	17.3 ± 19.4	21.1 ± 7.2	44.1 ± 2.4	45.9 ± 1.1	45.0 ± 2.6
5	25.6 ± 8.1	30.6 ± 14.1	39.3 ± 4.4	37.8 ± 3.0	35.1 ± 5.6
10	40.0 ± 12.6	37.4 ± 4.2	35.0 ± 14.7	40.3 ± 3.7	41.4 ± 4.7
50	66.2 ± 2.7	63.9 ± 4.4	64.3 ± 1.4	63.4 ± 2.3	64.2 ± 3.9
100	70.8 ± 1.8	71.3 ± 1.3	74.8 ± 1.8	71.1 ± 2.7	74.0 ± 1.0

B) DOC→CDDP schedule[b]

CDDP	DOC (nM)				
(μM)	0	1	5	10	50
0	-	53.5 ± 5.0	68.6 ± 1.4	73.3 ± 1.2	76.1 ± 1.9
1	16.0 ± 5.2	57.1 ± 4.9	71.5 ± 1.3	67.8 ± 8.6	73.8 ± 3.5
5	23.1 ± 11.8	49.1 ± 2.4	68.7 ± 3.4	66.1 ± 5.8	73.9 ± 3.2
10	26.7 ± 8.8	46.7 ± 6.7	62.2 ± 6.5	71.0 ± 2.3	70.5 ± 1.2
50	42.6 ± 1.1	52.1 ± 4.3	68.5 ± 3.3	69.3 ± 5.4	71.0 ± 2.8
100	44.6 ± 4.9	60.2 ± 2.7	71.2 ± 1.7	69.6 ± 2.0	70.7 ± 1.7

a) One representative result out of three independent experiments is shown, and the values represent mean ± SD of cytotoxicity in four to six replicate wells. b) Details of the treatment schedule are described in Materials and Methods.

Table 2. Cell growth inhibition by chemotherapeutic agents in RERF-LC-MS cell[a)]

A) CDDP→DOC schedule[b)]

CDDP	DOC (nM)				
(μM)	0	1	5	10	50
0	-	15.3 ± 13.8	13.8 ± 3.9	29.7 ± 8.1	32.8 ± 3.6
1	2.3 ± 3.2	21.1 ± 10.1	34.7 ± 7.8	37.8 ± 4.8	36.5 ± 7.5
5	24.2 ± 2.2	39.2 ± 7.1	50.3 ± 3.3	50.6 ± 1.7	51.9 ± 4.2
10	43.6 ± 3.3	45.5 ± 4.9	47.6 ± 5.7	55.5 ± 4.5	56.8 ± 2.6
50	88.7 ± 1.8	87.6 ± 1.0	89.1 ± 0.5	88.6 ± 1.0	88.9 ± 0.4
100	90.6 ± 1.0	90.4 ± 0.5	91.1 ± 0.2	91.2 ± 0.7	92.0 ± 0.4

B) DOC→CDDP schedule[b)]

CDDP	DOC (nM)				
(μM)	0	1	5	10	50
0	-	20.2 ± 5.3	35.5 ± 4.3	50.6 ± 5.5	55.8 ± 11.2
1	10.1 ± 3.6	29.6 ± 9.8	31.2 ± 6.5	50.0 ± 7.2	54.8 ± 9.7
5	22.7 ± 5.8	16.9 ± 7.8	34.6 ± 8.1	51.3 ± 6.2	56.0 ± 6.0
10	16.0 ± 3.6	17.8 ± 4.1	45.9 ± 9.4	52.8 ± 2.3	59.3 ± 2.3
50	40.3 ± 9.0	43.7 ± 16.8	59.9 ± 6.2	71.4 ± 2.2	80.6 ± 1.9
100	55.6 ± 2.2	60.1 ± 2.9	74.2 ± 1.9	77.9 ± 3.6	82.0 ± 2.0

a) One representative result out of three independent experiments is shown, and the values represent mean ± SD of cytotoxicity in four to six replicate wells. b) Details of the treatment schedule are described in Materials and Methods.

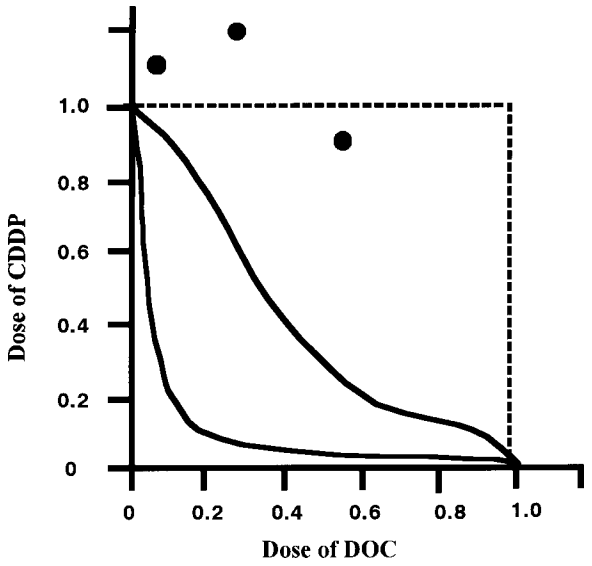

Fig. 2 Isobologram of combined effect of CDDP→DOC schedule in EBC-1. Representative data of isobologram analysis for EBC-1 cells, first with CDDP, then with DOC. Data points fell in the area from the protective to the subadditive.

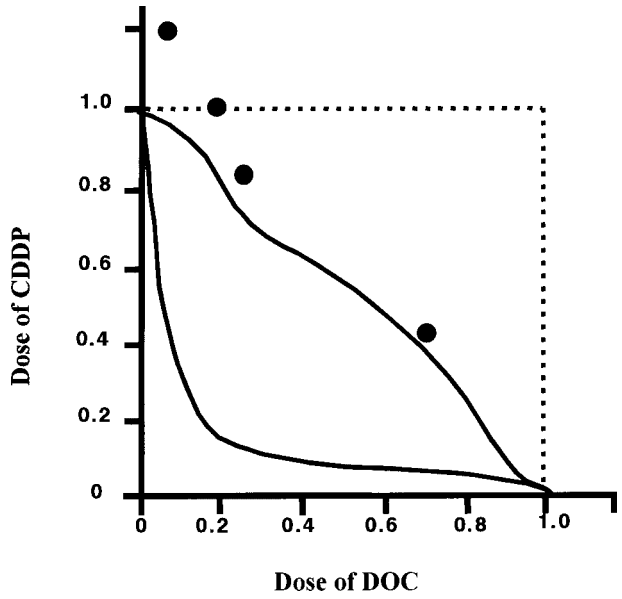

Fig. 3 Isobologram of combined effect of DOC→CDDP schedule in RERF-LC-MS cell. Representative data of isobologram analysis for MS cells, first with DOC, then with CDDP, and the data points also fell in the area from the protective to the subadditive.

late at G_2 / M phase of cell cycle (data not shown). However, longer exposure of DOC induced the apoptosis fraction and decreased the accumulation of the cells at G_2 / M phase (Fig. 4). As for the combination experiments of CDDP and DOC, the cells were treated with CDDP for first 24 h, then combined with DOC treatment for 48 h (Fig. 4). Although 48-h treatment with DOC alone markedly induced the apoptosis fractions, pretreatment of CDDP significantly blocked the following DOC-induced apoptosis fraction (Fig. 4 and Table 3). These findings suggested that the suppression of apoptosis might be one of the mechanisms for the antagonism of a drug combination.

On the contrary, when the cells were treated with DOC for the first 24 h, then combined with CDDP treatment for 48 h, the DOC-induced apoptosis was not inhibited by the CDDP treatment (Table 3). Taken together, we suggested that not only the suppression of apoptosis but other mechanisms may be involved in the mechanisms for the antagonistic effects of a drug combination.

DISCUSSION

Recent progress of chemotherapeutic agents suggested that the chemotherapy of taxans including paclitaxel (Taxol) or docetaxel (Taxotere) would be promising for the treatment of lung cancer in a clinical setting.[21,22] In addition, obtaining synergism and avoiding antagonism is one of the objectives in combination anticancer chemotherapy. In this study, we investigated the combination effect of CDDP and DOC against human lung cancer cell lines *in vitro*. We showed that the combination of CDDP and DOC exhibited the antagonistic effect of cytotoxicity against human lung cancer cell lines, and CDDP markedly blocked the DOC-induced

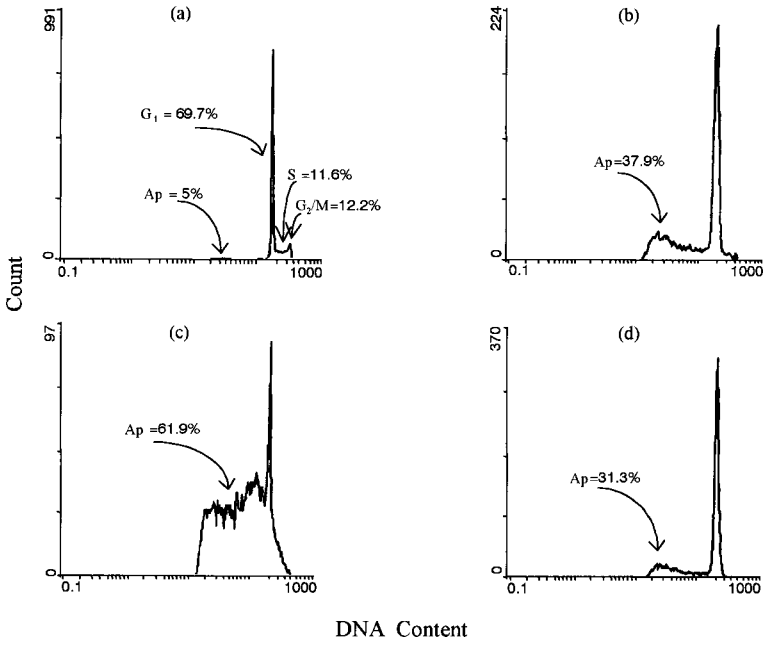

Fig. 4 **Apoptosis fractions of CDDP→DOC schedule in EBC-1 cell.** Pretreatment of CDDP significantly blocked the following DOC-induced apoptosis fraction. (a) Negative control, (b) 100 μM of CDDP exposure for 72 h as a control, (c) 5 nM of DOC exposure for 48 h as a control, (d) 100 μM of CDDP exposure for 72 h plus 5 nM of DOC exposure for 48 h (DOC was added 24 h after CDDP treatment). G_1, G_1 phase; S, S phase; G_2 / M, G_2 / M phase; Ap, Apoptosis fraction.

Table 3. Fractions of apoptosis and cell cycle in EBC-1 cell[a)]

Doses & Schedules	Apoptosis (%)	Cell cycle phase (%)		
		G_1	S	G_2/M
negative control	5.0	69.7	11.6	12.2
CDDP 100μM [b)]	37.9	52.5	6.6	1.8
DOC 5nM [c)]	61.9	23.2	10.5	3.0
CDDP 100μM→DOC 5nM	31.3	58.3	6.8	2.8
negative control	1.7	72.3	13.0	12.1
DOC 5nM [b)]	65.9	10.0	19.4	3.4
CDDP 100μM [c)]	13.2	32.2	35.6	17.1
DOC 5nM→CDDP 100μM	67.7	9.8	14.0	6.9

a) The experiments were repeated over three times and one representative result is shown. b) Drug exposure for 72 h as a control. c) Drug exposure for 48 h as a control.

apoptosis fraction. A previous report demonstrated that vinblastin, an antimitotic agent used for the treatment of lung cancer as a chemotherapeutic agent, blocked the binding of taxans to cells.[15] We speculate that CDDP may inhibit the specific binding of DOC to the lung cancer cell, resulting in the decrease of DOC-induced apoptosis.

The mechanisms of cancer cell death induced by chemotherapeutic agents have been discussed variously, and inductions of necrosis, apoptosis or both pathways have been proposed.[23,24] Previous reports showed that apoptosis triggered by chemotherapeutic agents can be regulated by a number of oncogenes and protein kinase signal transduction pathways in relation to the mechanism of chemosensitivity and resistance.[25,26] Therefore, the major mechanism of cell death depends on the chemotherapeutic agents and cell types, and the induction of apoptosis or the inhibition of apoptosis does not always correlate with the cell viability assessed by the MTT assay we used in the present study. However, the antagonistic effect of cytotoxicity combined with CDDP and DOC may be partly explained by the inhibition of DOC-induced apoptosis.

On the contrary, DOC-induced apoptosis was not inhibited when CDDP was added to the culture after DOC treatment. However, a first treatment of DOC in combination with a second treatment of CDDP showed a similar antagonistic effect of cytotoxicity as when the treatment regimen was reversed. Ma et al. reported that DOC inhibited DNA-adduct formation and intracellular accumulation of CDDP.[27] This may support the mechanism of the decrease of the cytotoxic activity observed in the combined treatment schedule of first-DOC and second-CDDP administration.

Our study suggested that the pharmacological interaction between DOC and CDDP would occur in tumor tissue. Several pharmacological studies in clinical settings have been under investigation, and this study may contribute to the designing of the clinical protocol for NSCLC patients.

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REFERENCES

1) Bissery, M.C., Guenard, D., Gueritte-Voegelein, F. and Lavelle, F.: Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. *Cancer Res.*, 51, 4845–4852 (1991).
2) Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P. and McPhail, A.T.: Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *J. Am. Chem. Soc.*, 93, 2325–2327 (1971).
3) Ringel, I. and Horwitz, S.B.: Studies with RP 56976 (taxotere): a semisynthetic analogue of taxol. *J. Natl. Cancer I.*, 83, 288–291 (1991).
4) Aoe, K., Kiura, K., Ueoka, H., Tabata, M., Matsumura, T., Chikamori, M., Matsushita, A., Kohara, H. and Harada, M.: Effect of docetaxel with cisplatin or vinorelbine on lung cancer cell lines. *Anticancer Res.*, 19, 291–299 (1999).
5) Riou, J.F., Naudin, A. and Lavelle, F.: Effects of Taxotere on murine and human tumor cell lines. *Biochem. Biophys. Res. Commun.*, 187, 164–170 (1992).
6) Zalcberg, J., Millward, M., Bishop, J., McKeage, M., Zimet, A., Toner, G., Friedlander, M., Barter, C., Rischin, D., Loret, C., James, R., Bougan, N. and Berille, J.: Phase II study of docetaxel and cisplatin in advanced non-small-cell lung cancer. *J. Clin. Oncol.*, 16, 1948–1953 (1998).

7) Spiridonidis, C.H., Laufman, L.R., Jones, J., Rhodes, V.A., Wallace, K. and Nicol, S.: Phase I study of docetaxel dose escalation in combination with fixed weekly gemcitabine in patients with advanced malignancies. *J. Clin. Oncol.*, 16, 3866–3873 (1998).
8) Georgoulias, V., Kouroussis, C., Androulakis, N., Kakolyris, S., Dimopoulos, M.A., Papadakis, E., Bouros, D., Apostolopoulou, F., Papadimitriou, C., Agelidou, A., Hatzakis, K., Kalbakis, K., Kotsakis, A., Vardakis, N. and Vlachonicolis, J.: Front-line treatment of advanced non-small-cell lung cancer with docetaxel and gemcitabine: a multicenter phase II trial. *J. Clin. Oncol.*, 17, 914–920 (1999).
9) Diaz, J.F. and Andreu, J.M.: Assembly of purified GDP-tubulin into microtubules induced by taxol and taxotere: reversibility, ligand stoichiometry, and competition. *Biochemistry*, 32, 2747–2755 (1993).
10) Diaz, J.F., Menendez, M. and Andreu, J.M.: Thermodynamics of ligand-induced assembly of tubulin. *Biochemistry*, 32, 10067–10077 (1993).
11) Andreu, J.M., Diaz, J.F., Gil, R., de Pereda, J.M., Garcia, d. L. M., Peyrot, V., Briand, C., Towns-Andrews, E. and Bordas, J.: Solution structure of Taxotere-induced microtubules to 3-nm resolution. The change in protofilament number is linked to the binding of the taxol side chain. *J. Biol. Chem.*, 269, 31785–31792 (1994).
12) Lavelle, F., Bissery, M.C., Combeau, C., Riou, J.F., Vrignaud, P. and Andre, S.: Preclinical evaluation of docetaxel (Taxotere). *Semin. Oncol.*, 22, 3–16 (1995).
13) Albain, K.S., Crowley, J.J., LeBlanc, M. and Livingston, R.B.: Survival determinants in extensive-stage non-small-cell lung cancer: the Southwest Oncology Group experience. *J. Clin. Oncol.*, 9, 1618–1626 (1991).
14) Bonomi, P.D., Finkelstein, D.M., Ruckdeschel, J.C., Blum, R.H., Green, M.D., Mason, B., Hahn, R., Tormey, D.C., Harris, J., Comis, R. and *et al.*: Combination chemotherapy versus single agents followed by combination chemotherapy in stage IV non-small-cell lung cancer: a study of the Eastern Cooperative Oncology Group. *J. Clin. Oncol.*, 7, 1602–1613 (1989).
15) Manfredi, J.J., Parness, J. and Horwitz, S.B.: Taxol binds to cellular microtubules. *J. Cell Biol.*, 94, 688–96 (1982).
16) Mosmann, T.: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65, 55–63 (1983).
17) Steel, G.G. and Peckham, M.J.: Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. *Int. J. Radiat. Oncol., Biol., Phys.*, 5, 85–91 (1979).
18) Kano, Y., Ohnuma, T., Okano, T. and Holland, J.F.: Effects of vincristine in combination with methotrexate and other antitumor agents in human acute lymphoblastic leukemia cells in culture. *Cancer Res.*, 48, 351–356 (1988).
19) Tsai, C.M., Gazdar, A.F., Venzon, D.J., Steinberg, S.M., Dedrick, R.L., Mulshine, J.L. and Kramer, B.S.: Lack of in vitro synergy between etoposide and cis-diamminedichloroplatinum (II). *Cancer Res.*, 49, 2390–2397 (1989).
20) Nicoletti, I., Migliorati, G., Pagliacci, M.C., Grignani, F. and Riccardi, C.: A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *J. Immunol. Methods*, 139, 271–279 (1991).
21) Rowinsky, E.K. and Donehower, R.C.: Paclitaxel (taxol). *New Engl. J. Med.*, 332, 1004–1014 (1995).
22) Fossella, F.V.: Overview of docetaxel (Taxotere) in the treatment of non-small cell lung cancer. *Semin. Oncol.*, 26, 4–8 (1999).
23) Eastman, A.: Activation of programmed cell death by anticancer agents: cisplatin as a model system. *Cancer Cells*, 2, 275–280 (1990).
24) Yee, C.S., Kamradt, J.M., Nielsen, L.C., Panvinchian, R. and Pienta, K.J.: Carboplatin-induced cell death in model prostate cancer systems. *Anticancer Res.*, 18, 4475–4482 (1998).
25) Darzynkiewicz, Z.: Apoptosis in antitumor strategies: modulation of cell cycle or differentiation. *J. Cell. Biochem.*, 58, 151–159 (1995).
26) Meterissian, S.H.: Apoptosis: its role in the progression of and chemotherapy for carcinoma. *J. Am. Coll. Surgeons*, 184, 658–666 (1997).
27) Ma, J., Verweij, J., Planting, A.S., Kolker, H.J., Loos, W.J., de, B.-D.M. van, d.B.M.E., Stoter, G. and Schellens, J.H.: Docetaxel and paclitaxel inhibit DNA-adduct formation and intracellular accumulation of cisplatin in human leukocytes. *Cancer Chemoth. Pharmacol.*, 37, 382–384 (1996).