some reports of the clinical evaluation of such a drug combination, namely irinotecan (CPT-11) and etoposide (VP-16) [12,24,25]. Although Masuda et al. [12] concluded that the combination regimen of CPT-11 and VP-16 was effective against refractory or relapsed SCLC, no further studies have been reported. In this study, we investigated the feasibility and effectiveness of the combination chemotherapeutic regimen of AMR (DNA topoisomerase II inhibitor) and TOP (DNA topoisomerase I inhibitor) in patients with relapsed or ED-SCLC.

The rationale for combining DNA topoisomerase I and II inhibitors is that such a combination of drugs would yield greater inhibition of the DNA topoisomerase activity resulting in more potent cytotoxicity, because each topoisomerase enzyme has some compensatory activity in the event of deficiency of the other. It has been reported that the cytotoxicity of such a drug combination increases when the drugs are administered sequentially [22,23]. Kim stressed the importance of the administration sequence in a preclinical study, and showed that administration of CPT-11 (topoisomerase I inhibitor) before doxorubicin (topoisomerase II inhibitor) resulted in a synergistic effect against human tumour xenografts in nude mice [23]. However, Masuda reported that administration of VP-16 (topoisomerase II inhibitor) before CPT-11 was also effective in a clinical study [12,25]. In this study, we administered TOP before AMR, and obtained favourable results. Therefore, clinically, the sequence of administration of the two drugs may not be very important.

The present study demonstrated that treatment with the drug combination of TOP and AMR is feasible in patients with relapsed or ED-SCLC. Negoro, et al. [14] reported the results of a phase I study of AMR monotherapy, with daily administration of the drug for three consecutive days. The MTD was 50 mg/m^2/day (150 mg/m^2/course), and the DLTs were leukopenia, neutropenia, thrombocytopenia and gastrointestinal toxicities. On the other hand, the MTD of TOP during 5 days' administration was estimated to be 1.5–2.0 mg/m^2/day, and the DLTs were reversible leukopenia and neutropenia [15,16]. Subsequently, the clinical effectiveness of a combination of DNA topoisomerase I and II inhibitors, that is, CPT-11 and VP-16, was reported by Karato [24] and Masuda [25]. In Karato's study [24], both drugs were administered on Days 1–3 with G-CSF support. The MTDs of VP-16/CPT-11 were 60/80 or 80/60 mg/m^2, and the DLTs were weight loss and diarrhoea. In Masuda's study [25], CPT-11 was administered on Days 1, 8 and 15, and VP-16 was given on Days 1–3 with G-SCF support. The MTD of CPT-11 was 90 mg/m^2 and that of VP-16 was 80 mg/m^2. The DLTs were diarrhoea and leukopenia. During treatment with the chemotherapeutic combination of TOP and AMR in our study, we determined the MTD of TOP and AMR to be 0.75 mg/m^2 and 50 mg/m^2, respectively. The DLT was almost limited to haematological toxicities and seemed severe, however, all these toxicities were reversible, and we finally considered the phase II dose to be the level 2 dose according to the initial definition for the recommended dose, although further investigation is needed to confirm its safety profiles in the following studies using larger cohorts.

In this study, the C_{max} and AUC of AMR increased in a dose-dependent manner, and statistical significance was not reached. However, the corresponding values of 13-OH-AMR varied markedly among the patients, perhaps attributable partly to our small patient population. However, Ohe et al. also demonstrated similar results with respect to 13-OH-AMR in red blood cells in a phase I/II trial of AMR and CDDP in 45 chemo-naïve patients with ED-SCLC [26]. Negoro, et al. [14] also documented that the plasma concentrations of 13-OH-AMR were very low as compared to those of AMR. Thus, it may be difficult to construct a limited sampling model for estimating the AUC of 13-OH-AMR in either single-agent therapy or combination therapy. The C_{max} and AUC of TOP were not significantly different among the first three dose levels, or between Days 2 and 3, which indicates that AMR did not influence the pharmacokinetics of TOP.

In the pharmacodynamic analysis, we demonstrated that the Cmax and AUC of AMR were correlated with the duration of grade 4 neutropenia. In addition, the mean C_{max} of TOP on Day 2 in seven responders was significantly higher than that in two non-responders. Concerning the relationship between the antitumour effect and pharmacokinetics of AMR, Noguchi et al. reported that the AUC of intracellular 13-OH-AMR was related to the anti-tumour effect of the drug [27]. However, these relationships were not observed in our study. It remains unknown why the Cmax of TOP on the previous day used together with AMR was associated with an objective response. Further investigation is warranted to confirm the role of pharmacokinetic and pharmacodynamic monitoring during treatment with the combination regimen of AMR and TOP.

Using CPT-11 and VP-16, a combination of DNA topoisomerase I and II inhibitors, Masuda et al. [12] reported favourable outcomes in cases of refractory or relapsed SCLC. Among the 24 assessable patients, complete response was observed in three (13%), while 14 (58%) patients showed a PR, with an overall response rate of 71%. The response rate was particularly high (80%) in patients with relapsed SCLC. In this study also, the PR rate in relapsed cancer patients was extremely high (80%). Kubota et al. [28] reported a high response rate of 88% to the CODE regimen in 17 relapsed SCLC patients, which was associated with an encouraging survival rate (MST: 245 days). Therefore, we may expect survival benefit with the use of this combination, and this should be confirmed in future studies.

5. Conclusion

In conclusion, this phase I study showed both the feasibility and effectiveness of the two-drug combination of TOP and AMR in patients with relapsed or ED-SCLC. Since this combination seems to be particularly effective for relapsed SCLC, a phase II trial of this drug regimen in this subset of patients (relapsed SCLC) is warranted.

References

[1] Noda K, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 2002;346:85–91.

[2] Livingston RB, Stephens RL, Bonnet JD, Grozea PN, Lehane DE. Long-term survival and toxicity in small cell lung cancer. Southwest Oncology Group study. Am J Med 1984;77:415–7.

[3] Johnson BE, Ihde DC, Bunn PA, Becker B, Walsh T, Weinstein ZR, et al. Patients with small-cell lung cancer treated with combination chemotherapy with or without irradiation. Data on potential cures, chronic toxicities, and late relapses after a five- to eleven-year follow-up. Ann Intern Med 1985;103:430–8.

[4] Ihde DC, Pass HI, Glatstein E. Small cell lung cancer. In: De Vita Jr VT, Hellman S, Rosenberg SA, editors. Cancer: principles and practice of oncology. 5th ed. Philadelphia: Lippincott-Raven; 1997. p. 911–49.

[5] Albain KS, Crowley JJ, Hutchins L, Gandara D, O'Bryan RM, Von Hoff DD, et al. Predictors of survival following relapse or progression of small cell lung cancer. Southwest Oncology Group Study 8605 report and analysis of recurrent disease data base. Cancer 1993;72:1184–91.

[6] Ettinger DS. New drugs for chemotherapy-naive patients with extensive-disease small cell lung cancer. Semin Oncol 2001;28:27–9.

[7] Yana T, Negoro S, Takada Y, Yokota S, Fukuoka M, The West Japan Lung Cancer Group. Phase II study of amrubicin (SM-5887), a 9-amino-anthracycline, in previously untreated patients with extensive stage small-cell lung cancer (ES-SCLC): A West Japan Lung Cancer Group Trial. Proc Am Soc Clin Oncol 1998;17:450c (abstr 1734).

[8] Schiller JH, Kim K, Hutson P, DeVore R, Glick J, Stewart J, et al. Phase II study of topotecan in patients with exensive-stage small-cell carcinoma of the lung: an Eastern Cooperative Oncology Group Trial. J Clin Oncol 1996;14:2345–52.

[9] Gupta RS, Gupta R, Eng B, Lock RB, Ross WE, Hertzberg RP, et al. Camptothecin-resistant mutants of Chinese hamster ovary cells containing a resistant form of topoisomerase I. Cancer Res 1998;48:6404–10.

[10] Eng WK, McCabe FL, Tan KB, Mattern MR, Hoffman GA, Woessner RD, et al. Development of a stable camptothecin-resistant subline of P388 leukemia with reduced topoisomerase I content. Mol Pharmacol 1990;38:471–80.

[11] Sugimoto Y, Tsukahara S, Oh-hara T, Liu LF, Tsuruno T. Elevated expression of DNA topoisomerase II in camptothecin-resistant human tumor cell lines. Cancer Res 1990;50:7962–5.

[12] Masuda N, Matsui K, Negoro S, Takifuji N, Takeda K, Yana T, et al. Combination of irinotecan and etoposide for treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 1998;16:3329–34.

[13] Mountain CF. Revisions in the international system for staging lung cancer. Chest 1997;111:1710–7.

[14] Negoro S, Fukuoka M, Nakamura S, Ikegami H, Sugiura T, Ariyoshi Y, et al. Phase I-II study of amrubicin (SM-5887), a novel 9-amino-antracycline, by IV administration for 3 consecutive days in patients with advanced non-small cell lung cancer. Proc Am Soc Clin Oncol 1995;14:361 [Abstract 1105].

[15] Kobayashi K, Hino M, Fukuoka M, Takeuchi K, Furuse K, Yoneda S, et al. Phase I studies of nogitecan hydrochloride for Japanese. Int J Clin Oncol 2002;7:177–86.

[16] Rowinsky EK, Grochow LB, Henricks CB, Ettinger DS, Forastiere AA, Hurowitz LA, et al. Phase I and pharmacologic study of topotecan: a novel topoisomerase I inhibitor. J Clin Oncol 1992;10:647–56.

[17] Shimoyama M. The Japanese edition of the National Cancer Institute-Common Toxicity Criteria (in Japanese). Gan To Kagaku Ryoho 1999;26:1084–109.

[18] Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2002;92:205–16.

[19] Kano Y, Suzuki K, Akutsu M, Suda K, Inoue Y, Yoshida M, et al. Effects of CPT-11 in combination with other anti-cancer agents in culture. Int J Cancer 1992;50:604–10.

[20] D'Arpa P, Beardmore C, Liu LF. Involvement of nucleic acid synthesis in cell killing mechanisms of topoisomerase poisons. Cancer Res 1990;50:6919–24.

[21] Kaufmann SH. Antagonism between camptothecin and topoisomerase II-directed chemotherapeutic agents in a human leukemia cell line. Cancer Res 1991;51:1129–36.

[22] Bertrand R, O'Connor PM, Kerrigan D, Pommier Y. Sequential administration of camptothecin and etoposide circumvents the antagonistic cytotoxicity of simultaneous drug administration in slowly glowing human colon carcinoma HT-29 cells. Eur J Cancer 1992;28A:743–8.

[23] Kim R, Hirabayashi N, Nishiyama M, Jinushi K, Toge T, Okada K. Experimental studies on biochemical modulation targeting topoisomerase I and II in human tumor xenografts in nude mice. Int J Cancar 1992;50:760–6.

[24] Karato A, Sasaki Y, Shinkai T, Eguchi K, Tamura T, Ohe Y, et al. Phase I study of CPT-11 and etoposide in patiets with refractory solid tumors. J Clin Oncol 1993;11:2030–5.

[25] Masuda N, Fukuoka M, Kudoh S, Matsui K, Kusunoki Y, Takada M, et al. Phase I and pharmacologic study of irinotecan and etoposide with recombinant human granulocyte colony-stimulating factor support for advanced lung cancer. J Clin Oncol 1994;12:1833–41.

[26] Ohe Y, Negoro S, Matsui K, Nakagawa K, Sugiura T, Takada Y, et al. Phase I–II study of amrubicin and cisplatin in previously untreated patients with extensive-stage small-cell lung cancer. Ann Oncol 2005;16:430–6.

[27] Noguchi T, Ichii S, Morisada S, Yamaoka T, Yanagi Y. In vivo efficacy and tumor-selective metabolism of amrubicin to its active metabolite. Jpn J Cancer Res 1998;89:1055–60.

[28] Kubota K, Nishiwaki Y, Kakinuma R, Hojo F, Matsumoto T, Ohmatsu H, et al. Dose-intensive weekly chemotherapy for treatment of relapsed small-cell lung cancer. J Clin Oncol 1997;15:292–6.

ONCOLOGY REPORTS 15: 837-842, 2006

The combination effect of amrubicin with cisplatin or irinotecan for small-cell lung cancer cells

NAGIO TAKIGAWA[1,2,3], MASAKO TAKEYAMA[2], TAKUO SHIBAYAMA[1,2], ATSUHIKO TADA[1,2], NORIKO KAWATA[1,2], CHIHARU OKADA[1,2], KEISUKE AOE[4], TOSHIYUKI KOZUKI[3], KATSUYUKI HOTTA[3], MASAHIRO TABATA[3], KATSUYUKI KIURA[3], HIROSHI UEOKA[4], MITSUNE TANIMOTO[3] and KIYOSHI TAKAHASHI[1]

[1]Department of Internal Medicine and [2]Clinical Research Institute, National Hospital Organization, Minami-Okayama Medical Center, 4066 Hayashima, Okayama 701-0304; [3]Department of Respiratory Medicine, Okayama University Hospital, 2-5-1 Shikata-cho, Okayama 700-8558; [4]Department of Respiratory Medicine, National Hospital Organization, Sanyo Hospital, 685 Higashikiwa, Ube 755-0241, Japan

Received September 26, 2005; Accepted November 21, 2005

Abstract. The single agent of amrubicin is active in untreated small-cell lung cancer (SCLC). Cytotoxicity of amrubicinol, the active form of amrubicin, was evaluated in a parent SCLC cell line (SBC-3); an active metabolite of irinotecan, 7-ethyl-10-hydroxy-camptothecin (SN-38)-resistant subline (SBC-3/SN-38); and cisplatin-resistant subline (SBC-3/CDDP) using AlamarBlue assay. Interaction of the combined drugs was evaluated by median-effect plot analysis, and the fraction of apoptotic cells was determined using flow cytometry. SBC-3/SN-38 was 34-fold more resistant to SN-38 and SBC-3/CDDP was 7.2-fold more resistant to cisplatin than parental SBC-3. However, these resistant sublines retained sensitivity to amrubicinol (1.8- and 1.7-fold, respectively). Simultaneous exposure of SBC-3/SN-38 cells to amrubicinol and cisplatin showed a synergistic effect. Simultaneous exposure of SBC-3/CDDP cells to amrubicinol and SN-38 displayed synergistic or additive effects. The two-drug combination produced an increase of apoptotic cells compared to each single agent alone in both resistant cells. These findings suggest that amrubicin alone and in combination with cisplatin or irinotecan is effective against SCLC refractory to irinotecan and/or cisplatin.

Introduction

More than 80% of patients with small-cell lung cancer (SCLC) receiving chemotherapy achieve an objective response; however, most responders eventually relapse because of drug resistance (1). Since a phase III study in patients with extensive disease (ED)-SCLC demonstrated that a combination regimen of cisplatin and irinotecan yielded a highly significant improvement in survival over a standard regimen consisting of cisplatin and etoposide (2), the combination may be considered the current standard treatment for ED-SCLC. However, the median survival time and 2-year survival rate were only 12.8 months and 19.5%, respectively (2). The development of irinotecan or cisplatin resistance in tumor cells is assumed to play a major role in these unsatisfactory results.

Amrubicin is a totally synthetic 9-aminoanthracyclin (3). Amrubicinol, its converted active form, has 10 to 100 times higher activity than amrubicin in cytotoxicity by inhibiting topoisomerase II (4,5). Antitumor activity of amrubicin was superior to that of the mother compound, adriamycin in human tumor xenografts (6). In addition, amrubicin had less toxicity, including cardiotoxicity, than adriamycin, in experimental animal models (7,8). Amrubicin was highly active (response rate, 78.8%; median survival time, 11.3 months) and well tolerated in a phase II study in untreated patients with ED-SCLC (9). The objectives of this study were to evaluate the antitumor activity of amrubicin for SCLC cells, especially for irinotecan- or cisplatin-resistant cells, and the combination effect of amrubicin with commonly used anticancer drugs against SCLC.

Materials and methods

Chemicals and reagents. Drugs in this study were provided by the following sources: amrubicin (SM5887) and amrubicinol (SM5887-13-OH) from Sumitomo Pharmaceuticals Co., Ltd., Osaka, Japan; irinotecan and 7-ethyl-10-hydroxycamptothecin (SN-38) from Yakult Honsha, Tokyo, Japan; etoposide and paclitaxel from Bristol-Myers Squibb, Tokyo, Japan; and cisplatin from Nippon Kayaku Kogyo Co., Ltd., Tokyo, Japan. Amrubicin, irinotecan and cisplatin were dissolved in 0.9% saline, and amrubicinol was dissolved in distilled

Correspondence to: Dr Nagio Takigawa, Department of Respiratory Medicine, Okayama University Hospital, 2-5-1 Shikata-cho, Okayama 700-8558, Japan
E-mail: ntakigaw@md.okayama-u.ac.jp

Key words: amrubicin, irinotecan, cisplatin, small-cell lung cancer, median-effect plot analysis

water. SN-38, etoposide, and paclitaxel were dissolved in dimethylsulfoxide. Drug solutions were stored at -20°C. AlamarBlue (UK Serotec Ltd., Oxford) was purchased from Dainippon Pharmaceutical Co. Ltd, Osaka, Japan.

Cell culture. The SBC-3 parent cell line was established from a bone marrow aspirate of a previously untreated patient with SCLC (10). The growth medium (RPMI-FBS) was RPMI-1640 supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). The SN-38-resistant subline (SBC-3/SN-38) (11) and cisplatin-resistant subline (SBC-3/CDDP) (12) were established by continuous exposure of the SBC-3 cells to increasing concentrations of SN-38 and cisplatin, respectively.

Assay of drug sensitivity. Drug sensitivity was determined using AlamarBlue assay (13). Briefly, 50 μl of RPMI-FBS containing serial concentrations of each chemotherapeutic agent was prepared in 96-well flat-bottomed microplates (Coster 3596; Corning Inc., Corning, NY, USA). The 50 μl of RPMI-FBS containing 500 cells for SBC-3, 1500 cells for SBC-3/SN-38 and 2000 cells for SBC-3/CDDP was then added to each well. Cells were incubated at 37°C for 96 h in a highly humidified incubator with 5% CO_2 and 95% air, and then 10 μl of AlamarBlue was added to each well. After incubation at 37°C for 5 h, the fluorescence of each well was measured using Fluoroskan Ascent (Labsystems Inc., Franklin, MA, USA) with 544 nm excitation and 590 nm emission. Fluorescence of a well without chemotherapeutic agents was used as the control, and a well containing only RPMI-FBS and AlamarBlue was used to determine the background. The percentage of surviving cells was calculated using the formula: [(mean fluorescence in 4 test wells - fluorescence in background wells)/(mean fluorescence in control wells - fluorescence in background wells)] x100. The drug concentration required to inhibit growth of tumor cells by 50% (IC_{50}) was determined by plotting the logarithm of drug concentration versus the percentage of surviving cells.

Table I. Drug sensitivity in the SBC-3 parent line, SN-38-resistant subline (SBC-3/SN-38), and cisplatin-resistant subline (SBC-3/CDDP).

	IC_{50} value (nM)		
	SBC-3	SBC-3/SN-38	SBC-3/CDDP
SN-38	4.1±1.5	139±16	13±4.5
R.R.		34	3.2
Cisplatin	345±39	120±15	2480±120
R.R.		0.35	7.2
Amrubicinol	33±16	60±26	57±20
R.R		1.8	1.7

IC_{50}, 50% inhibitory concentration; SD, standard deviation; R.R, relative resistance value (IC_{50} value of resistant cells/IC_{50} value of SBC-3 cells). Data are expressed as mean ± SD.

Determinations were carried out in quadruplicate for each experiment, and results were confirmed by 3 or more separate experiments. Relative resistance was calculated by dividing the IC_{50} value of resistant subline cells by the IC_{50} of SBC-3 cells.

Design for drug combination. The constant-ratio design for the combination assay is highly recommended as it allows the most efficient data analysis (14). After simultaneous exposure of the cells to two drugs for 96 h, growth inhibition was determined using AlamarBlue assay. Sequential exposure of two drugs was performed as follows. After exposure to the first drug for 24 h, cells were twice washed in drug-free medium, and the second drug was then added to the 96-well microplates for 24 h. At the end of exposure, the cells were washed in drug-free medium, re-incubated in drug-free medium for 48 h, and proliferation was measured with AlamarBlue. Experiments were repeated 3 times.

Table II. Combination effect of amrubicinol and other agents.

Cell line	Drugs	Combination index (mean ± SD)	
		IC_{70}	IC_{90}
SBC-3	AMR-OH + SN-38	1.2±0.1	1.0±0.02
	AMR-OH + CDDP	0.82±0.05	0.35±0.17
	AMR-OH + PTX	1.3±0.26	2.4±0.52
	AMR-OH + ETP	1.1±0.02	0.85±0.21
	AMR-OH → SN-38	1.0±0.02	1.1±0.25
	SN-38 → AMR-OH	1.5±0.32	2.2±0.17
	AMR-OH → CDDP	0.86±0.15	0.93±0.32
	CDDP → AMR-OH	0.93±0.12	1.0±0.06
SBC-3/CDDP	AMR-OH + SN-38	0.76±0.21	1.0±0.35
SBC-3/SN-38	AMR-OH + CDDP	0.99±0.17	0.89±0.24

AMR-OH, amrubicinol; CDDP, cisplatin; PTX, paclitaxel; ETP, etoposide.

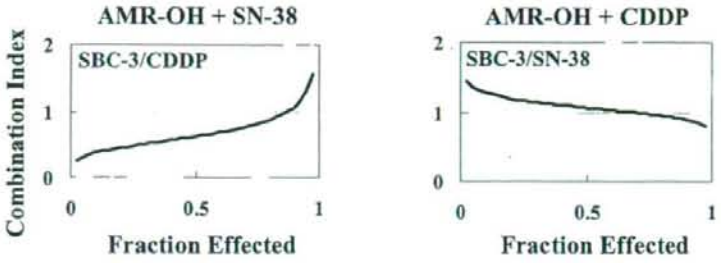

Figure 1. Combination index and surviving fraction of SBC-3/CDDP cells treated with amrubicinol (AMR-OH) in combination with SN-38 simultaneously for 96 h (left). Combination index and surviving fraction of SBC-3/SN-38 cells treated with AMR-OH in combination with cisplatin (CDDP) simultaneously for 96 h (right).

Median-effect principle for dose-effect analysis. The multiple drug effect analysis of Chou and Talaly, based on the median-effect principle, was used to calculate the combined drug effect (15). This method involved plotting dose-effect curves for each agent and its combination with other agents by using the median-effect equation: $fa/fu = (D/Dm)^m$ (equation 1).

In equation 1, D is the dose, Dm is the required dose for 50% inhibition of cell growth, *fa* is the fraction affected by dose D (e.g. 0.9 if cell growth is inhibited by 90%), *fu* is the unaffected fraction (therefore, fa = 1-fu), and *m* is a coefficient of the sigmoidicity of the dose-effect curve; $m=1$, $m>1$, and $m<1$ indicate hyperbolic, sigmoidal, and negative sigmoidal dose-effect curves, respectively, for an inhibitory drug. Thus, both potency (Dm) and shape (*m*) were taken into account as parameters in this method. Equation 2 was rearranged from equation 1 as follows: $D = Dm[fa/(1-fa)]^{1/m}$ (equation 2).

The Dm and m values were easily determined by the median-effect plot; x= log (D) versus y= log (*fa*/*fu*) was based on the logarithmic form of equation 1. In the median-effect plot, *m* was slope and log (Dm) was the x-intercept. Conformity of data to the median-effect principle could be readily manifested by the linear coefficient (r) of the median-effect plot. To obtain a reasonable *m* and *r*, non-linear points, usually at the lowest or the highest concentrations, were excluded. The 5 to 9 concentrations on a linear line were employed in this analysis. Computer programs based on the median-effect plot parameters and combination index equation have been used for data analysis in the present study (16).

Combination index for determining synergism and antagonism. The combination index (CI) isobologram equation was used for data analysis of the two-drug combination: $CI = (D)A/(Dx)A + (D)B/(Dx)B$ (equation 3).

$CI<1$, $CI=1$, and $CI>1$ indicate synergism, additive effect, and antagonism, respectively. Equation 3 dictates that drug A, i.e. (D)B in the numerators inhibit x% when drugs A and B are combined. (Dx)A and (Dx)B in denominators of equation 3 indicate doses of drug A and drug B alone, respectively, that also inhibit x%. Dx can be readily calculated from equation 2, where D is designated for x% inhibition. When equation 3 equals 1 (i.e. CI=1), it represents the classic isobologram equation. CI at the inhibitory concentration of 70% (IC_{70}) and 90% (IC_{90}) levels was used for determining synergism, additive effect, or antagonism.

Flow cytometry. Flow cytometry for cell cycle traverse perturbations was carried out after staining with propidium iodide using CycleTest Plus DNA Reagent kit (Becton-Dickinson Immunocytometry Systems, San Jose, CA, USA). Drug concentration was based on the IC_{50} value of a single drug. After 96 h simultaneous exposure to single drug or combined drugs, cells were stained according to the instruction manual. For sequential schedules, after 24 h of exposure to the first drug, cells were twice washed in drug-free medium, and the second drug was then added to cells for 24 h. At the end of exposure, cells were stained with propidium iodide. Flow cytometric analysis was performed on a FACSCalibur (Becton-Dickinson Immunocytometry Systems). Data were analyzed according to ModFit LT software (Verity Software House Inc, Topsham, ME, USA).

Results

Cytotoxicity of amrubicinol and other drugs. Values (mean ± standard deviation) for IC_{50} and relative resistance of SN-38, cisplatin, and amrubicinol for SBC-3, SBC-3/SN-38, and SBC-3/CDDP cells are shown in Table I. Although SBC-3/SN-38 was 34-fold more resistant to SN-38 and SBC-3/CDDP was 7.2-fold more resistant to cisplatin than the parental SBC-3, they retained sensitivity to amrubicinol with relative resistance values of 1.8 and 1.7, respectively. IC_{50} values of other drugs for SBC-3 cells were: amrubicin, 862±46 nM; irinotecan, 195±10.2 nM; etoposide, 270±170 nM; and paclitaxel, 0.55±0.25 nM.

Combination effect of amrubicinol with other drugs for SBC-3. To equalize the contribution of each drug, the ratio of IC_{50} value for each drug was used as the concentration ratio for the combination (14). Thus, concentration ratios of amrubicinol, SN-38, cisplatin, paclitaxel, and etoposide were designed to be relative ratios of 100: 10: 1000: 1:1000, respectively. CI values for SBC-3 cells treated with amrubicinol after 96 h simultaneous exposure to SN-38, paclitaxel, cisplatin or etoposide are shown in Table II. Amrubicinol and cisplatin showed a synergistic effect, however, amrubicinol and paclitaxel exerted an antagonistic

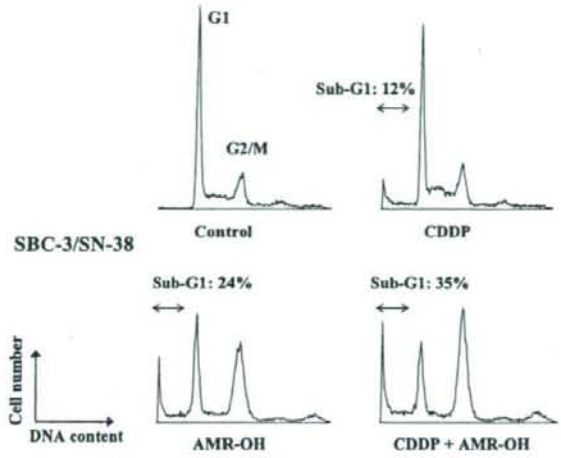

Figure 2. Effect of cisplatin (CDDP), amrubicinol (AMR-OH), or the combination of CDDP and AMR-OH induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3/SN-38 cells.

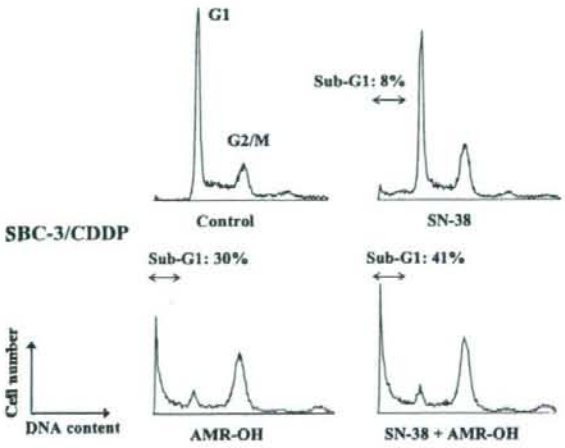

Figure 3. Effect of SN-38, amrubicinol (AMR-OH), or the combination of SN-38 and AMR-OH induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3/CDDP cells.

effect. At IC_{90}, the combination of amrubicinol and SN-38 showed an additive effect and that of amrubicinol and etoposide displayed a synergistic effect.

Combination effect of amrubicinol with SN-38 for SBC-3/CDDP and cisplatin for SBC-3/SN-38. CI values and the surviving fraction of SBC-3/CDDP cells treated by 96 h simultaneous exposure to amrubicinol and SN-38 are drawn in Fig. 1 (left). Based on IC_{50} values in resistant cells, the concentration ratio of amrubicinol and SN-38 was determined to be 5:1. CI values were 0.76±0.21 at IC_{70} and 1.0±0.35 at IC_{90}. Similarly, CI values and the surviving fraction of SBC-3/SN-38 cells treated by 96 h simultaneous exposure to amrubicinol and cisplatin are drawn in Fig. 1 (right). The concentration ratio of amrubicinol to cisplatin was 1:2.

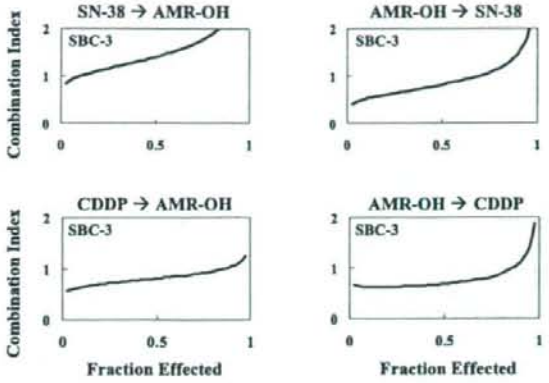

Figure 4. Combination index and surviving fraction of SBC-3 cells treated sequentially with SN-38 or cisplatin (CDDP) for 24 h followed by amrubicinol (AMR-OH) for 24 h and the reverse sequence.

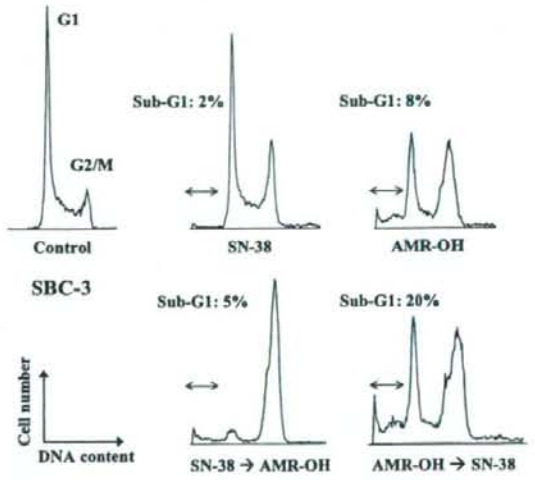

Figure 5. Effect of SN-38, amrubicinol (AMR-OH), SN-38 followed by AMR-OH, or AMR-OH followed by SN-38 induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3 cells.

CI values were 0.99±0.17 at IC_{70} and 0.89±0.24 at IC_{90}. Thus, the combination of amrubicinol with SN-38 showed synergistic or additive effects for cisplatin-resistant cells, and amrubicinol with cisplatin displayed a synergistic effect for SN-38-resistant cells. As shown in Fig. 2, an analysis of cell cycle traverse perturbations demonstrated that treating SBC-3/SN-38 cells with amrubicinol (50 nM) alone resulted in an accumulation of cells in the S+G2/M boundary and a measurable increase in the apoptotic cell population (sub-G1, 24%). Cisplatin (100 nM) alone increased apoptotic cells to 12%, however, the combination of these two drugs induced more apoptosis (35%). Similarly, treating SBC-3/CDDP cells with the combination of SN-38 (10 nM) and amrubicinol (50 nM) produced more apoptotic cells (sub-G1, 41%) than SN-38 alone (8%) or amrubicinol alone (30%) (Fig. 3).

Analysis of combination effect by exposure schedule of amrubicinol and SN-38 or cisplatin for SBC-3. CI values and

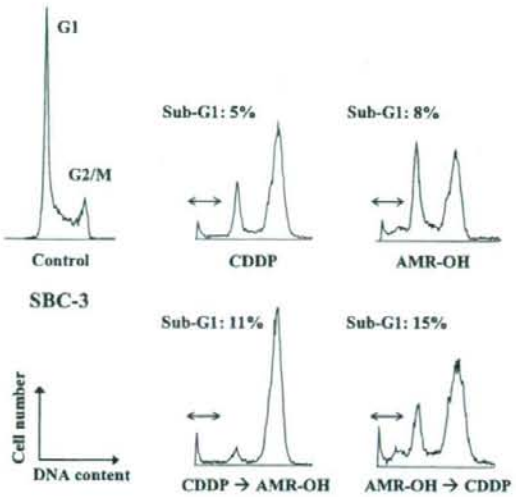

Figure 6. Effect of cisplatin (CDDP), amrubicinol (AMR-OH), CDDP followed by AMR-OH, or AMR-OH followed by CDDP induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3 cells.

the surviving fraction of SBC-3 cells treated sequentially with amrubicinol for 24 h followed by SN-38 or cisplatin for 24 h and those with a reverse sequence are shown in Fig. 4. The sequence of amrubicinol followed by SN-38 was more effective than the reverse sequence. As shown in Fig. 5, analysis of cell cycle traverse perturbations demonstrated that treatment of SBC-3 cells with amrubicinol alone resulted in an accumulation of cells in the S+G2/M boundary and a measurable increase in the apoptotic cell population (sub-G1, 8%). Treating the cells with SN-38 (5 nM) followed by amrubicinol (50 nM) resulted in no marked accumulation of cells at sub-G1 (5%), but the reverse sequence exposure produced a marked increase in apoptotic cells (20%). CI values after exposure to cisplatin followed by amrubicinol were 0.93±0.12 at IC_{70} and 1.0±0.06 at IC_{90}, and 0.86±0.15 at IC_{70} and 0.93±0.32 at IC_{90} for the reverse sequence. This combination of two drugs appears effective irrespective of sequence. Treatment with amrubicinol (50 nM) followed by cisplatin (500 nM) and the reverse sequence exposure increased the number of apoptotic cells (15% and 11%, respectively) as shown in Fig. 6.

Discussion

We have established adriamycin-resistant SBC-3/ADM (17), etoposide-resistant SBC-3/ETP (18), cisplatin-resistant SBC-3/CDDP (12), and SN-38-resistant SBC-3/SN-38 cells from SBC-3, which was derived from an untreated SCLC patient (11). Amrubicinol was found to be completely cross-resistant to adriamycin and etoposide in experiments using SBC-3/ADM and SBC-3/ETP cells (19). SBC-3/SN-38 cells had decreased topoisomerase I and II activity and over-expressed breast cancer-resistant protein compared to the SBC-3 cells (11). SBC-3/CDDP cells showed increased intracellular glutathione and glutathione S-transferase content and decreased intracellular accumulation of cisplatin (12). In the present study, SBC-3/SN-38 and SBC-3/CDDP retained sensitivity to amrubicinol. These results suggest that amrubicin may be effective for SCLC patients who were previously treated with cisplatin and irinotecan. In addition, the combination of amrubicinol and cisplatin showed a synergistic effect for SBC-3/SN-38 and that of amrubicinol and SN-38 displayed additive or synergistic effects for SBC-3/CDDP. In a phase II study, the combination of amrubicin and cisplatin was reported to be highly effective for untreated ED-SCLC (20). A combination of amrubicin and irinotecan was feasible and effective in some patients with relapsed non-small cell lung cancer in our phase I study (21). The present study suggests that combination of amrubicin and cisplatin or irinotecan is also worth evaluating in relapsed SCLC patients.

Amrubicin had additive effects in combination with cisplatin for several human tumor cells, including lung cancer cells, by isobologram analysis (22,23). The present study confirmed those results using SBC-3, as both simultaneous and sequential combinations of the two drugs displayed synergistic or additive effects by median-effect plot analysis. In addition, flow cytometric analysis showed that exposure of the two drugs produced an increase of apoptotic cells compared to that for each single agent. It was difficult to draw a conclusion about the effect of the combination of amrubicinol and SN-38. However, sequential exposure of amrubicinol followed by SN-38 may be considered for further studies since: i) CI values after simultaneous exposure of amrubicinol and SN-38 were 1.2 at IC_{70} (antagonistic) and 1.0 at IC_{90} (additive); ii) the effect of SN-38 followed by amrubicinol was antagonistic; and iii) CI values after sequential exposure of amrubicinol followed by SN-38 were 1.0 at IC_{70} (additive) and 1.1 at IC_{90} (antagonistic), and this sequence produced a marked increase in apoptotic cells. Amrubicinol had an additive effect with etoposide for T-cell leukemia cells and osteosarcoma cells, although the effects were antagonistic at IC_{70} and synergistic at IC_{90} for SBC-3 (22). To our knowledge, the combination of amrubicinol with paclitaxel, which had an antagonistic effect in this study, has not been reported. More cell lines should be investigated to further evaluate these combinations.

The mechanisms of drug interaction between amrubicinol and other drugs have not been elucidated. Flow cytometry data in the present study suggested the presence of apoptotic cells based on the sub-G1 peak. Biochemical analysis for apoptotic cell death should be carried out for further investigation. Yamauchi *et al* reported that cisplatin enhanced the topoisomerase II inhibitory effect of amrubicinol and amrubicinol enhanced the formation of cisplatin-induced DNA interstrand cross-links (23). A combination of topoisomerase I inhibitors and topoisomerase II inhibitors is thought reasonable because reciprocal enhancement of one enzyme in the resistant cell lines develops an inhibitory effect on the other enzyme (24). However, the effectiveness of a combination and administration schedule has been a controversial issue in clinical trials to date (25). Thus, additional research will be needed to establish a rationale for the combination of irinotecan and amrubicin.

The combination of irinotecan and cisplatin is accepted as the standard treatment for ED-SCLC (2). Concurrent

chemoradiotherapy consisting of cisplatin, etoposide and thoracic radiotherapy followed by cisplatin and irinotecan is considered to be very active in limited disease SCLC (26). The present study indicated that further studies are warranted on amrubicin alone and in combination with cisplatin or irinotecan in relapsed SCLC patients.

References

1. Doyle LA: Mechanisms of drug resistance in human lung cancer cells. Semin Oncol 20: 326-337, 1993.
2. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S, Saijo N; Japan Clinical Oncology Group: Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 346: 85-91, 2002.
3. Ishizumi K, Ohashi N and Tanno N: Streospecific total synthesis of 9-aminoanthracyclines: (+)-9-amino-9-deoxydaunomycin and related compounds. J Org Chem 50: 4477-4485, 1987.
4. Yamaoka T, Hanada M, Ichii S, Morisada S, Noguchi T and Yanagi Y: Cytotoxicity of amrubicin, a novel 9-aminoanthracycline, and active metabolite amrubicinol on human tumor cells. Jpn J Cancer Res 89: 1061-1067, 1998.
5. Hanada M, Mizuno S, Fukushima A, Saito Y, Noguchi T and Yamaoka T: A new antitumor agent amrubicin induces cell growth inhibition by stabilizing topoisomerase II-DNA complex. Jpn J Cancer Res 89: 1229-1238, 1998.
6. Morisada S, Yanagi Y, Noguchi T, Kashiwazaki Y and Fukui M: Antitumor activities of a novel 9-aminoanthracycline (SM5887) against mouse experimental tumors and human tumor xenografts. Jpn J Cancer Res 80: 69-76, 1989.
7. Suzuki T, Minamide S, Iwasaki T, Yamamoto H and Kanda H: Cardiotoxicity of a new anthracycline derivative (SM-5887) following intravenous administration to rabbits: comparative study with doxorubicin. Invest New Drugs 15: 219-225, 1997.
8. Noda T, Watanabe T, Kohda A, Hosokawa S and Suzuki T: Chronic effects of a novel synthetic anthracycline derivative (SM-5887) on normal heart and doxorubicin-induced cardiomyopathy in beagle dogs. Invest New Drugs 16: 121-128, 1998.
9. Yana T, Negoro Y, Takada S, Yokota M and Fukuoka M: Phase II study of amrubicin (SM-5887), a 9-amino-anthracycline, in previously untreated patients with extensive stage small-cell lung cancer (ES-SCLC): a West Japan Lung Cancer Group Trial. Proc Am Soc Clin Oncol 17: 450, 1998.
10. Miyamoto H: Establishment and characterization of an adriamycin-resistant subline of human small cell lung cancer cells. Acta Med Okayama 40: 65-73, 1986.
11. Chikamori M, Takigawa N, Kiura K, Tabata M, Shibayama T, Segawa Y, Ueoka H, Ohnoshi T and Tanimoto M: Establishment of a 7-ethyl-10-hydroxy-camptothecin-resistant human small-cell lung cancer cell line. Anticancer Res 24: 3911-3916, 2004.
12. Moritaka T, Kiura K, Ueoka H, Tabata M, Segawa Y, Shibayama T, Takigawa N, Ohnoshi T and Harada M: Cisplatin-resistant human small cell lung cancer cell line shows collateral sensitivity to vinca alkaloids. Anticancer Res 18: 927-933, 1998.
13. Ahmed SA, Gogal RM Jr and Walsh JE: A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H]thymidine incorporation assay. J Immunol Methods 170: 211-224, 1994.
14. Chang TT and Chou TC: Rational approach to the clinical protocol design for drug combinations: a review. Acta Paediatr Taiwan 41: 294-302, 2000.
15. Chou TC and Talaly P: A simple generalized equation for the analysis of multiple inhibitions of Michaelis-Menten kinetic systems. J Biol Chem 252: 6438-6442, 1977.
16. 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.
17. Kiura K, Ohnoshi T, Tabata M, Shibayama T and Kimura I: Establishment of an adriamycin-resistant subline of human small cell lung cancer showing multifactorial mechanisms of resistance. Acta Med Okayama 47: 191-197, 1993.
18. Takigawa N, Ohnoshi T, Ueoka H, Kiura K and Kimura I: Establishment and characterization of an etoposide-resistant human small cell lung cancer cell line. Acta Med Okayama 46: 203-212, 1992.
19. Takigawa N, Ohnoshi T, Ueoka H, Kiura K and Kimura I: Comparison of antitumor activity of new anthracycline analogues, ME2303, KRN8602, and SM5887 using human lung cancer cell lines. Acta Med Okayama 46: 249-256, 1992.
20. Ohe Y, Negoro S, Matsui K, Nakagawa K, Sugiura T, Takada Y, Nishiwaki Y, Yokota S, Kawahara M, Saijo N, Fukuoka M and Ariyoshi Y: Phase I-II study of amrubicin and cisplatin in previously untreated patients with extensive stage small cell lung cancer. Ann Oncol 16: 430-436, 2005.
21. Hotta K, Takigawa N, Kiura K, Tabata M, Umemura S, Ogino A, Uchida A, Bessho A, Segawa Y, Shinkai T, Nogami N, Harita S, Okimoto N, Ueoka H and Tanimoto M: Phase I study of irinotecan and amrubicin in patients with advanced non-small-cell lung cancer. Anticancer Res 25: 2429-2434, 2005.
22. Takagi T, Yazawa Y, Suzuki K, Yamauchi Y and Kano Y: Effects of 13-hydroxy SM5887 in combination with other anticancer agents on human tumor cell lines. Invest New Drugs 14: 357-363, 1996.
23. Yamauchi S, Kudoh S, Kimura T, Hirata K and Yoshikawa J: Additive effects of amrubicin with cisplatin on human lung cancer cell lines. Osaka City Med J 48: 69-76, 2002.
24. Rubin EH, Li TK, Duann P and Liu LF: Cellular resistance to topoisomerase poisons. Cancer Treat Res 87: 243-260, 1996.
25. Xu Y and Villalona-Calero MA: Irinotecan: mechanisms of tumor resistance and novel strategies for modulating its activity. Ann Oncol 13: 1841-1851, 2002.
26. Mitsuoka S, Kudoh S, Takada Y, Matsui K, Eguchi K, Ichinose Y, Nakagawa K, Takada M, Saito H and Ariyoshi Y: Phase II study of cisplatin, etoposide and concurrent thoracic radiotherapy (TRT) followed by irinotecan and cisplatin in patients with limited stage small-cell lung cancer (SCLC); updated results of WJTOG9902. Proc Am Soc Clin Oncol 22: 7044, 2004.

Is the Importance of Achieving Stable Disease Different between Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors and Cytotoxic Agents in the Second-Line Setting for Advanced Non-small Cell Lung Cancer?

Takayasu Kurata, MD, Keitaro Matsuo, MD,† Minoru Takada, MD,‡ Masaaki Kawahara, MD,‡ Masahiro Tsuji, MD,§ Yuka Matsubara, MD,§ Nagahiro Otani, MD,§ Shigeki Matsuyama, MD,§ Kenya Muraishi, MD,§ Tetsuya Fujita, MD,§ Masato Ishikawa, MD,§ Keita Koyano, MD,§ Isamu Okamoto, MD,* Taroh Satoh, MD,* Kenji Tamura, MD,* Kazuhiko Nakagawa, MD,* and Masahiro Fukuoka, MD**

Background: It is controversial whether achieving stable disease leads to a survival benefit and whether the importance of achieving stable disease differs between cytotoxic agents and molecular targeted agents. To examine these questions, the authors retrospectively reviewed phase II and III studies in the second-line setting for advanced non-small cell lung cancer using epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) and cytotoxic agents separately.

Methods: The authors chose 45 trials for the chemotherapy group and nine for the EGFR TKI group by searching the PubMed database. All nine trials in the EGFR TKI group concern gefitinib and erlotinib.

Results: The median survival time increased 0.0375 month with each 1% increase in stable disease rate ($p = 0.039$), and each 1% increase in response rate resulted in 0.0744 ($p < 0.001$) month of median survival time in the analysis combined with both cytotoxic agents and EGFR TKIs. Main and interaction terms for EGFR TKI treatment were not statistically significant. With respect to time to progression, only response rate showed a statistically significant relationship with survival.

Conclusions: To obtain response seems to be more important than to achieve stable disease for both cytotoxic agents and EGFR TKIs, although achieving stable disease is still valuable. The relationship between survival and response or stable disease appears similar for cytotoxic agents and EGFR TKIs.

*Department of Medical Oncology, Kinki University School of Medicine, Osaka, Japan; †Department of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan; ‡National Hospital Organization, Kinki-Chuo Chest Medical Center; and §Kinki-Chuo Oncology Meeting, Osaka, Japan.

Address for correspondence: Takayasu Kurata, MD, Department of Medical Oncology, Kinki University School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan. E-mail: kurata@hp.pref.hyogo.jp

ISSN: 1556-0864/06/0107-0684

Key Words: Stable disease, Response rate, Non-small cell lung cancer, Second-line setting, Epidermal growth factor receptor, Tyrosine kinase inhibitors.

(*J Thorac Oncol.* 2006;1: 684–691)

In 1995, a meta-analysis demonstrated a modest survival benefit for cisplatin-based chemotherapy compared with best supportive care as first-line therapy in patients with locally advanced or metastatic non-small cell lung cancer (NSCLC).[1] Equal survival improvement is provided by introducing several new agents with novel mechanisms and significant activity against NSCLC such as taxanes, gemcitabine, and vinorelbine, when used in combination with a platinum agent.[2–4] However, most patients relapse following platinum-based chemotherapy, leading to poor survival. Until recently, the role of second-line chemotherapy was not well defined because most patients had a poor performance status by the time of relapse. However, as newer agents in combination with platinum agents have increased, the number of patients with durable antitumor effects and the number of patients for second-line chemotherapy have increased. Therefore, second-line chemotherapy for advanced NSCLC is becoming increasingly important. Several chemotherapy agents have been evaluated in the second-line setting. Among them, docetaxel was the first agent to show a survival benefit and an improvement in quality of life in two large phase III studies[5,6] and has been approved as a second-line agent. A recent randomized phase III study reported that pemetrexed (a multitargeted antifolate, Alimta; Eli Lilly & Co., Indianapolis, IN) had comparable activity and better symptom relief than docetaxel.[7] Both of these cytotoxic agents demonstrated response rates of less than 10%, but both agents have demonstrated survival benefits and an improvement in quality of life. This indicates that it is important to achieve stable disease and objective response for second-line cytotoxic agents.

The molecular targeted agents are attractive because they promise to produce specific cytostatic action with a resultant mild toxicity profile. In many tumors, overexpression of the epidermal growth factor receptor (EGFR) is associated with a poor prognosis and chemoresistance,[8,9] and it is common in NSCLC.[10–12] The low-molecular-weight EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib are the most advanced agents in clinical trials. The results of a recent phase III study in the second-line setting showed that erlotinib significantly improved survival compared with best supportive care,[13] although the overall response rate was only 9% on the erlotinib arm.

Because of their mechanism of action, it might be more important to achieve stable disease for most molecular targeted agents than for their cytotoxic counterparts. However, evaluating stable disease in clinical trials is very difficult, as patients with stable disease are not a homogeneous population.

Based on this background, we hypothesized that not only objective response but also stable disease could lead to survival benefit, in particular, with molecular targeted agents. Therefore, we retrospectively reviewed phase II and randomized phase III studies in the second-line setting using EGFR TKIs and cytotoxic agents separately to evaluate our hypothesis and ascertain whether the importance of achieving stable disease was different between EGFR TKIs and cytotoxic agents.

METHODS

Search and Selection for Trials

Data concerning response rates, rates of stable disease, time to progression, and survival from all published studies including phase II and randomized phase III studies assessing the activity of EGFR TKIs and cytotoxic agents in the second-line setting were identified electronically. We performed the search for trials through a computer-based search of the PubMed database using the following terms: "NSCLC," "chemotherapy (second or pretreated)," "advanced," "not radiation," "not adjuvant," "randomized controlled trial," "human," and "English," in the chemotherapy group. In the EGFR TKI group, we used the following terms: "NSCLC," "clinical trial," "human," "English," and the name of the EGFR TKI (e.g., gefitinib, referred from the review of Wendy et al.[14]). All trials that had been reported by September 30, 2004, were targeted. However, because there was no phase III study in the EGFR TKI group, only one abstract from the *Proceedings of the American Society of Clinical Oncology*, by Shepherd et al., was added. Among the retrieved studies, we excluded the trials that had missing outcomes data. We also excluded phase I/II studies. When we examined randomized phase III and randomized phase II studies, if both arms (experimental and reference arms) included cytotoxic agents or EGFR TKIs, both were included in our analysis.

Statistical Analysis

All the analyses were performed with Stata version 8 (Stata Corp., College Station, TX). Multiple linear regression analysis was applied to examine impacts on the proportion of subjects who responded and achieved stable disease on survival (median survival time [MST] and time to progression [TTP]). Scales in the models were percentages and months for proportion of subjects and survival, respectively. Two models were examined: model 1, including response rate and stable disease rate or disease control rate (response rate plus stable disease rate) as explanatory variables; and model 2, including EGFR TKI usage (yes/no) and interaction terms between EGFR TKI usage and response/stable disease rate or disease control rate in addition to model 1. In the models, each study was weighted by the number of subjects in an intent-to-treat analysis setting in each study. Thereafter, we chose model 1 based on the significance of interaction terms. To further evaluate the impact of stable disease rate considering response rate, we chose a linear regression model for residual (the observed median survival minus fitted median survival in the response rate only model) as a dependent variable with stable disease rate as a responsible variable. This approach was applied to MST and TTP separately (Figures 1 and 2). The statistical significance was defined as a value of $p < 0.05$, and adjustment for multiple comparison was not considered because of the exploratory setting of this study.

RESULTS

Study Characteristics

As a result of our search, we identified 219 references and chose 45 trials for the chemotherapy group and nine trials for the EGFR TKI group. The baseline characteristics of the 45 trials and nine trials are shown in Tables 1 and 2, respectively. There are four randomized phase II and three phase III studies for cytotoxic agents, and two randomized phase II studies and one phase III study for EGFR TKIs. In the analysis of cytotoxic agents, docetaxel, pemetrexed, other agents, and many types of combination regimens are included. In the analysis of EGFR TKIs, only monotherapies of gefitinib and erlotinib were detected. The median number of enrolled patients per study was 40 (range, 17–288) for the cytotoxic agents and 103 (range, 31–488) for the analysis of EGFR TKIs.

Median Survival Time

As shown in Table 3, both rate of stable disease and response rate were statistically significanty associated with MST in model 1 in the analysis that combined both cytotoxic agents and EGFR TKIs. The coefficient 0.0375 ($p = 0.039$) for stable disease in model 1 indicates that MST increases by 0.0375 month for each 1% increase in stable disease rate. Similarly, each 1% increase in response rate is associated with an increase of 0.0744 month in MST ($p < 0.001$). This trend was similarly observed in model 2, which considered the interaction between EGFR TKI treatment and two response parameters. As interaction terms for EGFR TKI treatment were not statistically significant, one may interpret that the relationship between survival and response rate or stable disease rate is not different between EGFR TKI and cytotoxic chemotherapy. We therefore took model 1 as the model

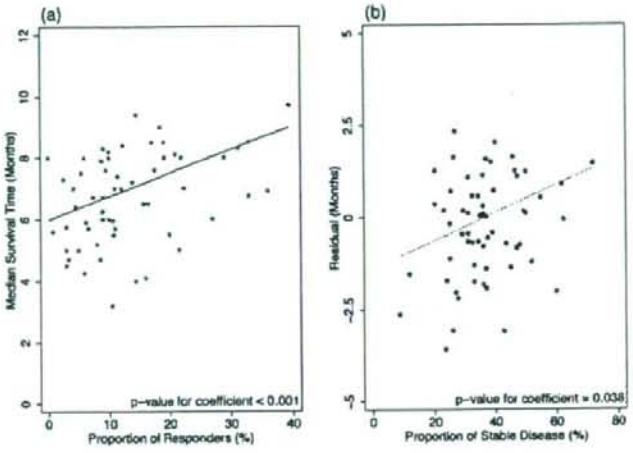

FIGURE 1. Scatterplot for MST and response/stable disease rates. (*A*) The observed MST corresponding to the percentage of responders. (*B*) The residuals (observed MST minus fitted MST in the model for *A*). The figure indicates that both response rate and stable disease rate significantly influence the prolongation of MST.

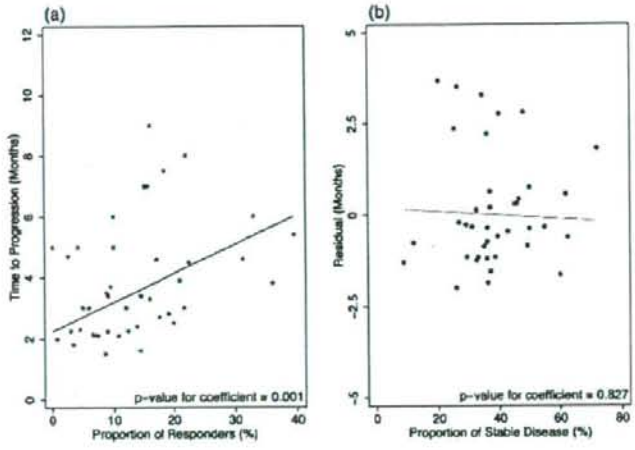

FIGURE 2. Scatterplot for TTP and response/stable disease rates. (*A*) The observed median TTP corresponding to the percentage of responders. (*B*) The residuals (observed TTP minus fitted TTP in the model for *A*). The figure indicates that the response rate but not the stable disease rate significantly influences the prolongation of TTPs.

explaining associations between MST and response variables. Figure 1A is a graphic presentation of observed MSTs corresponding to response rates with the fitted line. Figure 1B presents how well the stable disease rate explains the residual by the response rate only model. Both figures indicate that the response rate and the stable disease rate significantly contribute to MST prolongation. The coefficient for the disease control rate in model 1 was 0.05, indicating that a 1% increase in the disease control rate prolongs MST by 0.05 month ($p < 0.001$). Similar results regarding EGFR TKI terms are listed in Table 3.

Time to Progression

Table 4 shows similar analyses as MST for TTP considering stable disease rate and response rate. Contrary to MST analyses, only response rate showed a statistically significant association with TTP. The coefficient 0.0954 ($p = 0.001$) for response rate in model 1 indicates that TTP increases 0.0954 month with each 1% increase in response rates. Nonsignificant coefficient for stable disease rates indicates lack of impact of this factor on TTP after response rate has been accounted for. As interaction terms for EGFR TKI treatment were not statistically significant, we took model 1 as the model explaining associations between TTP and response variables. Figure 2 is a similar graphic presentation of observed TTPs. Although Figure 2A shows that response rate significantly influences the TTPs, there is no apparent association between TTPs and stable disease rate (Figure 2B). As shown in Table 4, disease control rate was not significantly associated with prolongation of TTP in model 1 and model 2. EGFR TKI interaction terms were not statistically significant.

DISCUSSION

Since the introduction of molecular targeted agents (especially epidermal growth factor receptor inhibitors) in clinical trials in recent years, the importance of achieving stable disease has become an important issue. For these

TABLE 1. Characteristics of the Trials with Cytotoxic Agents in the Second-Line Setting for NSCLC

Author	Phase	Regimen	No. (ITT)	RR (%)	SD (%)	DCR (%)	TTP (mo)	MST (mo)
Stewart et al., 1996[15]	II	Paclitaxel + hydroxyurea	30	3	52	55	—	5
Georgoulias et al., 1997[16]	II	Paclitaxel + gemcitabine	26	29	25	54	—	8
Gridelli et al., 1999[17]	II	Gemcitabine	30	20	60	80	2.5	5.5
Crino et al., 1999[18]	II	Gemcitabine	83	19	31	50	—	8.5
Stathopoulos et al., 1999[19]	II	Paclitaxel + cisplatin	36	38.9	58.3	97.2	—	—
Perng et al., 2000[20]	II	Docetaxel	14	28.6	—	—	4.75	11.7
Mattson et al., 2000[21]	II	Docetaxel	72	13.8	29.3	43.1	2.4	7.2
Rosati et al., 2000[22]	II	Paclitaxel + cisplatin + gemcitabine	26	27	27	54	—	6
Sculier et al., 2000[23]	II	Gemcitabine	77	6	27.7	33.7	—	4.25
Gridelli et al., 2000[24]	II	Docetaxel	23	21.7	8.7	30.4	3	5
Hainsworth et al., 2000[25]	II	Gemcitabine + vinorelbine	55	16.4	43.6	60	—	6.5
Shepherd et al., 2000[5]	III	Docetaxel	55	5.5	47.3	52.8	—	7.5
		Docetaxel	49	6.3	37.5	43.8	—	5.9
Fossella et al., 2000[6]	III	Docetaxel	125	10.8	33	43.8	2.1	5.5
		Docetaxel	125	6.7	36	42.7	2.13	5.7
		Vinorelbine/ifosfamide	123	0.8	31	31.8	1.98	5.6
Kosmas et al., 2001[26]	II	Gemcitabine + vinorelbine	43	33	37	70	6	8.5
Hainsworth et al., 2001[27]	II	Docetaxel + gemcitabine	40	10	48	58	6	6
		Docetaxel + vinorelbine	23	0	40	40	5	8
Agelaki et al., 2001[28]	II	Vinorelbine + carboplatin	37	16	30	46	9	—
Kakolyris et al., 2001[29]	II	Cisplatin + irinotecan	44	22	20	42	8	8
Huisman et al., 2001[30]	II	Cisplatin + epirubicin	27	33	33	66	—	6.75
Pectasides et al., 2001[31]	II	Gemcitabine + vinorelbine	39	2.6	35.9	38.5	4.7	7.3
Lilenbaum et al., 2001[32]	II	Docetaxel	30	10	20	30	—	8
Kosmas et al., 2001[33]	II	Gemcitabine + docetaxel	40	22.5	32.5	55	4.5	7
Kakolyris et al., 2001[34]	II	Docetaxel + gemcitabine	32	15.6	34.4	50	7	6.5
Spiridonidis et al., 2001[35]	II	Docetaxel + gemcitabine	40	32.5	—	—	—	8.1
Juan et al., 2001[36]	II	Paclitaxel	40	39.47	39.47	78.94	5.4	9.7
Chen et al., 2002[37]	II	Docetaxel + gemcitabine	36	36.1	36.11	72.21	3.8	6.9
Gonzalez et al., 2002[38]	II	Irinotecan + vinorelbine	35	9	39	48	—	6.25
Rinaldi et al., 2002[39]	II	Topotecan + gemcitabine	35	11	23	34	—	7
Socinski et al., 2002[40]	II	Paclitaxel	62	8.1	37	45.1	—	5.2
Herbst et al., 2002[41]	II	Gemcitabine + vinorelbine	36	17	50	67	4.6	8.5
Sculier et al., 2002[42]	II	Paclitaxel	67	3	24	27	—	4.5
Thongprasert et al., 2002[43]	II	Docetaxel	34	10.7	47	57.2	—	5.95
Han et al., 2003[44]	II	Irinotecan + capecitabine	37	11.4	34.3	45.7	—	7.4
Chen et al., 2003[45]	II	Docetaxel + ifosfamide	17	31.3	62.5	93.8	4.6	8.3
Font et al., 2003[46]	II	Irinotecan + docetaxel	51	6	37	43	3	8
Chen et al., 2003[47]	II	Vinorelbine + cisplatin	22	9.5	61.9	71.4	3.7	7.6
Smit et al., 2003[48]	II	Pemetrexed	45	4.5	36	40.5	2.3	6.4
		Pemetrexed	36	14.3	26	40.3	1.6	4
Chen et al., 2003[49]	II	Gemcitabine + vinorelbine	50	10	72	82	5	8.2
Dongiovanni et al., 2004[50]	II	Paclitaxel + gemcitabine	34	12	50	62	3	7
Georgoulias et al., 2003[51]	II	Irinotecan + gemcitabine	76	18.4	26.3	44.7	7.5	9
		Irinotecan	71	4.2	25.3	29.5	5	7
Park et al., 2003[52]	II	Gemcitabine + vinorelbine	38	21	55	76	3.9	8.1
Serke et al., 2003[53]	II	Docetaxel	36	11	25	36	—	5.7
Hanna et al., 2003[7]	III	Pemetrexed	283	9.1	45.8	54.9	3.4	8.3
		Docetaxel	288	8.8	46.4	55.2	3.5	7.9
Ceresoli et al., 2003[54]	II	Paclitaxel	53	15	21	36	7	—
Ardizzoia et al., 2003[55]	II	Docetaxel	42	10.5	23.5	34	—	3.2
Quoix et al., 2003[56]	II	Docetaxel	93	8.6	37.1	45.7	1.5	4.7
		Docetaxel	89	7.4	49.4	56.8	2.1	6.7

ITT, intention to treat; RR, response rate; SD, stable disease; DCR, disease control rate; TTP, time to progression; MST, median survival time.

TABLE 2. Characteristics of the Trials with EGFR TKIs in the Second-Line Setting for NSCLC

Author	Phase	Regimen	No. (ITT)	RR (%)	SD (%)	DCR (%)	MST (mo)
Gridelli et al., 2000[57]	II	Gefitinib	59	3.4	11.8	15.2	4.7
Cappuzzo et al., 2003[58]	II	Gefitinib	63	15.9	42.8	58.7	4.1
Pallis et al., 2003[59]	II	Gefitinib	31	3	29	32	5.75
Fukuoka et al., 2003[60]	II	Gefitinib	103	17.5	35.9	53.4	7.6
		Gefitinib	109	19.1	32.4	51.5	8
Kris et al., 2003[61]	II	Gefitinib	106	12	31	43	7
		Gefitinib	115	9	31	40	6
Shepherd et al., 2004[62]	III	Erlotinib	488	9	35	44	6.7
Pérez-Soler et al., 2004[63]	II	Erlotinib	57	12.3	38.6	50.9	8.4
Cappuzzo et al., 2004[64]	II	Gefitinib	106	14.4	26.8	41.2	9.4
Cappuzzo et al., 2000[65]	II	Gefitinib	40	5	45	50	5

ITT, intention to treat; RR, response rate; SD, stable disease; DCR, disease control rate; TTP, time to progression; MST, median survival time.

TABLE 3. Multiple Regression Models for Predicting MST by Study Parameters

	Model 1			Model 2		
	Coefficient	SE	*p* Value	Coefficient	SE	*p* Value
Models evaluating SD/RR and interactions with EGFR TKIs use No. 1*						
SD (%)	0.0375	0.0178	0.039	0.0500	0.0188	0.01
RR (%)	0.0744	0.0181	<0.001	0.0669	0.0190	0.001
SD_EGFR interaction	—	—	—	−0.0967	0.0703	0.175
RR_EGFR_interaction	—	—	—	0.1082	0.0591	0.073
EGFR TKI	—	—	—	2.2773	2.5364	0.373
_cons	4.6156	0.6532	<0.001	4.1579	0.7617	<0.001
			$R^2 = 0.214$			$R^2 = 0.284$
Models evaluating DCR and an interaction with EGFR TKIs use No. 2†						
DCR (%)	0.0501	0.0119	<0.001	0.0559	0.0132	<0.001
DCR_EGFR_interaction	—	—	—	−0.0226	0.0466	0.629
EGFR TKI	—	—	—	1.3146	2.0593	0.526
_cons	4.4323	0.6003	<0.001	4.0573	0.7019	<0.001
			$R^2 = 0.19$			$R^2 = 0.204$

*Coefficients for SD and RR denote increase of MST in months for 1% increase in SD/RR (model 1).
†Coefficients for DCR denote increase of MST in months for 1% increase in DCR (model 1).
SD, stable disease; RR, response rate; DCR, disease control rate.

agents, stabilization of disease without tumor shrinkage may represent a meaningful benefit. This phenomenon has been derived from two randomized phase II studies (Iressa Dose Evaluation in Advanced Lung Cancer [IDEAL]-1 and IDEAL-2).[60,61] In IDEAL-2, the median survival time of patients achieving stable disease was 9.4 months versus 5.2 months for those with progressive disease.[61] Moreover, when survival and symptom improvement were analyzed together, the median survival time for patients achieving stable disease with symptom improvement was 12.8 months versus 4.8 months for those without symptom improvement.

In contrast, the importance of achieving stable disease has been evaluated for cytotoxic agents. Docetaxel significantly improved overall survival compared with best supportive care as second-line therapy despite the overall response rate of only 6%.[5] In this study, 42.7% of patients achieved stable disease, which suggests that docetaxel also confers clinical benefit by producing stable disease.

In this retrospective review, we investigated the relationship between response rates and survival benefit and between the rates of stable disease and survival benefit in second-line treatment of NSCLC using both cytotoxic agents and EGFR TKIs. The more the rates of response and stable disease increase, the more the improvement of overall survival is obtained in the analysis that combined both cytotoxic agents and EGFR TKIs. However, as shown in Table 3, for both cytotoxic agents and EGFR TKIs, the survival improvement for a 1% increase in response rate is higher than for a 1% increase in stable disease rate. Moreover, for time to progression, only response rate showed a statistically significant association with TTP. These results indicate that it is more important to increase response rates than to achieve

TABLE 4. Multiple Regression Models for Predicting TTP by Study Parameters

	Model 1			Model 2		
	Coefficient	SE	*p* Value	Coefficient	SE	*p* Value
Models evaluating SD/RR and interactions with EGFR TKIs use No. 1*						
SD (%)	−0.0050	0.0229	0.828	−0.0248	0.0292	0.402
RR (%)	0.0954	0.0265	0.001	0.0963	0.0291	0.002
SD_EGRF_interaction	—	—	—	0.0297	0.0353	0.406
RR_EGFR_interaction	—	—	—	−0.0344	0.0391	0.385
EGFR TKIs	—	—	—	−1.9322	1.3858	0.172
_cons	2.4205	0.9348	0.014	3.5861	1.2925	0.009
			$R^2 = 0.183$			$R^2 = 0.325$
Models evaluating DCR and an interaction with EGFR TKIs use No. 2†						
DCR (%)	0.0281	0.1430	0.057	0.0166	0.0197	0.405
DCR_EGFR_interaction	—	—	—	0.0088	0.0210	0.677
EGFR TKIs	—	—	—	−1.5120	1.3021	0.253
_cons	1.9636	0.8734	0.03	2.8927	1.2334	0.024
			$R^2 = 0.047$			$R^2 = 0.148$

*Coefficients for SD and RR denote increase of TTP in months for 1% increase in SD/RR (model 1).
†Coefficients for DCR denote increase of TTP in months for 1% increase in DCR (model 1).
SD, stable disease; RR, response rate; DCR, disease control rate.

stable disease to improve overall survival for both cytotoxic agents and EGFR TKIs in the second-line setting, although increasing stable disease rates is still valuable.

In our analysis, we could not find a significant difference between cytotoxic agents and EGFR TKIs in terms of the relationship between survival and response and stable disease rate, as interaction terms for EGFR TKI treatment were not statistically significant. As a result, one may infer that the effect on survival of increasing response rates and stable disease rates is similar for cytotoxic agents and EGFR TKIs. However, this interpretation requires cautions on two points. First, our review contains many heterogeneous phase II studies with greatly different registered numbers of cases, and many heterogeneous patient characteristics with a greatly different administered number of regimens before these studies. The method of evaluating response is also different. These may possibly lead to a false conclusion. Moreover, the main effect of EGFR TKI was large but not statistically significant, indicating no evidence of a difference between EGFR TKIs and cytotoxic agents in terms of survival. However, there are very few EGFR TKI studies included in this review, and therefore the ability to detect such an effect may be low. Second, evaluating stable disease in clinical trials is very difficult, as patients with stable disease are not a homogeneous population. The Response Evaluation Criteria in Solid Tumors study defined stable disease as the longest diameter of tumor size from a less than 30% decrease to a less than 20% increase.[65] True disease stabilization inhibits tumor growth and metastasis and may be associated with improvement of survival, symptoms, and quality of life. However, it is difficult to distinguish true stable disease from nonstable disease. Therefore, it is crucial to classify a category of stable disease in the future.

CONCLUSIONS

In conclusion, our review indicated that although it is appropriate to adapt disease control rates to assess the effect of agents in the second-line setting, which is a new concept often used by clinical trials for molecular targeted agents, to obtain response seems to be more important than to achieve stable disease when new agents are developed, although achieving stable disease is still valuable. The relationship between survival and response and stable disease appears similar for cytotoxic agents and EGFR TKIs.

REFERENCES

1. Non-Small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: A meta-analysis using updated data on individual patients from 52 randomized clinical trials. *BMJ* 1995;311: 899–909.
2. Fossella F, Pereira JR, von Pawel J, et al. Randomized, multinational, phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for advanced non-small-cell lung cancer: The Tax 326 Study Group. *J Clin Oncol* 2003;21:3016–3024.
3. Kelly K, Crowley J, Bunn PA, et al. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small-cell lung cancer: A Southwest Oncology Group trial. *J Clin Oncol* 2001;19:3210–3218.
4. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002;346:92–98.
5. Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small cell lung cancer previously treated with platinum-based chemotherapy. *J Clin Oncol* 2000;18:2095–2103.
6. Fossella FV, DeVore R, Kerr RN, et al. Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. *J Clin Oncol* 2000;18:2354–2362.
7. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* 2004;22:1589–1597.

8. Fujino S, Enokibori T, Tezuka N, et al. A comparison of epidermal growth factor receptor levels and other prognostic parameters in non-small cell lung cancer. *Eur J Cancer* 1996;32A:2070–2074.
9. Pavelic K, Banjac Z, Pavelic J, et al. Evidence for a role of EGF receptor in the progression of human lung carcinoma. *Anticancer Res* 1993;13: 1133–1137.
10. Veale D, Kerr N, Gibson GJ, et al. Characterization of epidermal growth factor receptor in primary human non-small cell lung cancer. *Cancer Res* 1989;49:1313–1317.
11. Sekine I, Takami S, Guang SG, et al. Role of epidermal growth factor receptor overexpression, K-ras point mutation and c-myc amplification in the carcinogenesis of non-small cell lung cancer. *Oncol Rep* 1998;5: 351–354.
12. Rusch V, Klimstra D, Linkov I, et al. Aberrant expression of p53 or the epidermal growth factor receptor is frequent in early bronchial neoplasia and coexpression precedes squamous cell carcinoma development. *Cancer Res* 1995;55:1365–1372.
13. Shepherd FA, Pereira J, Ciuleanu TE, et al. A randomized placebo-controlled trial of erlotinib in patients with advanced non-small cell lung cancer (NSCLC) following failure of 1st line or 2nd line chemotherapy: A National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) trial (Abstract 7022). *Proc Am Soc Clin Oncol* 2004;.
14. Parulekar WR, Eisenhauer EA. Phase I trial design for solid tumor studies of targeted, non-cytotoxic agents: Theory and practice. *J Natl Cancer Inst* 2004;96:990–997.
15. Stewart DJ, Tomiak EM, Goss G, et al. Paclitaxel plus hydroxyurea as second line therapy for non-small cell lung cancer. *Lung Cancer* 1996; 15:115–123.
16. Georgoulias V, Kourousis C, Kakolyris S, et al. Second-line treatment of advanced non-small cell lung cancer with paclitaxel and gemcitabine: A preliminary report on an active regimen. *Semin Oncol* 1997;24(4 Suppl 12):61–66.
17. Gridelli C, Perrone F, Gallo C, et al. Single-agent gemcitabine as second-line treatment in patients with advanced non small cell lung cancer (NSCLC): A phase II trial. *Anticancer Res* 1999;19:4535–4538.
18. Crino L, Mosconi AM, Scagliotti G, et al. Gemcitabine as second-line treatment for advanced non-small-cell lung cancer: A phase II trial. *J Clin Oncol* 1999;17:2081–2085.
19. Stathopoulos GP, Rigatos S, Malamos NA. Paclitaxel combined with cis-platin as second-line treatment in patients with advanced non-small cell lung cancers refractory to cis-platin. *Oncol Rep* 1999;6:797–800.
20. Perng RP, Shih JF, Chen YM, et al. A phase II study of single-agent docetaxel chemotherapy for non-small cell lung cancer. *Jpn J Clin Oncol* 2000;30:429–434.
21. Mattson K, Bosquee L, Dabouis G, et al. Phase II study of docetaxel in the treatment of patients with advanced non-small cell lung cancer in routine daily practice. *Lung Cancer* 2000;29:205–216.
22. Rosati G, Rossi A, Nicolella G, et al. Second-line chemotherapy with paclitaxel, cisplatin and gemcitabine in pre-treated sensitive cisplatin-based patients with advanced non-small cell lung cancer. *Anticancer Res* 2000;20:2229–2234.
23. Sculier JP, Lafitte JJ, Berghmans T, et al. A phase II trial testing gemcitabine as second-line chemotherapy for non small cell lung cancer: The European Lung Cancer Working Party. *Lung Cancer* 2000;29:67–73.
24. Gridelli C, Frontini L, Barletta E, et al. Single agent docetaxel plus granulocyte-colony stimulating factor (G-CSF) in previously treated patients with advanced non small cell lung cancer: A phase II study and review of the literature. *Anticancer Res* 2000;20:1077–1084.
25. Hainsworth JD, Burris III, HA, Litchy S, et al. Gemcitabine and vinorelbine in the second-line treatment of nonsmall cell lung carcinoma patients: A Minnie Pearl Cancer Research Network phase II trial. *Cancer* 2000;88:1353–1358.
26. Kosmas C, Tsavaris N, Panopoulos C, et al. Gemcitabine and vinorelbine as second-line therapy in non-small-cell lung cancer after prior treatment with taxane+platinum-based regimens. *Eur J Cancer* 2001; 37:972–978.
27. Hainsworth JD, Burris IIIHA Billings IIIFT et al. Weekly docetaxel with either gemcitabine or vinorelbine as second-line treatment in patients with advanced nonsmall cell lung carcinoma: Phase II trials of the Minnie Pearl Cancer Research Network. *Cancer* 2001;92:2391–2398.
28. Agelaki S, Bania H, Kouroussis C, et al. Second-line treatment with vinorelbine and carboplatin in patients with advanced non-small cell lung cancer: A multicenter phase II study. *Lung Cancer* 2001;34(Suppl 4):77–80.
29. Kakolyris S, Kouroussis Ch, Souglakos J, et al. Cisplatin and irinotecan (CPT-11) as second-line treatment in patients with advanced non-small cell lung cancer. *Lung Cancer* 2001;34(Suppl 4):71–76.
30. Huisman C, Biesma B, Postmus PE, et al. Accelerated cisplatin and high-dose epirubicin with G-CSF support in patients with relapsed non-small-cell lung cancer: Feasibility and efficacy. *Br J Cancer* 2001; 85:1456–1461.
31. Pectasides D, Kalofonos HP, Samantas E, et al. An out-patient second-line chemotherapy with gemcitabine and vinorelbine in patients with non-small cell lung cancer previously treated with cisplatin-based chemotherapy: A phase II study of the Hellenic co-operative Oncology Group. *Anticancer Res* 2001;21:3005–3010.
32. Lilenbaum RC, Schwartz MA, Seigel L, et al. Phase II trial of weekly docetaxel in second-line therapy for nonsmall cell lung carcinoma. *Cancer* 2001;92:2158–2163.
33. Kosmas C, Tsavaris N, Vadiaka M, et al. Gemcitabine and docetaxel as second-line chemotherapy for patients with nonsmall cell lung carcinoma who fail prior paclitaxel plus platinum-based regimens. *Cancer* 2001;92:2902–2910.
34. Kakolyris S, Papadakis E, Tsiafaki X, et al. Docetaxel in combination with gemcitabine plus rhG-CSF support as second-line treatment in non-small cell lung cancer: A multicenter phase II study. *Lung Cancer* 2001;32:179–187.
35. Spiridonidis CH, Laufman LR, Carman L, et al. Second-line chemotherapy for non-small-cell lung cancer with monthly docetaxel and weekly gemcitabine: A phase II trial. *Ann Oncol* 2001;12:89–94.
36. Juan O, Albert A, Ordono F, et al. Low-dose weekly paclitaxel as second-line treatment for advanced non-small cell lung cancer: A phase II study. *Jpn J Clin Oncol* 2001;32:449–454.
37. Chen YM, Perng RP, Lin WC, et al. Phase II study of docetaxel and gemcitabine combination chemotherapy in non-small-cell lung cancer patients failing previous chemotherapy. *Am J Clin Oncol* 2002;25:509–512.
38. Gonzalez Cao M, Aramendia JM, Salgado E, et al. Second-line chemotherapy with irinotecan and vinorelbine in stage IIIB and IV non-small-cell lung cancer: A phase II study. *Am J Clin Oncol* 2002;25:480–484.
39. Rinaldi DA, Lormand NA, Brierre JE, et al. A phase II trial of topotecan and gemcitabine in patients with previously treated, advanced nonsmall cell lung carcinoma. *Cancer* 2002;95:1274–1278.
40. Socinski MA, Schell MJ, Bakri K, et al. Second-line, low-dose, weekly paclitaxel in patients with stage IIIB/IV nonsmall cell lung carcinoma who fail first-line chemotherapy with carboplatin plus paclitaxel. *Cancer* 2002;95:1265–1273.
41. Herbst RS, Khuri FR, Lu C, et al. The novel and effective nonplatinum, nontaxane combination of gemcitabine and vinorelbine in advanced nonsmall cell lung carcinoma: Potential for decreased toxicity and combination with biological therapy. *Cancer* 2002;95:340–353.
42. Sculier JP, Berghmans T, Lafitte JJ, et al. European Lung Cancer Working Party: A phase II study testing paclitaxel as second-line single agent treatment for patients with advanced non-small cell lung cancer failing after a first-line chemotherapy. *Lung Cancer* 2002;37:73–77.
43. Thongprasert S, Cheewakriangkrai R, Napapan S. Docetaxel as second-line chemotherapy for advanced non-small cell lung cancer. *J Med Assoc Thai* 2002;85:1296–1300.
44. Han JY, Lee DH, Kim HY, et al. A phase II study of weekly irinotecan and capecitabine in patients with previously treated non-small cell lung cancer. *Clin Cancer Res* 2003;9:5909–5914.
45. Chen YM, Shih JF, Lee CS, et al. Phase II study of docetaxel and ifosfamide combination chemotherapy in non-small-cell lung cancer patients failing previous chemotherapy with or without paclitaxel. *Lung Cancer* 2003;39:209–214.
46. Font A, Sanchez JM, Taron M, et al. Weekly regimen of irinotecan/docetaxel in previously treated non-small cell lung cancer patients and correlation with uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) polymorphism. *Invest New Drugs* 2003;21:435–443.
47. Chen YM, Lee CS, Lin WC, et al. Phase II study with vinorelbine and cisplatin in advanced non-small cell lung cancer after failure of previous chemotherapy. *J Chin Med Assoc* 2003;66:241–246.
48. Smit EF, Mattson K, von Pawel J, et al. ALIMTA (pemetrexed diso-

dium) as second-line treatment of non-small-cell lung cancer: A phase II study. *Ann Oncol* 2003;14:455–460.
49. Chen YM, Perng RP, Lee CS, et al. Phase II study of gemcitabine and vinorelbine combination chemotherapy in patients with non-small-cell lung cancer not responding to previous chemotherapy. *Am J Clin Oncol* 2003;26:567–570.
50. Dongiovanni V, Addeo A, Berruti A, et al. A phase II trial of weekly paclitaxel and gemcitabine in non-small cell lung cancer patients previously treated with platinum and vinorelbine. *Anticancer Res* 2004;24: 2567–2572.
51. Georgoulias V, Kouroussis C, Agelidou A, et al. Irinotecan plus gemcitabine vs irinotecan for the second-line treatment of patients with advanced non-small-cell lung cancer pretreated with docetaxel and cisplatin: A multicentre, randomised, phase II study. *Br J Cancer* 2004;91:482–488.
52. Park YH, Lee JC, Kim CH, et al. Gemcitabine and vinorelbine as second-line therapy for non-small cell lung cancer after treatment with paclitaxel plus platinum. *Jpn J Clin Oncol* 2004;34:245–249.
53. Serke M, Schoenfeld N, Loddenkemper R. Weekly docetaxel as second-line chemotherapy in advanced non-small cell lung cancer: Phase II trial. *Anticancer Res* 2004;24:1211–1216.
54. Ceresoli GL, Gregorc V, Cordio S, et al. Phase II study of weekly paclitaxel as second-line therapy in patients with advanced non-small cell lung cancer. *Lung Cancer* 2004;44:231–239.
55. Ardizzoia A, Acquati M, Fagnani D, et al. Second line therapy with weekly low-dose docetaxel for pretreated non-small-cell lung carcinoma patients: A multicenter Italian phase II study. *Lung* 2004;182:1–8.
56. Quoix E, Lebeau B, Depierre A, et al. Randomised, multicentre phase II study assessing two doses of docetaxel (75 or 100 mg/m2) as second-line monotherapy for non-small-cell lung cancer. *Ann Oncol* 2004;15:38–44.
57. Gridelli C, Maione P, Castaldo V, et al. Gefitinib in elderly and unfit patients affected by advanced non-small-cell lung cancer. *Br J Cancer* 2003;89:1827–1829.
58. Cappuzzo F, Gregorc V, Rossi E, et al. Gefitinib in pretreated non-small-cell lung cancer (NSCLC): Analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. *J Clin Oncol* 2003;21:2658–2663.
59. Pallis AG, Mavroudis D, Androulakis N, et al. ZD1839, a novel, oral epidermal growth factor receptor-tyrosine kinase inhibitor, as salvage treatment in patients with advanced non-small cell lung cancer: Experience from a single center participating in a compassionate use program. *Lung Cancer* 2003;40:301–307.
60. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2003;21:2237–2246.
61. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: A randomized trial. *JAMA* 2003;290:2149–2158.
62. Perez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non–small-cell lung cancer. *J Clin Oncol* 2004;22:3238–3247.
63. Cappuzzo F, Magrini E, Ceresoli GL, et al. Akt phosphorylation and gefitinib efficacy in patients with advanced non-small-cell lung cancer. *J Natl Cancer Inst* 2004;96:1133–1141.
64. Cappuzzo F, Bartolini S, Ceresoli GL, et al. Efficacy and tolerability of gefitinib in pretreated elderly patients with advanced non-small-cell lung cancer (NSCLC). *Br J Cancer* 2004;90:82–86.
65. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205–216.

Invest New Drugs
DOI 10.1007/s10637-006-9012-9

PHASE II STUDIES

Phase II study of amrubicin in previously untreated patients with extensive-disease small cell lung cancer: West Japan Thoracic Oncology Group (WJTOG) study

Takashi Yana · Shunichi Negoro · Minoru Takada · Soichiro Yokota · Yoshiki Takada · Takahiko Sugiura · Hidehiko Yamamoto · Toshiyuki Sawa · Masaaki Kawahara · Nobuyuki Katakami · Yutaka Ariyoshi · Masahiro Fukuoka

Received: 28 January 2005 / Accepted: 5 September 2006

Summary *Purpose:* To evaluate the efficacy and safety of amrubicin, (+)-(7*S*, 9*S*)-9-acetyl-9-amino-7-[(2-deoxy-β-D-*erythro*-pentopyranosyl)oxy]-7,8,9,10-tetrahydro-6,11-dihydroxy-5,12-naphthacenedione hydrochloride, in previously untreated patients with extensive-disease small cell lung cancer (SCLC).

Patients and methods: A total of 35 previously untreated patients with extensive-disease SCLC were entered into the study. Amrubicin was given by daily intravenous infusion at 45 mg/m^2/day for 3 consecutive days, every 3 weeks. Unless there was tumor regression of 25% or greater after the first cycle, or 50% or greater after the second cycle, treatment was switched to salvage chemotherapy in combination with etoposide (100 mg/m^2, days 1, 2, and 3) and cisplatin (80 mg/m^2, day 1).

Results: Of the 35 patients entered, 33 were eligible and assessable for efficacy and toxicity. Of the 33 patients, 3 (9.1%) had a complete response (95% confidence interval [CI], 1.9–24.3%) and 22 had a partial response, for an overall response rate of 75.8% (95% CI, 57.7–88.9%). Median survival time was 11.7 months (95% CI, 9.9–15.3 months), and 1-year and 2-year survival rates were 48.5% and 20.2%, respectively. The most common toxicity was hematologic. Non-hematologic toxicity of grade 3 or 4 was only seen in 3 patients with anorexia (9.1%) and 1 patient with alopecia (3.0%). Salvage chemotherapy was administered to only 6 patients.

T. Yana · M. Takada
Department of Internal Medicine, Osaka Prefectural Habikino Hospital, Habikino

S. Negoro
Department of Clinical Oncology, Osaka City General Hospital, Osaka

S. Yokota
Department of Internal Medicine, National Toneyama Hospital for Chest Diseases, Toyonaka

Y. Takada
Department of Radiology, Hyogo Medical Center for Adults, Akashi

T. Sugiura
Department of Internal Medicine, Aichi Cancer Center Hospital, Nagoya

H. Yamamoto
Department of Internal Medicine, Aso Iizuka Hospital, Iizuka

T. Sawa
Division of Respiratory Medicine, Gifu Municipal Hospital, Gifu

M. Kawahara
Department of Internal Medicine, National Kinki Central Hospital for Chest Disease, Sakai

N. Katakami
Division of Pulmonary Medicine, Kobe City General Hospital, Kobe

Y. Ariyoshi
Department of Internal Medicine, Prefectural Aichi Hospital, Okazaki

M. Fukuoka
Department of Medical Oncology, Kinki University School of Medicine, Osakasayama, Japan

T. Yana (✉)
Division of Respiratory Medicine, KKR Otemae Hospital, 1-5-34, Otemae, Chuo-ku, Osaka 540-0008, Japan
e-mail: t-yana@otemae.gr.jp

Conclusion: Amrubicin was active for extensive-disease SCLC with acceptable toxicity. Further studies in combination with other agents for SCLC are warranted.

Keywords Amrubicin · Small cell lung cancer · Anthracycline · Previously untreated patients · Phase II study

Introduction

Small cell lung cancer (SCLC) is a major cause of cancer deaths and accounts for 15 to 20% of all lung cancers [1]. Although this cancer is initially highly responsive to chemotherapy, the vast majority of patients will ultimately relapse and die of recurrent disease within 2 years [2]. Recently, combination chemotherapy with irinotecan and cisplatin for extensive-disease SCLC produced more survival benefit than etoposide and cisplatin, the worldwide standard regimen since 1981 [3, 4]. Median survival time and 2-year survival rate of the standard regimen is 12.8 months and 19.5%, respectively. Clearly, new and more effective agents against SCLC are needed.

Amrubicin is a totally synthetic 9-aminoanthracycline, (+)-(7*S*, 9*S*)-9-acetyl-9-amino-7-[(2-deoxy-β-D-*erythro*-pentopyranosyl)oxy]-7, 8, 9, 10-tetrahydro-6, 11-dihydroxy-5,12-naphthacenedione hydrochloride, with a chemical structure similar to that of doxorubicin (Fig. 1) [5]. Amrubicin showed more potent antitumor activity than doxorubicin in several human tumor xenografts implanted in nude mice [6]. Acute toxicity of amrubicin is qualitatively similar to that of doxorubicin [7], however, amrubicin shows almost no delayed toxicity (e.g. cardiotoxicity) [8, 9].

Amrubicin is converted to an active metabolite, amrubicinol, by reduction of its C-13 ketone group to a hydroxy group. *In vitro* cytotoxic activity of amrubicinol was almost equipotent to that of doxorubicin and 20 to 220 times more potent than that of its parent compound, amrubicin [10]. Amrubicinol is considered to be closely associated with the efficacy and toxicity of amrubicin [11].

Despite their similarity in chemical structure, amrubicin has a different mode of action to doxorubicin [12]. Amrubicin and its active metabolite, amrubicinol, are inhibitors of DNA topoisomerase II. Amrubicin and amrubicinol exert cytotoxic effects by stabilizing topoisomerase II-mediated cleavable complexes, while doxorubicin does not inhibit this step of the catalytic cycle of topoisomerase II at concentrations for which it demonstrates cytotoxicity. Doxorubicin is a potent DNA intercalator, and its cytotoxicity is thought to be mainly due to this. Amrubicin and amrubicinol are about one-tenth weaker DNA intercalators than doxorubicin. Therefore, they are similar to etoposide in terms of inhibition of topoisomerase II by stabilizing the cleavable complexes, although etoposide does not show any DNA intercalating activity.

In a phase I–II study in patients with non-small cell lung cancer, amrubicin was administered as a 5-min intravenous infusion for 3 consecutive days [13]. The maximum tolerated dose (MTD) was 50 mg/m^2/day and the dose-limiting toxicities were leukopenia, neutropenia, thrombocytopenia, and gastrointestinal complications. The recommended dose for the phase II study was 45 mg/m^2/day for 3 consecutive days every 3 weeks.

Based on these experimental data and preliminary clinical reports indicating that amrubicin may be active against lung cancer, the West Japan Thoracic Oncology Group (WJTOG) evaluated it for use in SCLC. The WJTOG conducted a phase II study in previously untreated extensive-disease SCLC patients as a first-line therapy. Salvage chemotherapy with etoposide and cisplatin and an early cessation rule were set in place as precautionary measures.

Patients and methods

Eligibility criteria

Eligibility criteria included histologically or cytologically proven small cell lung cancer with extensive-disease defined as distant metastasis and/or disease involving the

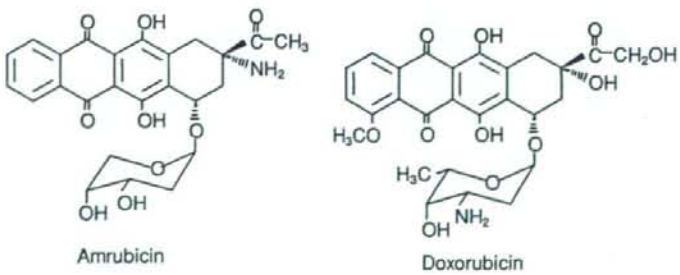

Fig. 1 Chemical structures of amrubicin and doxorubicin

contralateral hilar lymph nodes; no prior treatment; life expectancy of at least 2 months; the Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; at least one bidimensionally measurable lesion; age less than 80; adequate organ function, such as white blood cell (WBC) count of 4000×10^6/L or greater, hemoglobin level 10 g/dL or greater, platelet count 100×10^9/L or greater, AST and ALT less than 100 IU/L, bilirubin level 1.5 mg/dL or less, creatinine concentration 1.2 mg/dL or less, electrocardiogram (ECG) findings within normal range, and left ventricular ejection fraction (LVEF) of echocardiogram 60% or greater. All patients gave written informed consent. Ineligibility criteria were: brain or bone metastases requiring radiation; continuous long-term treatment with non-steroidal anti-inflammatory drugs and glucocorticoids; pulmonary fibrosis; serious complications and other active malignancy; or pregnant or nursing subjects.

This study was approved by the institutional review boards at each participating center.

Study design

Amrubicin (Sumitomo Pharmaceuticals Co., Ltd, Osaka, Japan) was dissolved in 20 mL normal saline and administered once intravenously as a 5-min infusion at a dose of 45 mg/m^2/day on days 1 to 3, every 3 weeks.

Before treatment, all patients underwent a medical history, physical examination, hematology and serum biochemistry tests, urinalysis, ECG, LVEF, and baseline tumor measurements (chest radiography, CT scans, bone scintigraphy, and other measurements as appropriate). All measurable and assessable lesions were evaluated within 2 weeks before treatment. ECG and LVEF were undertaken within 1 month before treatment.

Complete and differential blood cell counts, platelet counts, hematocrit analysis, biochemical analysis including AST, ALT, alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, serum bilirubin, albumin, total protein, and electrolyte levels (Na, K, Cl, and Ca), and urinalysis (including protein, glucose, urobilinogen, and occult blood) were performed weekly as a rule. When severe myelosuppression was observed, complete and differential blood cell counts plus platelet counts were performed 2 times or more per week. ECG was undertaken every treatment cycle and LVEF every other cycle. Chest radiography and CT scans were carried out every cycle as a rule.

Subjective and objective symptoms were observed and recorded as appropriate.

Dose modifications were made according to WBC and platelet counts. If the WBC count nadir was lower than $1{,}000 \times 10^6$/L for 4 days or longer and/or the platelet count nadir was lower than 50×10^9/L, a dose reduction of 5 mg was stipulated in the subsequent treatment course. Treatment was postponed until the WBC and platelet counts recovered to $\geq 3{,}000 \times 10^6$/L and $\geq 100 \times 10^9$/L, respectively.

In patients who demonstrated tumor regression of 25% or greater after the first course of chemotherapy, amrubicin treatment was continued. After the second course, patients had to have achieved tumor regression of 50% or greater to continue to receive the drug up to a maximum of 6 courses. Treatment of combination chemotherapy with etoposide (100 mg/m^2 on days 1, 2, and 3) and cisplatin (80 mg/m^2 on day 1) was recommended for patients who failed to fulfill any of the above criteria.

Evaluation of response and toxicity

Response was assessed according to the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14], which are virtually identical to those of the World Health Organization [15]. A complete response (CR) was defined as disappearance of all lesions for a minimum of 4 weeks. A partial response (PR) was defined as a 50% or greater decrease in the sum of the products of the diameters of measurable lesions for a minimum period of 4 weeks and no new lesions. No change (NC) was defined as a decrease in the tumor mass of less than 25% or any increase of less than 25%. Progressive disease (PD) was defined as an increase in the size of any measurable lesion by 25% or greater or the appearance of new lesions.

Toxicity grading was recorded based on the side effect record form in the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14].

Statistical analyses

The estimated sample size was 30 to guarantee that the lower limits of 95% confidence interval would be at least 20% at 40% of expected response rate. An early cessation rule was in place to terminate the study if at least 4 responses had not been seen among 15 patients evaluated. Median overall survival was estimated using the product-limit (Kaplan-Meier) method [16].

Results

Patient characteristics

Of 35 patients entered into this study between May 1995 and January 1997, 33 patients were eligible and assessable for efficacy and toxicity. There were 2 ineligible patients because of serious complications before treatment (cardiac

Table 1 Patient characteristics

Patient characteristics	No. of patients (N = 33)	%
Age (years)		
Median	66	
Range	42–78	
Sex		
Male	29	87.9
Female	4	12.1
Performance status (ECOG)		
0	5	15.2
1	26	78.8
2	2	6.1
Stage		
IIIB	1	3.0
IV	32	97.0
Prior therapy		
No	33	100

ECOG: Eastern Cooperative Oncology Group.

failure and aggravation of hepatitis, respectively), and they did not receive amrubicin. Characteristics of the 33 eligible patients are shown in Table 1. Of the 33 patients, 13 (39%) were 70 years of age or older, 88% were male, and 94% had an ECOG performance status of 0 or 1.

Efficacy

Response to amrubicin is shown in Table 2. The early cessation rule was not imposed to terminate the study, as 10 responses were seen after 15 patients were enrolled. Of 33 patients, 3 achieved a complete response, giving a CR rate of 9.1% (95% CI, 1.9–24.3%), and 22 a partial response, for an overall response rate of 75.8% (95% CI, 57.7–88.9%). Of 7 patients, 6 experiencing no change under amrubicin treatment were switched to salvage chemotherapy. Of these, 2 had partial responses and the others had no change.

The overall survival curve is shown in Fig. 2. Median survival time was 11.7 months (95% CI, 9.9–15.3 months), and 1-year and 2-year survival rates were 48.5% (95% CI, 31.4–65.5%) and 20.2% (95% CI, 6.4–34.4%), respectively.

Toxicity

The major observed toxicity was hematologic, as shown in Table 3. All patients experienced leukopenia and neutropenia. Grade 3 or 4 leukopenia occurred in 51.5% of patients and grade 3 or 4 neutropenia in 84.8%. Anemia and thrombocytopenia were observed in 78.8% and 39.4% of patients, respectively, both with a frequency of grade 3 or 4 of 21.2%. Despite the severe hematologic toxicity of amrubicin, there was no febrile neutropenia or treatment-related death during the entire treatment of 33 patients. Granulocyte colony-stimulating factor (G-CSF) was used in 55 (40%) of a total of 136 cycles, in 13 patients (39%). Most hematologic toxicity in this trial was well-controlled without dose reduction: 88% of the total treatment cycles were delivered at the planned dosage of amrubicin, 45 mg/m^2/day.

Non-hematologic toxicities observed in more than 10% of patients were anorexia (54.5%), nausea and vomiting

Table 2 Response to amrubicin

No. of assessable patients	Response (No. of patients)				CR rate, % (95% CI)	Response rate, % (95% CI)
	CR	PR	NC	PD		
33	3	22	7	1	9.1 (1.9–24.3)	75.8 (57.7–88.9)

CR: complete response; PR: partial response; NC: no change; PD: progressive disease; 95% CI: 95% confidence interval.

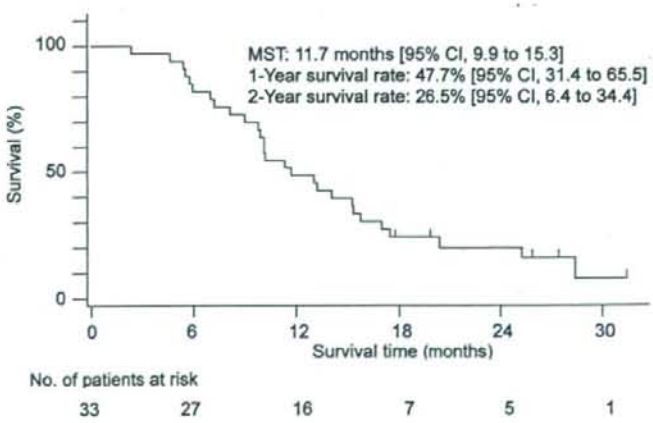

Fig. 2 Overall survival of patients with extensive-disease small cell lung cancer treated with amrubicin. MST: median survival time; 95% CI: 95% confidence interval