not allowed. If grade 4 leukopenia, grade 4 neutropenia, or febrile neutropenia was noted, the use of granulocyte colony-stimulating factor (G-CSF) was permitted.

2.4. Assessment of antitumour activity

The standard response evaluation criteria in solid tumors was used to evaluate the responses [18]. Complete and partial response (PR) were confirmed by two observations not less than 4 weeks apart.

2.5. Pharmacokinetic analysis

Blood samples for pharmacokinetic analysis were obtained during the second and third days of the first cycle, from an indwelling venous catheter placed in the arm contralateral to that used for the drug infusion. Five milliliters of blood were collected in heparinised tubes before the drug administration, at the end of the TOP infusion, and 0.5, 3, 8 and 23 h after the end of the TOP infusion on both Days 2 and 3 in the first cycle. After centrifugation, the plasma specimens were stored at −80 °C until the assays. The plasma concentrations of AMR, amrubicinol (13-OH-AMR: active form of AMR) and TOP were measured by high-performance liquid chromatography (HPLC). The area under the plasma concentration–time curve (AUC) was calculated using WINNONLIN Standard Edition, Version 1.5. Differences in the pharmacokinetic parameters among three dose levels in the first cycle were evaluated by the Kruskal–Wallis test, and those between Days 2 and 3 in the first cycle were evaluated by Mann–Whitney's *U*-test. The correlations between the pharmacokinetic parameters and the clinical toxicities or responses were assessed with Spearman's rank test. Statistical analyses were performed using the STATVIEW 5.0 program (Brainpower, Calabasas, CA). A *p*-value of less than 0.05 was considered to denote statistical significance.

3. Results

3.1. Patients' characteristics

Nine patients with relapsed or ED-SCLC were enrolled between April and November 2003. There were eight men and one woman, with a median age of 62 years (range, 51–75 years). All patients had a good performance status (PS 0 in five patients and PS 1 in four patients). Five patients (56%) had received prior chemotherapy (CDDP + VP-16 in three, CDDP + CPT-11 in one, and carboplatin + VP-16 in one). Three patients had sensitive disease and two had refractory disease.

A total of 24 chemotherapy cycles were administered. Three patients (33%) received only one cycle of chemotherapy, because of unacceptable toxicity (two patients) or the patient's refusal to undergo further treatment (one patient). At the first dose level (TOP 0.75 mg/m^2, AMR 30 mg/m^2), one patient developed DLT (grade 3: diarrhoea, stomatitis and febrile neutropenia, grade 4: leukopenia, neutropenia lasting for more than 4 days and thrombocytopenia). At the second dose level (TOP 0.75 mg/m^2, AMR 35 mg/m^2), one patient developed DLT (grade 4 neutropenia lasting for more than 4 days). At the third dose level (TOP 0.75 mg/m^2, AMR 40 mg/m^2), all three patients experienced DLT (grade 4 neutropenia lasting for more than 4 days in one, grade 4 neutropenia lasting for more than 4 days and grade 3 febrile neutropenia in one patient each, and grade 4 thrombocytopenia in one). Therefore, the third dose level was deemed to be MTD, and the recommended doses for the phase II study were the second dose levels, that is, 0.75 mg/m^2 for TOP, and 35 mg/m^2 for AMR.

Table 1 Grade 2 or more severe haematological toxicity (all courses)

Toxicity	Grade	Dose level		
		1	2	3
No. of treated patients		3	3	3
No. of courses evaluated		7	9	8
No. of courses in which toxicity was encountered (%)				
	2	0	1 (11%)	1 (13%)
Leukopenia	3	6 (86%)	8 (89%)	3 (38%)
	4	1 (14%)	0	4 (50%)
	2	1 (14%)	0	2 (25%)
Neutropenia	3	2 (29%)	3 (33%)	0
	4	4 (57%)	6 (67%)	6 (75%)
	2	1 (14%)	4 (44%)	0
Thrombocytopenia	3	1 (14%)	0	5 (63%)
	4	1 (14%)	0	0
	2	1 (14%)	5 (56%)	3 (38%)
Anaemia	3	1 (14%)	2 (22%)	2 (25%)
	4	2 (29%)	0	1 (13%)

3.2. Haematological toxicity

The main toxicity of this drug combination was myelosuppression. Analysis of the toxicity during all courses of chemotherapy is shown in Table 1. Grade 3 or 4 leukopenia was observed during all the seven courses (100%) at the first dose level, eight courses (89%) at the second dose level, and seven courses (88%) at the third dose level. Similarly, grade 3 or 4 neutropenia was also frequently observed, necessitating G-CSF administration in eight patients. Grade 3 or 4 thrombocytopenia was observed less frequently at the first and second dose level, however it was observed during five courses (63%) at the third dose level, with two patients requiring platelet transfusion. Although grade 3 or 4 anaemia was observed less frequently, three patients required red blood cell transfusion.

3.3. Non-haematological toxicity

The non-haematological toxicities observed are summarised in Table 2. Febrile neutropenia occurred during one course (14%) at the first dose level, two courses (22%) at the second dose level, and four courses (50%) at the third dose level, however, it was reversible in all cases with only appropriate supportive care. Other toxicities, including diarrhoea, were mild, and did not require any intensive management.

There seemed to be different severity in toxicity profiles in patients with or without prior chemotherapy; grade 4 neutropenia and leucopenia were observed in 5 (38%) of 13 courses versus none of 11 courses in previously treated and untreated patients, respectively. Additionally, febrile neutropenia occurred in only patients with prior chemotherapy (7 [54%] of 13 courses versus none of 11 courses, respectively). However, in our study, pretreated patients tended to be incidentally accrued at higher dose level, which might be rather contributed to the difference in severity of toxicity profiles than prior chemotherapy itself was.

3.4. Antitumour activity

All patients were assessable for response. Although none of the cases showed complete response, six patients (67%),

Table 2 Grade 2 or more severe non-haematologic toxicity (all courses)

Toxicity	Grade[a]	Dose level		
		1	2	3
No. of treated patients		3	3	3
No. of courses evaluated		7	9	8
No. of courses in which toxicity was encountered (%)				
Febrile neutropenia	3	1 (14%)	2 (22%)	4 (50%)
Nausea/vomiting	2	0	1 (11%)	0
	3	0	0	0
Hepatotoxicity	2	1 (14%)	0	0
	3	0	0	0
Infection	2	0	0	0
	3	0	1 (11%)	0
Diarrhoea	2	0	1 (11%)	0
	3	1 (14%)	0	0

[a]No grade 4 or more severe toxicities were observed.

Table 3 Pharmacokinetic parameters of the drugs at dose levels 1–3

		Level 1 (AMR 30 mg/m^2) [number of points: 3]	Level 2 (AMR 35 mg/m^2) [number of points: 3]	Level 3 (AMR 40 mg/m^2) [number of points: 3]	p
AMR	C_{max} (ng/mL)	319.4 ± 109.5	401.6 ± 76.1	447.5 ± 33.5	0.49
	AUC (ng h/mL)	1195.6 ± 445.5	1615.1 ± 194.6	1849.8 ± 90.2	0.58
13-OH-AMR	C_{max} (ng/mL)	23.2 ± 13.3	28.9 ± 2.5	28.3 ± 2.5	0.73
	AUC (ng h/mL)	196.2 ± 169.7	191.2 ± 95.3	299.4 ± 88.2	0.67
TOP (day 2)	C_{max} (ng/mL)	20.3 ± 2.9	21.6 ± 7.9	18.8 ± 7.5	0.73
	AUC (ng h/mL)	64.2 ± 5.1	54.3 ± 15.7	45.1 ± 5.9	0.25
TOP (day 3)	C_{max} (ng/mL)	22.1 ± 1.7	15.0 ± 1.1	16.8 ± 1.7	0.09
	AUC (ng h/mL)	71.4 ± 6.7	53.2 ± 6.2	56.5 ± 1.9	0.19

Each data represents the mean values and standard errors. *Abbreviations*: AMR, amrubicin; TOP, topotecan; C_{max}, maximum concentration; AUC, area under the plasma concentration–time curve.

Table 4 Pharmacokinetic parameters of topotecan on days 2 and 3

	Day 2 (topotecan alone) [number of points: 9]	Day 3 (topotecan combined with amrubicin) [number of points: 9]	p
Parameters			
T_{max} (h)	0.5	0.5	
C_{max} (ng/mL)	20.2±3.3	18.0±1.3	0.83
AUC (ng h/mL)	54.5±5.8	60.4±3.9	0.23

Each data represents the mean values and standard errors. Abbreviations: C_{max}, maximum concentration; AUC, area under the plasma concentration–time curve.

including one receiving only the first dose level, showed PR. It is worthy of note that 4 out of the 5 (80%) relapsed patients showed PR, although only 2 out of 4 (50%) chemo-naïve patients showed PR. The median time to progression was 4.0 (95% CI: 0.8–6.8) months.

3.5. Pharmacokinetic and pharmacodynamic analysis

Pharmacokinetic parameters were determined in samples obtained on the second and third days of the first cycle in all nine patients. The maximum concentration (C_{max}) and AUC of AMR increased in a dose-dependent manner, although statistical significance was not reached (Table 3). The C_{max} and AUC of TOP were almost comparable among the first three dose levels, suggesting that the AMR dose did not influence the pharmacokinetics of TOP (Table 3). The C_{max} and AUC of

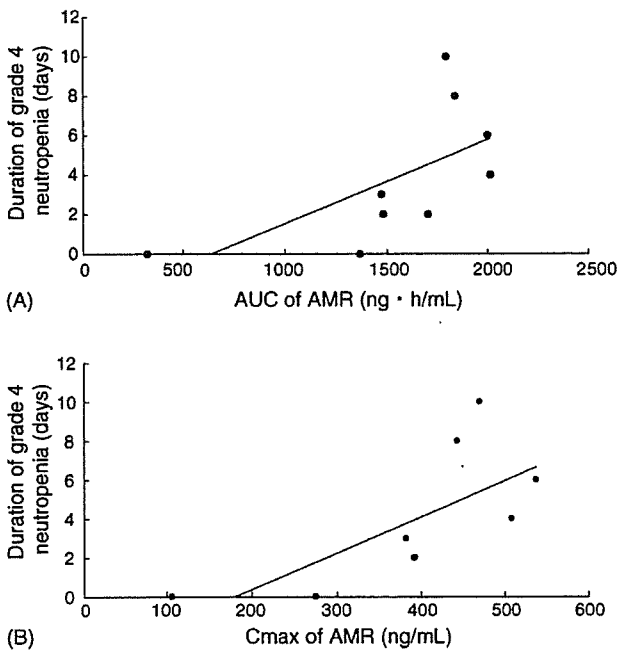

Fig. 1 (A) The correlation between the area under the plasma concentration–time curve (AUC) of AMR (amrubicin) on day 2 and the duration of grade 4 neutropenia in the first cycle (Spearman rank test, $p=0.0288$), and (B) the correlation between the maximum concentration (C_{max}) of AMR on day 2 and the duration of grade 4 neutropenia in the first cycle (Spearman rank test, $p=0.0225$).

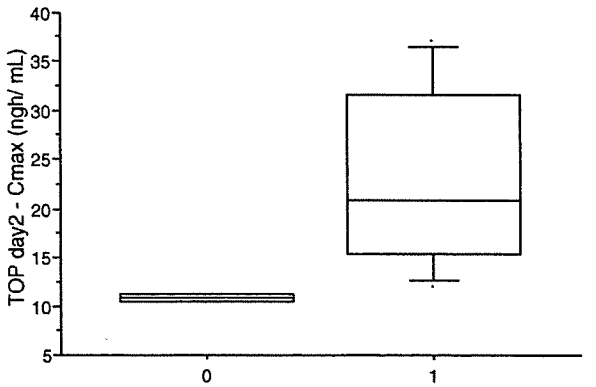

Fig. 2 Correlation between the maximum concentration (C_{max}) of topotecan on day 2 and objective tumour response in the first cycle. "0" denotes stable disease and progressive disease and "1" denotes partial response. The mean C_{max} of seven responders and two non-responders were 22.9±3.6 and 10.9±0.4, respectively (Mann–Whitney's U-test, $p=0.0404$).

13-OH-AMR were not significantly different even with dose escalation of AMR. 13-OH-AMR was not detectable in any of the samples collected from the first patient and two of the samples collected from the second patient at the first dose level, in three samples collected from the two patients at the second dose level, and in one sample collected from the patients at the third dose level, although AMR was detectable in all of these samples. However, the serum concentrations of 13-OH-AMR were higher than 20 ng/mL (minimum detectable value) in all the other patients. We also evaluated differences in the pharmacokinetic parameters of TOP between Day 2 (TOP alone) and Day 3 (TOP plus AMR), in order to investigate the effect of concurrent administration of AMR on the pharmacokinetics of TOP. As listed in Table 4, there were no significant differences. In the correlation of toxicity profiles with the pharmacokinetic parameters, the AUC and C_{max} of AMR were correlated with the duration of grade 4 neutropenia ($p=0.0288$ and 0.0225, respectively; Fig. 1A and B). In addition, the mean C_{max} of TOP on Day 2 in 7 responders (22.9±3.6) was significantly higher than that in 2 non-responders (10.9±0.4, $p=0.0404$; Fig. 2).

4. Discussion

Although the combined use of DNA topoisomerase I and II inhibitors is theoretically attractive, preclinical studies have demonstrated mixed results [19,20–23]. There have been

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.

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

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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

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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.

		Combination index (mean ± SD)	
Cell line	Drugs	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.

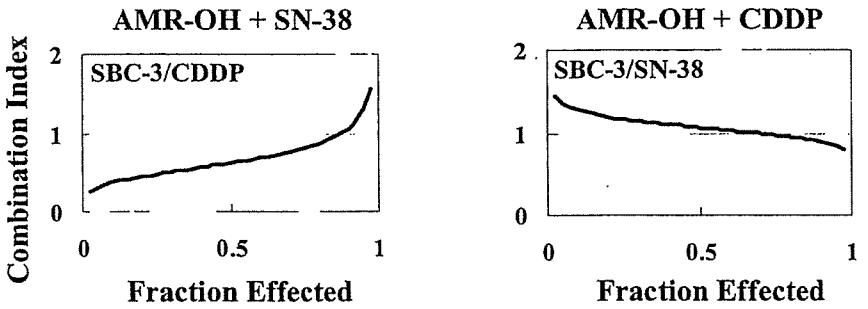

Figure 1. Combination index and surviving fraction of SBC-3/CDDP cells treated with amrubicinol (AMR-OH) in combination with SN-38 simultaneously for 96 h (left). Combination index and surviving fraction of SBC-3/SN-38 cells treated with AMR-OH in combination with cisplatin (CDDP) simultaneously for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

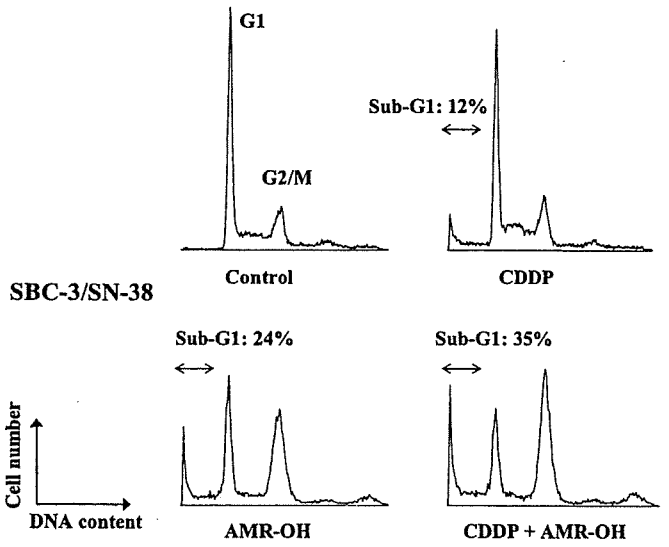

Figure 2. Effect of cisplatin (CDDP), amrubicinol (AMR-OH), or the combination of CDDP and AMR-OH induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3/SN-38 cells.

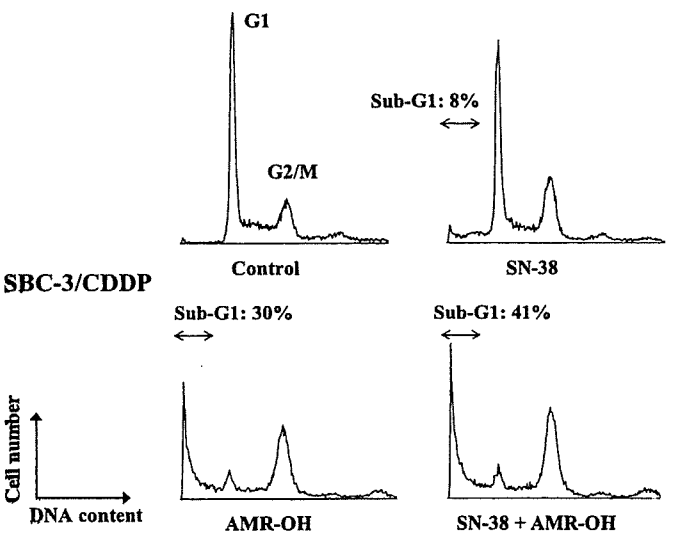

Figure 3. Effect of SN-38, amrubicinol (AMR-OH), or the combination of SN-38 and AMR-OH induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3/CDDP cells.

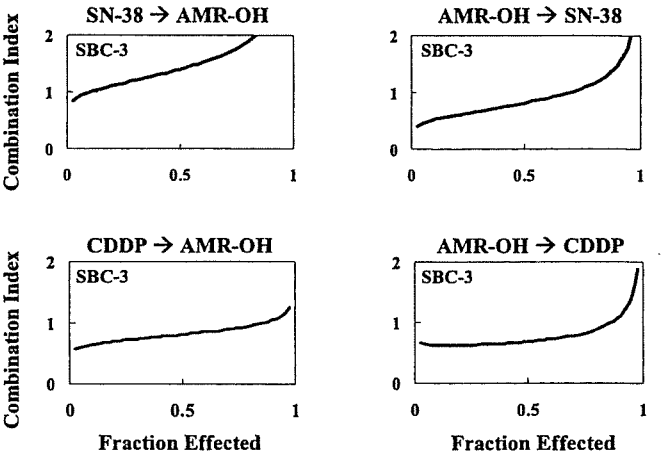

Figure 4. Combination index and surviving fraction of SBC-3 cells treated sequentially with SN-38 or cisplatin (CDDP) for 24 h followed by amrubicinol (AMR-OH) for 24 h and the reverse sequence.

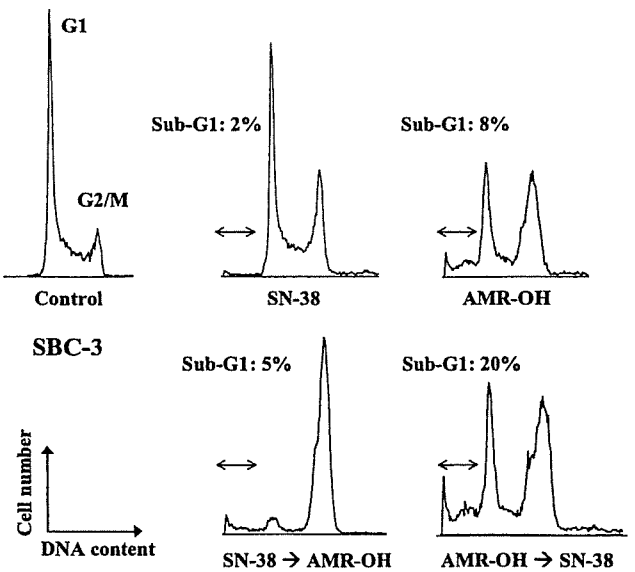

Figure 5. Effect of SN-38, amrubicinol (AMR-OH), SN-38 followed by AMR-OH, or AMR-OH followed by SN-38 induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3 cells.

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. 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

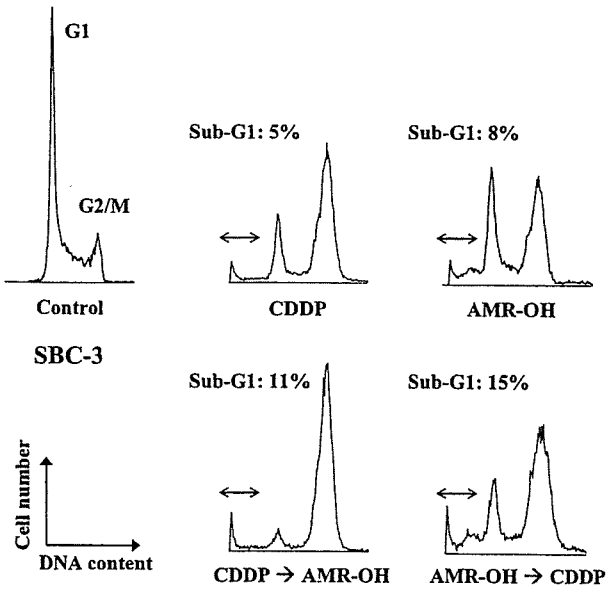

Figure 6. Effect of cisplatin (CDDP), amrubicinol (AMR-OH), CDDP followed by AMR-OH, or AMR-OH followed by CDDP induced cell cycle traverse perturbations and apoptosis (% cells in sub-G1 fraction) in SBC-3 cells.

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.

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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?

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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.

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

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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

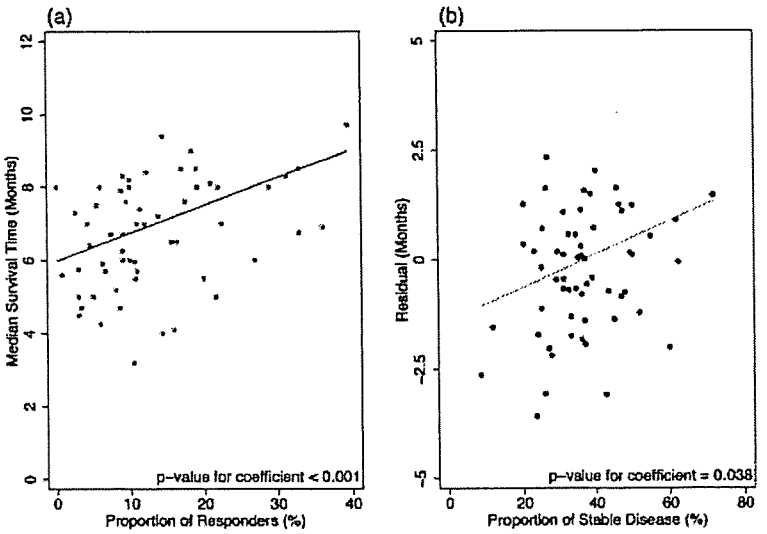

FIGURE 1. Scatterplot for MST and response/stable disease rates. (*A*) The observed MST corresponding to the percentage of responders. (*B*) The residuals (observed MST minus fitted MST in the model for *A*). The figure indicates that both response rate and stable disease rate significantly influence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

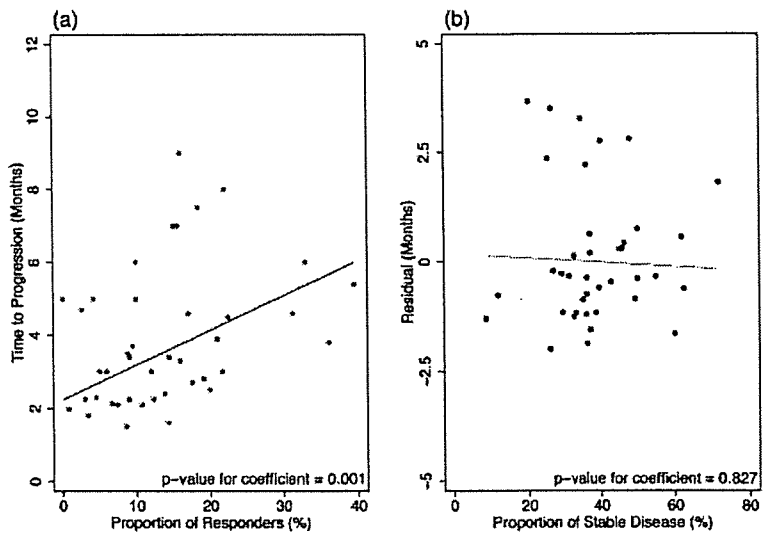

FIGURE 2. Scatterplot for TTP and response/stable disease rates. (*A*) The observed median TTP corresponding to the percentage of responders. (*B*) The residuals (observed TTP minus fitted TTP in the model for *A*). The figure indicates that the response rate but not the stable disease rate significantly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

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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

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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.

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