Table 1 Patient Characteristics

Characteristic	BLM	OK-432	PE
All patients	36	34	35
Eligible patients	35	33	34
Age (years)			
Median	64	60.5	61
Range	44–75	31–73	39–75
Sex			
Male	24	21	24
Female	12	13	11
PS (ECOG)[a]			
0	2	4	2
1	30	27	28
2	4	3	5
≥10% weight loss within 6 m			
No	33	27	31
Yes	3	7	4
Histology			
Adenocarcinoma	29	32	32
Squamous cell	4	1	3
Large cell	1	1	0
Other	1	0	0
TNM (N factor)			
N0	14	14	14
N1	2	0	2
N2	16	13	11
N3	3	7	8
Stage			
IIIB	23	17	25
IV	12	17	10

[a] At the time of reexpansion of the affected lung.

patients (5.7%) in the BLM arm had pneumonitis induced by BLM and one of them had treatment-related death. One patient in the PE group did not receive systemic chemotherapy due to elevation of serum creatinine. Other reasons for noncompletion of the protocol treatment were two patient refusals in each for the OK-432 and the PE arms, and one patient in the PE arm who could not receive sufficient drainage due to self-removal of the drain 48 h after intrapleural therapy.

Table 2 Treatment compliance

Variable	BLM	OK-432	PE
Eligible patients	35	33	34
No therapy	1	2	1
End of study protocol	18	19	14
Progressive disease	14	11	16
Toxicity	1	0	1
Death	1	0	0
Patient refusal	0	1	1
Insufficient drainage	0	0	1

Toxicities for intrapleural therapy in the three arms are listed in Table 3. Hematological toxic events were well tolerated in the three arms. Grade 4 nonhematological toxicity was not found in the three arms. Grade 2–3 chest pain occurred almost equally in the three arms. Grade 2–3 fever and nausea/vomiting occurred most frequently in the OK-432 arm (59.4%) and the PE arm (50.0%), respectively.

3.3. PPFS and OS

All eligible patients in the three arms were included in the survival analysis. PPFS and OS data are shown in Figs. 2 and 3, respectively. Median PPFS for the BLM arm was 20.9 weeks (95% confidence interval (CI), 4.7–25.9 weeks); for the OK-432 arm, 27.9 weeks (95% CI, 18.6–50.0 weeks); and for the PE arm, 18.4 weeks (95% CI, 4.4–41.4 weeks). The 4-week PPFS rate, which was the primary endpoint of this study, was 68.6% for the BLM arm (95% CI, 53.2–84.0%); 75.8% for the OK-432 arm (95% CI, 61.1–90.4%); and 70.6% for the PE arm (95% CI, 55.3–85.9%). The median survival time (MST) for the BLM arm was 32.1 weeks (95% CI, 21.6–37.9 weeks); 48.1 weeks for the OK-432 arm (95% CI, 26.7–58.4 weeks); and 45.7 weeks for the PE arm (95% CI, 34.4–57.1 weeks). The 48-week survival rate for the BLM arm was 29.9% (95% CI, 14.4–45.3%); 51.1% for the OK-432 arm (95% CI,

Table 3 Toxicity (JCOG grade) for Intrapleural Therapy

	BLM (n=35)				OK-432 (n=32)				PE (n=34)			
	1	2	3	4	1	2	3	4	1	2	3	4
Leukocytes	3	3	0	1	1	0	1	0	8	3	2	1
Neutrophils	1	0	2	1	0	0	1	0	5	5	1	2
Hemoglobin	3	5	3	ND	3	6	1	ND	6	6	3	ND
Platelet	0	0	1	0	0	0	0	0	1	1	0	0
AST	8	0	0	0	15	2	0	0	6	0	0	0
ALT	11	0	0	0	14	7	0	0	10	2	0	0
Serum creatinine	1	0	0	0	0	0	0	0	4	1	0	0
Chest pain	10	5	4	0	15	8	1	0	13	6	1	0
Fever	12	13	0	0	6	18	1	0	9	7	2	0
Nausea/vomiting	7	3	0	ND	15	0	0	ND	10	13	4	ND

Abbreviation: ND, not defined.

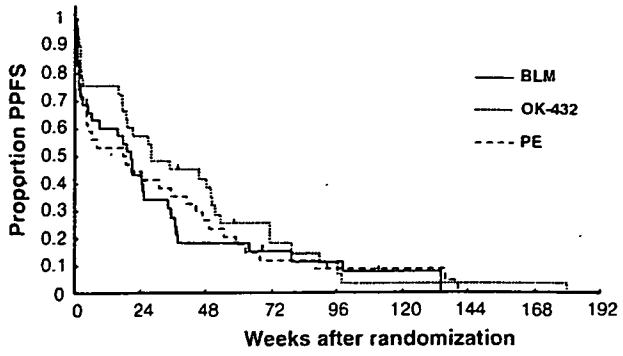

Fig. 2 Pleural progression-free survival (PPFS) in all eligible patients ($n = 102$).

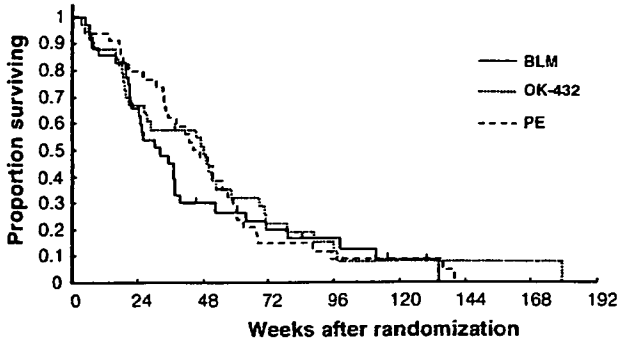

Fig. 3 Overall survival in all eligible patients ($n = 102$).

34.0–68.3%); and 47.1% for the PE arm (95% CI, 30.3–63.8%). Both the PPFS and OS for the OK-432 arm were superior to those for other two arms; however, the outcomes did not differ significantly between groups.

4. Discussion

To date, numerous chemical agents for treatment of MPE have been studied. These were antibiotics, antineoplastic agents, biological response modifiers (BRMs) and others that showed varied degrees of chemical sclerosis. Among them, BLM and talc are most frequently used for the management of MPE [5,7,17,18]. BLM is an antineoplastic antibiotic used in sclerotherapy with a success rate of 63–85% [7,8,18–21]. Talc applied as either slurry or poudrage is superior to other commonly used sclerosing agents with a success rate of 71–100% [5,7,22–24]. Because talc has not been available commercially in Japan and the use of talc was considered controversial at the beginning of this study because of severe complications, such as acute respiratory distress syndrome [25,26], we selected BLM as the sclerosing agent. A recent report demonstrated that the safety of talc pleurodesis and that acute respiratory distress syndrome can be avoided by using large-particle talc applied as thoracoscopic poudrage [27]. The thoracoscopic pleurodesis with talc is now considered to be the gold standard treatment for MPE [28,29].

OK-432 has been used as a BRM for gastric and lung cancer [9,10,30,31]. OK-432 has been reported to be effective in controlling MPE in two prospective randomized trials. One study reported a 73% success rate with OK-432 compared to 41% with mitomycin C treatment ($p = 0.03$) [11]. The other comparison found OK-432 70% effective compared to 46% in BLM subjects (statistical data not reported) [12]. OK-432 has been reported to induce various cytokines, such as tumor necrosis factor-α, interferon-γ, interleukin (IL)-1, IL-8 and IL-12 [32] and also to enhance cytotoxicity against tumor cells [33,34]. It is suggested that the main therapeutic effects of OK-432 for malignant effusion depend on increased expression of intercellular adhesion molecule-1 on tumor cells induced by interferon-γ [35].

Intrapleural combination chemotherapy is focused on achieving higher concentrations in the pleural cavity with less toxicity than systemic chemotherapy [36]. Two phase II studies with intrapleural cisplatin and cytarabine had success rates of 49% [2] and 73% [37]. Tohda et al. [13] reported that intrapleural instillation of cisplatin and etoposide for NSCLC with MPE resulted in a 46.2% overall response rate and the MST of 8 months was found to be improved, compared with previous reports for NSCLC with MPE of 3–6 months [11,18,38]. The reason for this was assumed to be that intrapleural combination chemotherapy of cisplatin and etoposide produced systemic as well as local effects. The overall response rates of intrapleural combination chemotherapy are variable and there are no prospective randomized studies compared modality of intrapleural combination chemotherapy with that of sclerotherapy.

There have been several special problems raised in the clinical trials for MPE, such as patient selection, response criteria, treatment procedures, short life expectancy, small sample sizes, and different endpoints [2–7,11,39]. To minimize the bias of patient selection, NSCLC patients with MPE who had received no prior therapy were entered into this study. Furthermore, justifiable and simplified response criteria and whether further treatment was required or not, as suggested by Ruckdeschel [18] and Rusch [40] were used and single intrapleural instillation of each agent was permitted to allow uniform estimation of responses. In many trials, successful pleurodesis was determined by assessing clinical and radiological findings. The positive response criteria have been defined generally as no pleural re-accumulation, 50% less effusion than that observed in the baseline radiograph taken immediately after the procedure, or no requirement for further thoracentesis. To determine the efficacy, we used the criterion that a decrease in effusion over one-quarter of the treated lung provides a stricter assessment of chemical pleurodesis that may relieve the symptoms of MPE. The position rotation after intrapleural instillation was recommended traditionally because it was thought to allow the agents to be distributed thoroughly throughout the entire pleural space. In contrast, studies using tetracycline and talc [41,42] demonstrated that rotation does not affect the overall intrapleural dispersion. It is unclear whether rotation is beneficial or not when applying the agents used in this study. Because a previous phase II study [13] showed that etoposide remains for a long period (β-phase half-life = 62.53 h) in intrapleural fluids, we applied the longer duration of clamping in the PE arm (72 h) than the other two arms (3 h) to provide enough exposure to the cancer cells. We found no major safety concerns such as excess pleural effusion as a result of the longer duration of clamping.

In this study, all three regimens were feasible. One treatment-related death occurred in the BLM arm 9 weeks after intrapleural instillation of BLM. Treatment compliance

rates for both intrapleural and systemic therapy was 50% (51 of the 102 eligible patients). This study lacks sufficient power to demonstrate differences between treatment arms; however, the OK-432 arm seemed to demonstrate modest benefit compared with the other two arms in terms of PPFS. It is assumed that the favorable efficacy in the OK-432 arm suggests that OK-432 has clinically meaningful activity for controlling MPE in NSCLC patients. NSCLC patients with MPE have been treated as patients with stage IV disease even when without metastasis, and systemic chemotherapy should be recommended when they have a good PS [43]. We prescribed systemic PE chemotherapy regimens, which were considered one of the standard regimens at the beginning of the study, following successful pleurodesis. However, we expect that platinum-based systemic combination chemotherapy regimens with several active new chemotherapeutic agents such as taxanes (paclitaxel and docetaxel), vinorelbine, gemcitabine and irrinotecan, which are the current standard treatment options for patients with advanced NSCLC, should enhance the survival benefit more than PE regimens.

This is the first fully reported randomized study that has evaluated the efficacy of intrapleural therapy for previously untreated patients with NSCLC and compliance with sequential systemic chemotherapy. As the results of this study demonstrate that intrapleural therapy with OK-432 shows a tendency to be more effective than BLM or PE in the management of MPE in NSCLC, in terms of PPFS, further studies are needed to compare OK-432 with talc.

Conflict of interest

None declared.

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EGFR exon 20 insertion mutation in Japanese lung cancer

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Summary Mutations of the epidermal growth factor receptor (*EGFR*) gene have been reported in non-small cell lung cancer (NSCLC), especially in female, never smoker patients with adenocarcinoma. Some common somatic mutations in *EGFR*, including deletion mutations in exon 19 and leucine to arginine substitution at amino acid position 858 (L858R) in exon 21, have been examined for their ability to predict sensitivity to gefitinib or erlotinib. On the other hand, previous report has shown that the insertion mutation at exon 20 is related to gefitinib resistance. We investigated the exon 20 *EGFR* mutation statuses in 322 surgically treated non-small cell lung cancer cases. Two hundred and five adenocarcinoma cases were included. The presence or absence of *EGFR* mutations of kinase domains was analyzed by direct sequences. *EGFR* insertion mutations at exon 20 were found from 7 of 322 (2.17%) lung cancer patients. We also detected the 18 deletion type mutations in exon 19, and 25 L858R type mutations in exon 21. There was a tendency towards higher exon 20 insertion ratio in never smoker (never smoker 4.4% versus smoker 1.3%, $p = 0.0996$) and female (female 4.5% versus male 1.3%, $p = 0.0917$). Two exon 20 insertion cases were treated with gefitinib and failed to response.

EGFR insertion mutation in exon 20 could not be ignored from Japanese lung cancers.

1. Introduction

Lung cancer is a major cause of death from malignant diseases, due to its high incidence, malignant behavior and lack of major advancements in treatment strategy [1]. There are much accumulated evidences that epidermal growth fac-

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tor receptor (*EGFR*) and its family members are strongly implicated in the development and progression of numerous human tumors, including lung cancer [2,3]. The *EGFR* tyrosine kinase inhibitor, gefitinib, was approved in Japan for the treatment of non-small cell lung cancer (NSCLC) since 2002. Original two reports showed that *EGFR* mutations statuses at ATP binding pockets in NSCLC patients were correlated with the clinico-pathological features related to good response to gefitinib [4,5]. These *EGFR* mutations are predominantly found in Japanese lung cancer patients (about 25–40%) [4,6–9] when compared to USA patients (about 8–10%) [4,5,7,10] or European patients [7,11]. Actually, *EGFR* mutations in lung cancer have been correlated with clinical response to gefitinib therapy *in vivo* and *in vitro* [4,5,10]. Although many *EGFR* mutations have been reported, not all have been associated with responsiveness to gefitinib. The two most common *EGFR* mutations that have been identified, representing 85–90% of *EGFR* mutations, are the *EGFR* exon 19 deletion that eliminates a leucine–arginine–glutamate–alanine motif in the tyrosine kinase domain of *EGFR* and a thymine to guanine transversion that results in an arginine for leucine substitution at amino acid 858 (L858R). These two mutants responded significantly better for gefitinib therapy than other types of mutants [12,13]. However, Greulich et al. showed transformation by an exon 20 insertion, made cells resistant to gefitinib or erlotinib [14]. To determine the *EGFR* mutation status and correlation with clinico-pathological features in Japanese lung carcinoma, we investigated exon 20 insertion mutation status by direct sequences. The findings were compared to the clinico-pathological features of lung cancer.

2. Material and methods

2.1. Patients

The study group included 295 lung cancer patients who had undergone surgery at the Department of Surgery II, Nagoya City University Medical School between 1994 and 2005. We have also investigated *EGFR* mutation status for 27 lung cancer patients who had undergone surgery followed by treated with gefitinib at the National Hospital Organization, Kinki-chuo Chest Medical Center. Gefitinib was used after lung cancer recurrence, and clinical outcome was shown in reference [9]. The lung tumors were classified according to the general rule for clinical and pathological record of lung cancer in Japan [15]. All tumor samples were immediately frozen and stored at −80 °C until assayed. Written informed consent was obtained from the patients, and the institutional ethics committee of the Nagoya City University Medical School approved the study.

2.2. PCR assays for *EGFR* mutations

Genomic DNA was extracted using Wizard SV Genomic DNA Purification Systems (Promega) according to the manufacturers' instructions. The primers for exon 20 sequencing were designed with Primer Express 2.0 software (Applied Biosystems). The sequences of the primer sets used in the assay are: forward ACTTCACAGCCCTGCGTAAAC, and reverse: ATGGGACAGGCACTGATTTGT. The sequence results of exon 20 about 131 of 322 cases were already reported [4,16]. The cycling conditions were as follows: initial denaturation at 94 °C for 10 min, followed by 35 cycles at 94 °C for 30 s, 64 °C for 30 s, 72 °C for 60 s. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA). Amplified cDNAs were separated on 1% agarose gels, and the bands were visualized by ethidium bromide and photographed under ultraviolet transillumination. These samples were sequenced by ABI prism 3100 analyzer (Applied Biosystems Japan Ltd., Tokyo, Japan) and analyzed by BLAST and chromatograms by manual review form forward and reverse, both side.

2.3. Statistical analysis

Statistical analyses were done using the Mann–Whitney *U*-test for unpaired samples and Wilcoxon's singed rank test for paired samples. Linear relationships between variables were determined by means of simple linear regression. Correlation coefficients were determined by rank correlation using Spearman's test and χ^2 test. The overall survival of lung cancer patients was examined by the Kaplan–Meier methods, and differences were examined by the Log-rank test. All analysis was done using the Stat-View software package (Abacus Concepts Inc. Berkeley, CA), and was considered significant when the *p*-value was less than 0.05.

3. Results

3.1. *EGFR* gene mutation status in Japanese lung cancer patients

The clinical and pathological characteristics of the 322 lung cancer patients are as follows: 234 (72.7%) were males and 88 were females. Two hundred and five (63.7%) were diagnosed as adenocarcinoma, and 117 were diagnosed as other types of carcinoma. Two hundred and thirty-one (71.7%) were smokers and 90 were non-smokers (one unknown). Of 295 lung cancer patients from Nagoya City University, 167 (56.6%) were stage I.

Most of the sequencing results about exon 18, 19 and 21 were already reported [4,16,17]. In exon 19, 18 patients had the deletion type mutation. In exon 18 or exon 21, 29 patients had the missense point mutations (2 G719S, 1 G719C, 25 L858R and 1 L861Q). Of these 47 patients, 17 were males and 30 were females. Thirty were non-smokers and 17 were smokers. Forty-three patients had adenocarcinoma, one had squamous cell carcinoma and three had adenosquamous cell carcinoma. Thus *EGFR* mutation status at exon 18, 19 or 21 was significantly correlated with gender ($p < 0.0001$), tobacco-smoking ($p < 0.0001$) and pathological subtypes (adenocarcinoma versus non-adenocarcinoma, $p < 0.0001$).

For exon 20, 7 patients had the insertion mutations (Table 1). These mutations were exclusively associated with other *EGFR* mutation. Three were males and four were females. Four were non-smokers and three were smokers. Six patients had adenocarcinoma and one had squamous

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Table 1 Clinico-pathological features of 322 lung cancer patients

Factors	*EGFR* exon 20 mutations		
	Mutation patients	Wild type patients	*p*-Value
Mean age (65.5 ± 9.3; years)	7	315	
Age			
≦60	1 (1.1%)	94 (98.9%)	0.6783
>60	6 (2.6%)	221 (97.4%)	
Gender			
Male	3 (1.3%)	231 (98.7%)	0.0917
Female	4 (4.5%)	84 (95.5%)	
Pathological subtypes			
Adeno	6 (3.0%)	197 (97.0%)	0.2666
Non-adeno	1 (0.8%)	118 (99.2%)	
Differentiation			
Well	4 (3.5%)	111 (96.5%)	0.4236
Moderately or poorly	2 (1.5%)	128 (98.5%)	
Lymph node metastasis			
N0	4 (1.9%)	205 (98.1%)	>0.9999
N+	2 (2.3%)	84 (97.7%)	
Smoking status			
Smoker	3 (1.3%)	228 (98.7%)	0.0996
Non-smoker	4 (4.5%)	86 (95.5%)	
Pathological stages			
I	4 (2.4%)	164 (97.6%)	0.7025
II–IV	2 (1.6%)	125 (98.4%)	

Adeno, adenocarcinoma; N+, lymph node metastasis positive.

cell carcinoma. Two were moderately differentiated, and four were well differentiated (one unclassified). There was a tendency towards higher exon 20 insertion mutation ratio in never smoker (never smoker 4.4% versus smoker 1.3%, $p=0.0996$) and female (female 4.5% versus male 1.3%, $p=0.0917$). Two female patients had 774_776 insertion NPH (2320-2328 insertion AACCCCCAC) mutations reported as D7 mutation by Shigematsu et al. (Fig. 1) [7]. A female patient had 770_772 insertion ASV (2308-2316 insertion GCCAGCGTG) mutation reported as D1 mutation by Shigematsu et al. [7]. A male patient had 771_773 insertion SVD (2311-19 insertion GCGTGGACA) mutation reported by Sonobe et al. [18]. A male patient had 772_773 insertion V (2312-14 insertion GGT) reported by Thomas et al. [19]. Two patients had 772_773insertion N (2312-14 insertion AAC) mutations (Fig. 1).

3.2. Relationship between clinical course of patients with lung cancer and *EGFR* mutations

The overall survival of 322 lung cancer patients with follow-up through December 30, 2006, was studied in reference to the *EGFR* mutation status. The prognosis from patients with exon 20 insertion mutation ($n=7$, 2 were dead) and the patient without exon 20 insertion mutation *EGFR* ($n=315$, 102 were dead) was not significantly different (Log-rank test, $p=0.7186$, Breslow–Gehan–Wilcoxon test, $p=0.8593$) (Fig. 2). Eighteen patients received adjuvant chemotherapy (five were with cisplatin base, seven were with calboplatin base and six were with Uracil-Ftegafur). Even if the 18 patients were excluded for survival analysis, the prognosis from patients with exon 20 mutation and without mutation was not significantly different ($p=0.7215$).

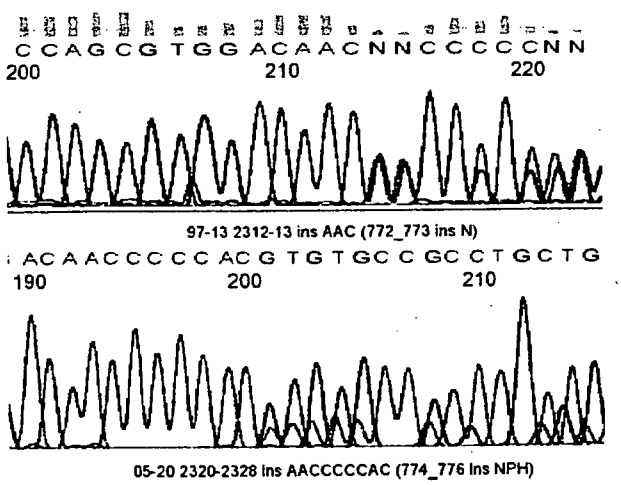

Fig. 1 Two patients had 774_776 insertion NPH (2320-2328 insertion AACCCCCAC) mutations reported as D7 mutation (upper). Two patients had 772_773insertion N (2312-14 insertion AAC) mutations (below).

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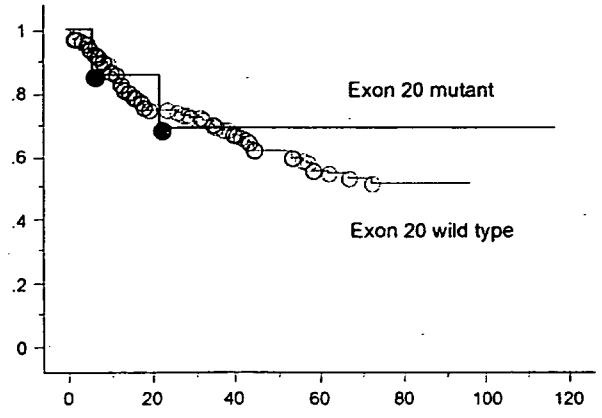

Fig. 2 The prognosis from patients with exon 20 insertion mutation ($n=7$, 2 were dead) and the patient without exon 20 insertion mutation *EGFR* ($n=315$, 92 were dead) was not significantly different (Log-rank test, $p=0.7186$, Breslow–Gehan–Wilcoxon test, $p=0.8593$).

3.3. Clinical course of two recurrent lung cancer patients treated with gefitinib

Case 1: 58-year-old adenocarcinoma woman with no history of smoking underwent surgery at Kinki-chuo Chest Medical Center. A molecular analysis revealed 772_773 insertion N (2312-14 insertion AAC) mutations at *EGFR* exon 20. Three years later, the recurrent lung cancer was treated with gemcitabine, vinorelbine and Uracil-Ftegafur in addition to radiotherapy. Because the treatment failed, gefitinib treatment was started at 2004. The patient died from progressive disease about 6 months after gefitinib administration. Case 2: 72-year-old adenocarcinoma man with no history of smoking underwent surgery at Nagoya City University Hospital. A molecular analysis revealed 772_773 insertion V (2312-14 insertion GGT) at *EGFR* exon 20 (Fig. 3), and wild type at *Kras* codon 12/13. Multiple lung metastasis were treated with Uracil-Ftegafur, however, the treatment failed. Gefitinib treatment was started at 2005. But the tumor size was increased (Fig. 3) and the treatment was quitted at 3 months.

4. Discussion

We obtained findings that exon 20 insertion type *EGFR* mutations tend to be higher in female gender and never smoker, as like as other *EGFR* mutation subtypes [8–14]. From the original three papers published by Lynch et al., Paez et al. and Pao et al., there was no *EGFR* exon 20 insertion subtypes. Shigematsu et al. reported that 12 of 617 (1.9%) had exon 20 insertion mutation, however, 356 of 617 patients were either from Japan or Taiwan [11]. Sonobe et al. reported that the 2 of 154 cases (1.3%) had *EGFR* exon 20 insertion mutations. These data suggested that *EGFR* mutations at exon 20 might be also higher in East Asian. More interestingly, patients with exon 20 mutation did not respond to gefitinib therapy.

Although many reports have identified more than 30 different mutations in the tyrosine kinase domains of *EGFR*, the vast majority of which can be grouped into three major types, including in-frame deletion at exon 19, single-nucleotide substitution at exon 18 or 21 and in-frame duplication at exon 20 [8–14]. To date, only the L858R missense mutation in exon 21 and deletions in exon 19 have been proven to be activating mutations [4,5,10,14]. On the

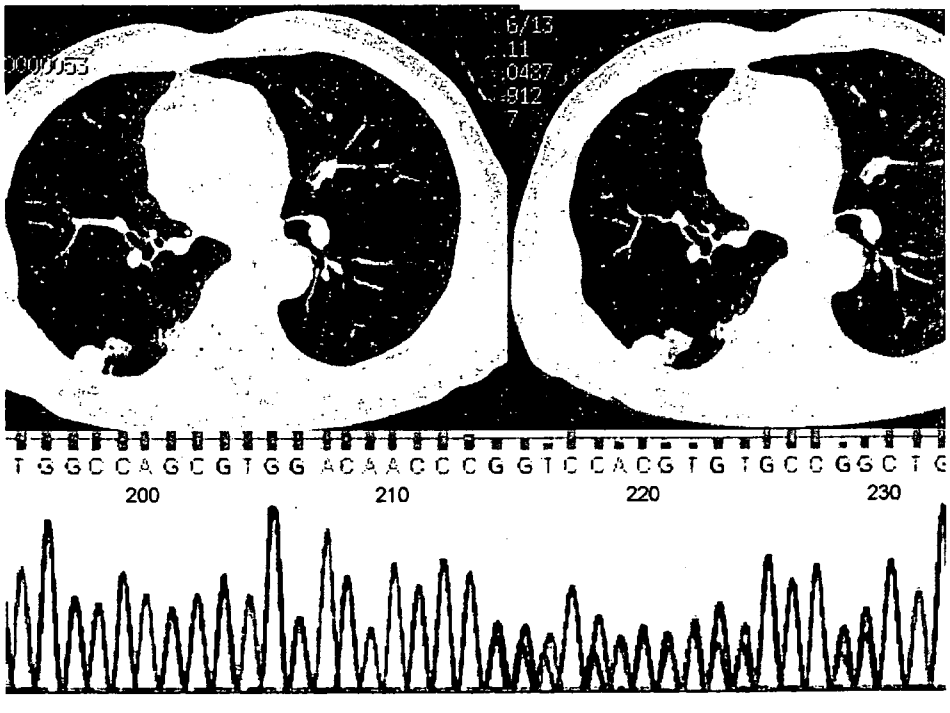

Fig. 3 CT examination before (left) and after (right) gefitinib therapy revealed increased tumor size. A molecular analysis revealed 772_773 insertion V (2312-14 insertion GGT) at *EGFR* exon 20 (below).

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other hands, Greulich et al. reported that transformation by the D770_N771 ins NPG (exon 20) *EGFR* insertion mutant was remarkably insensitive to gefitinib and erlotinib, as inhibition of colony growth in soft agar required exposure to 100-fold higher concentrations (>1 mM) of these agents than was required to inhibit colony formation by cells expressing the *EGFR* missense mutants or deletion mutant [14]. No significant inhibition of anchorage-independent growth of cells expressing D770_N771ins NPG *EGFR* was observed at 3 mM gefitinib or erlotinib [14]. Greulich et al. also reported that all three lung adenocarcinoma patients with known exon 20 insertion mutants of *EGFR* have failed to show a clinical response to treatment and have instead achieved only stable disease with erlotinib [14]. *In vitro* analysis, cells expressing the *EGFR* deletion and insertion mutants formed colonies in soft agar with a higher efficiency than that of cells expressing the missense mutants, comparable to the colony formation efficiency of cells expressing polyoma middle T antigen, suggested these mutants were oncogenic [14]. Interestingly, the irreversible *EGFR* inhibitor CL-387, 785 [20] is more effective than gefitinib or erlotinib for inhibition of colony formation by cells expressing the exon 20 insertion mutant [14]. CL-387, 785 had an even greater effect on colony formation by cells expressing L858R [14], and this compound was previously found to be active against *EGFR* containing the exon 20 point mutation T790M, associated with resistance to gefitinib and erlotinib [21]. Thus the distinct inhibitor sensitivity of various *EGFR* mutants argues that therapies may need to be targeted against specific mutant forms of a protein, whereas generalized inhibition of a particular oncogenic target may not be sufficient.

Conflict of interest

None declared.

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Invest New Drugs (2007) 25:253–258
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

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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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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

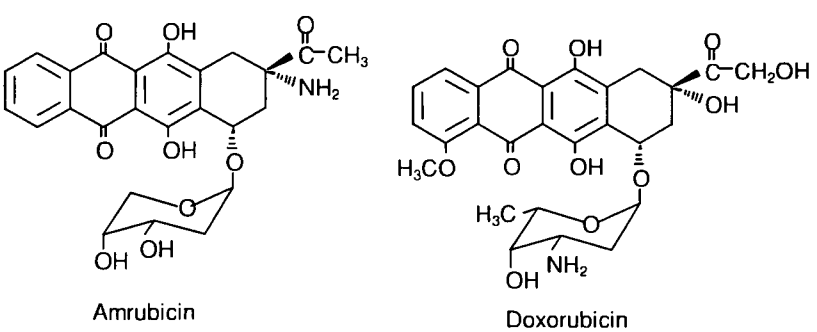

Fig. 1 Chemical structures of amrubicin and doxorubicin

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.

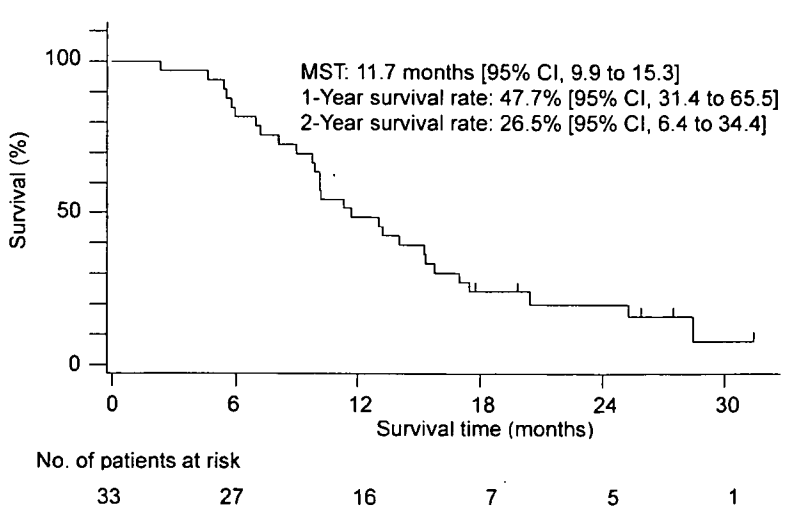

Fig. 2 Overall survival of patients with extensive-disease small cell lung cancer treated with amrubicin. MST: median survival time; 95% CI: 95% confidence interval

Table 3 Main treatment-related toxicity of amrubicin

Toxicity	No. of assessable patients	Toxicity grade others					
		1	2	3	4	≥ 1	≥ 3
		(No. of patients)				Frequency (%)	
Hematologic toxicity							
Anemia (hemoglobin)	33	12	7	6	1	78.8	21.2
Leukopenia	33	5	11	13	4	100	51.5
Neutropenia	33	1	4	14	14	100	84.8
Thrombocytopenia	33	3	3	1	6	39.4	21.2
Non-hematologic toxicity							
Stomatitis	33	2	1	0	0	9.1	0
Anorexia	33	12	3	3	–[a]	54.5	9.1
Nausea and vomiting	33	12	7	0	–[a]	57.6	0
Diarrhea	33	6	0	0	0	18.2	0
Fever	33	3	7	0	0	30.3	0
Phlebitis	33	1	1	0	0	6.1	0
Alopecia	33	11	8	1	–[a]	60.6	3.0
Total bilirubin elevation	33	1	1	0	0	6.1	0
AST elevation	33	5	0	0	0	15.2	0
ALT elevation	33	8	1	0	0	27.3	0
ALP elevation	33	1	0	0	0	3.0	0
BUN elevation	33	2	0	0	0	6.1	0
Others[b]	Headache, 1/33[c]; Rash, 1/33; Constipation, 1/33; Interstitial pneumonia, 1/33; Rhinorrhagia, 1/33; ECG abnormality, 3/32						

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; BUN: blood urine nitrogen; ECG: electrocardiogram.

[a]Toxicity grade was not defined for these toxicities.

[b]Toxicities which were not graded.

[c]Proportion of number of reported patients to number of observed patients.

(57.6%), diarrhea (18.2%), fever (30.3%), alopecia (60.6%), AST increase (15.2%), and ALT increase (27.3%). Most of these were mild (≤grade 2), with only 3 patients (9.1%) experiencing grade 3 anorexia and 1 patient grade 3 alopecia (3.0%). A single patient developed interstitial pneumonia after the second cycle of treatment; however, it was reversibly recovered by steroid therapy and cessation of amrubicin treatment. ECG abnormality was observed in 3 patients (9.4%; supraventricular extrasystole, prolonged QT interval, and T wave flattening in 1 patient each), which did not need any treatment. No LVEF decrease was observed.

Discussion

Results of this phase II study demonstrate that amrubicin is an extremely active agent against extensive-disease SCLC. The complete response rate was 9.1% (95% CI, 1.9–24.3%), overall response rate 75.8% (95% CI, 57.7–88.9%), and median survival time 11.7 months (95% CI, 9.9–15.3 months). These results are comparable or even superior to those of the standard combination regimen of cisplatin and etoposide, used as the gold standard of extensive-disease SCLC therapy since 1981 and remaining unchanged over the last 2 decades [4].

SCLC is sensitive to cytotoxic anticancer agents. Of anticancer drugs developed before 1990, a number of agents with response rates of 20% or greater for SCLC were listed as active drugs [17]. Of these drugs, etoposide, cisplatin, carboplatin, doxorubicin, cyclophosphamide, and vincristine, are still currently used as important constituents of combination regimens in the treatment of SCLC. In addition, several drugs with significant activity for SCLC have been developed since 1990. Irinotecan showed a response rate of 33% to 47% even in previously treated patients who are generally less sensitive to chemotherapy [18, 19]. Recently a new combination regimen of irinotecan plus cisplatin was demonstrated to be significantly superior to standard regimen of etoposide plus cisplatin in median survival time (12.8 months vs. 9.4 months, $P = 0.002$) [3]. In addition, topotecan, paclitaxel, docetaxel, and gemcitabine are reported to have response rates of 26% to 41% for extensive-disease SCLC patients without previous treatment [20–24]. Compared to these agents, amrubicin demonstrated a much higher response rate (75.8%) in this study, indicating it is a promising novel agent with potential to overcome the therapeutic plateau of SCLC.

The major toxicity of amrubicin was hematologic. Grade 3 or 4 leukopenia was frequently observed in 51.5% of patients and grade 3 or 4 neutropenia in 84.8% of patients. Despite such severe hematologic toxicity, 88% of the total treatment cycles could be delivered without dose reduction and non-hematologic toxicities were mild. Although anorexia (54.5%) and nausea and vomiting (57.6%) were frequently observed, there were no episodes of grade 3 or 4 toxicity, except for 3 patients (9.1%) with grade 3 anorexia and 1 patient (3.0%) with grade 3 alopecia. A single patient developed interstitial pneumonia; however, this was reversible with steroid therapy. ECG abnormalities were observed in 3 patients, but they were each reviewed by a medical cardiologist and judged not to be clinically significant. No LVEF decrease was observed. Results show that the toxic profiles of amrubicin are acceptable and favorable in the treatment of extensive-disease SCLC, although due to its hematologic toxicity, in particular neutropenia, G-CSF support is needed.

In conclusion, amrubicin is a very active and promising agent with acceptable toxicity for patients with SCLC. Further studies are warranted in combination with other agents for this disease.

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