

**Fig. 5.** Upper panel: preparation of recombinant human full-length  $\alpha$ -enolase protein:  $\alpha$ -Eno (1–434 a.a.), C-terminal deletion mutant  $\alpha$ -enolase proteins:  $\alpha$ -Eno1 (1–334 a.a.),  $\alpha$ -Eno2 (1–234 a.a.) and  $\alpha$ -Eno3 (1–134 a.a.). Lower panel: recombinant proteins were stained with Coomassie brilliant blue or probed with anti-6  $\times$  His antibody, anti-enolase antibody or non-small cell lung cancer sera.

as immunogens, and other types of protein processing in tumor tissue.<sup>(21)</sup> In the present study we used normal recombinant proteins to confirm the immunogenicity of  $\alpha$ -enolase in NSCLC patients. In addition, although a past study reported that expression of  $\alpha$ -enolase was downregulated in NSCLC tissues,<sup>(22)</sup> IHC staining used in our study showed that  $\alpha$ -enolase expression is commonly increased in malignant lung tissue from NSCLC patients compared to the expression in non-malignant lung tissue, which is consistent with other previous reports.<sup>(23–27)</sup> Interestingly, IHC staining also showed not only cytoplasmic but also membranous immunoreactivity. Moreover, flow cytometric analysis demonstrated expression of  $\alpha$ -enolase on the surface of lung cancer cells. We think that enhanced expression of  $\alpha$ -enolase on cancer-cell surface might be one reason for autoantigenicity, and might be required for induction of autoantibody responses. However,  $\alpha$ -enolase expression alone is insufficient for autoantibody production as increased expression was also found in these patients without autoantibodies against  $\alpha$ -enolase. Further study is necessary to investigate the detailed mechanisms involved in this autoantibody response.

It is widely accepted that the propensity for glycolysis is enhanced in cancer cells because of increased cell proliferation. In fact,  $\alpha$ -enolase, a key enzyme in the glycolysis pathway, was found to be overexpressed in 18 cancers.<sup>(25)</sup> Furthermore, although the mechanism of its surface expression and orientation on the membrane are not yet clearly understood, it is known that the C-terminal a.a. of  $\alpha$ -enolase, lysine, is exposed at the cell surface and is involved in binding to plasminogen, which is then activated and converted to plasmin.<sup>(28)</sup> Once plasmin is stabilized at the cell surface, it in turn induces fibrinolysis.<sup>(20)</sup> In response to the upregulation of  $\alpha$ -enolase expression, progression of the fibrinolytic system is markedly accelerated, and the resultant increase in local fibrinolysis may contribute to cancer cell invasion and metastasis. This is consistent with our finding that there was a tendency for autoantibodies against  $\alpha$ -enolase to be more prevalent in patients with advanced NSCLC (stage III/IV) than in stage I/II cases.

Tumor markers for NSCLC are potentially useful for both diagnostic and therapeutic practice. To date, a variety of NSCLC

tumor markers have been identified and the most extensively investigated circulating protein markers include CEA, CYFRA 21-1, SCC antigen, NSE and CA125.<sup>(6)</sup> The percentages of NSCLC patients who have elevated serum protein levels of the above markers are 26%–42% for CEA, 51%–74% for CYFRA 21-1, 20%–32% for SCC, 28–32% for NSE, and 46–55% for CA125, with variations depending on histology and stage.<sup>(5–8)</sup> However, because elevated serum protein levels of these markers have been observed in tumors other than NSCLC, their sensitivity and specificity are not satisfactory and their clinical applicability is limited. Our study demonstrated that when ‘Mean OD<sub>healthy control sera</sub> + 3 SD<sub>healthy control sera</sub>’ was used as the cut-off point, autoantibodies against  $\alpha$ -enolase occurred in 27.7% of NSCLC patients but not in those with SCLC, gastrointestinal cancer or MAC infection of the lungs. Moreover, combined detection of autoantibodies against  $\alpha$ -enolase, CEA and CYFRA 21-1 enhanced sensitivity for the diagnosis of NSCLC. These results suggest that using autoantibodies against  $\alpha$ -enolase has potential as a clinical biomarker for serological screening of NSCLC. Further large-scale validation studies will be needed to determine the sensitivity, specificity and positive prognostic value of this marker in real-world screening scenarios.

Autoantibodies against  $\alpha$ -enolase have been detected in some autoimmune diseases,<sup>(20)</sup> and Fujii *et al.* report that in Hashimoto’s encephalopathy one of these autoantibodies recognizes the N-terminal region of  $\alpha$ -enolase.<sup>(29)</sup> Because our results show this same recognition in NSCLC, further experiments are warranted to compare epitopes in  $\alpha$ -enolase as detected in NSCLC and in autoimmune diseases to determine its utility as a biomarker for NSCLC.

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# Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan

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**Background:** To compare the efficacy and toxicity of three platinum-based combination regimens against cisplatin plus irinotecan (IP) in patients with untreated advanced non-small-cell lung cancer (NSCLC) by a non-inferiority design.

**Patients and methods:** A total of 602 patients were randomly assigned to one of four regimens: cisplatin 80 mg/m<sup>2</sup> on day 1 plus irinotecan 60 mg/m<sup>2</sup> on days 1, 8, 15 every 4 weeks (IP); carboplatin AUC 6.0 min × mg/mL (area under the concentration–time curve) on day 1 plus paclitaxel 200 mg/m<sup>2</sup> on day 1 every 3 weeks (TC); cisplatin 80 mg/m<sup>2</sup> on day 1 plus gemcitabine 1000 mg/m<sup>2</sup> on days 1, 8 every 3 weeks (GP); and cisplatin 80 mg/m<sup>2</sup> on day 1 plus vinorelbine 25 mg/m<sup>2</sup> on days 1, 8 every 3 weeks (NP).

**Results:** The response rate, median survival time, and 1-year survival rate were 31.0%, 13.9 months, 59.2%, respectively, in IP; 32.4%, 12.3 months, 51.0% in TC; 30.1%, 14.0 months, 59.6% in GP; and 33.1%, 11.4 months, 48.3% in NP. No statistically significant differences were found in response rate or overall survival, but the non-inferiority of none of the experimental regimens could be confirmed. All the four regimens were well tolerated.

**Conclusion:** The four regimens have similar efficacy and different toxicity profiles, and they can be used to treat advanced NSCLC patients.

**Key words:** carboplatin, cisplatin, gemcitabine, irinotecan, non-small-cell lung cancer, paclitaxel, randomized phase III study, vinorelbine

## Introduction

Nearly 60 000 patients in Japan died of lung cancer in 2004, and the mortality rate is still increasing [1]. Even old-generation cisplatin-based chemotherapy provides a survival benefit and symptom relief in patients with inoperable non-small-cell lung cancer (NSCLC) [2]. Several anticancer agents including irinotecan, paclitaxel, docetaxel, gemcitabine, and vinorelbine, were developed in the 1990s and most of them have mechanisms of action that differ from those of the old-generation agents [3–7]. The combinations of platinum and these new agents developed in the 1990s are more useful against advanced NSCLC than old-generation combination

chemotherapy, and doublets of platinum and new-generation anticancer agents are considered standard chemotherapy regimens for advanced NSCLC, although no consistent standard regimens have yet been established [8–17].

Two phase III studies comparing cisplatin plus irinotecan (IP) with cisplatin plus vindesine for advanced NSCLC have been conducted in Japan [18, 19]. Fukuoka et al. [20] reported the results of a combined analysis of the 358 eligible stage IV patients in these studies. They carried out a multivariate analysis using the Cox regression model with adjustment for well-known prognostic factors, and the Cox regression analysis demonstrated that treatment with IP was one of significant independent favorable factor. Based on their data, we selected IP for the reference arm in our study.

The Ministry of Health, Labour and Welfare of Japan approved the prescription of paclitaxel, gemcitabine, and

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vinorelbine for NSCLC in 1999 and requested a phase III study to confirm the efficacy and safety of these agents. The Japanese investigators and the pharmaceutical companies decided to conduct a four-arm randomized phase III study for NSCLC, the so-called FACS, Four-Arm Cooperative Study. The purpose of the study was to compare the efficacy and toxicity of three platinum-based combination regimens, carboplatin plus paclitaxel (TC), cisplatin plus gemcitabine (GP), cisplatin plus vinorelbine (NP), with IP as the reference arm.

## patients and methods

### patient selection

Patients with histologically and/or cytologically documented NSCLC were eligible for participation in the study. Each patient had to meet the following criteria: clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy, such as malignant pleural effusion, pleural dissemination, malignant pericardiac effusion, or metastatic lesion in the same lobe), at least one target lesion >2 cm, no prior chemotherapy, no prior surgery and/or radiotherapy for the primary site, age 20–74 years, Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, adequate hematological, hepatic and renal functions, partial pressure of arterial oxygen ( $\text{paO}_2$ )  $\geq 60$  torr, expected survival >3 months, able to undergo first course treatment in an inpatient setting, and written informed consent. The study was approved by the Institutional Review Board at each hospital. Written informed consent was obtained from every patient.

### treatment schedule

All patients were randomly assigned to one of the four treatment groups by the central registration office by means of the minimization method. Stage, PS, gender, lactate dehydrogenase (LDH) and albumin values, and institution were used as adjustment variables. The first group received the reference treatment, 80  $\text{mg/m}^2$  of cisplatin on day 1 and 60  $\text{mg/m}^2$  of irinotecan on days 1, 8, and 15, and the cycle was repeated every 4 weeks. The second group received 200  $\text{mg/m}^2$  of paclitaxel (Bristol-Myers K.K., Tokyo, Japan) over a 3-h period followed by carboplatin at a dose calculated to produce an area under the concentration–time curve of 6.0  $\text{min} \times \text{mg/mL}$  on day 1 and the cycle was repeated every 3 weeks. The third group received 80  $\text{mg/m}^2$  of cisplatin on day 1 and 1000  $\text{mg/m}^2$  of gemcitabine (Eli Lilly Japan K.K., Kobe, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. The fourth group received 80  $\text{mg/m}^2$  of cisplatin on day 1 and 25  $\text{mg/m}^2$  of vinorelbine (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. Each treatment was repeated for three or more cycles unless the patient met the criteria for progressive disease or experienced unacceptable toxicity.

### response and toxicity evaluation

Response was evaluated according to the Response Evaluation Criteria in Solid Tumors, and tumor markers were excluded from the criteria [21]. Objective tumor response in all responding patients was evaluated by an external review committee with no information on the treatment group. Toxicity grading criteria in National Cancer Institute Common Toxicity Criteria Ver 2.0 were used to evaluate toxicity.

### quality of life assessment

Quality of life (QoL) was evaluated by means of the Functional Assessment of Cancer Therapy—Lung (FACT-L) Japanese version and the QoL Questionnaire for Cancer Patients Treated with Anticancer Drugs (QoL-ACD), before treatment, immediately before the second cycles of chemotherapy, and 3 and 6 months after the start of treatment [22–24].

### statistical analysis and monitoring

The primary end point of this study was overall survival (OS), and the secondary end points were response rate, response duration, time to progressive disease (TTP), time to treatment failure (TTTF), adverse event, and QoL. The 1-year survival rate of the control group in this study was estimated to be 43% based on the data in published papers, and the 1-year survival rate in the other treatment group was expected to be 50%. The lower equivalence limit for 1-year survival rate was set as ‘–10%’. The criterion for the non-inferiority of each treatment was a lower limit of the two-sided 95% confidence interval (CI) of the 1-year survival rate of treatment minus that of control larger than the lower equivalence limit. Because the non-inferiority of each treatment versus the control was to be evaluated independently, a separate null hypothesis was stated for each treatment, and for that reason no multiple comparison adjustment was included in the study. Based on the above conditions and binomial distribution, 135 patients were needed per arm for a one-sided Type I error of 2.5% and 80.0% power. In view of the possibility of variance inflation due to censoring, the sample size was set at 600 (150 per arm).

Central registration with randomization, monitoring, data collection, and the statistical analyses were independently carried out by a contract research organization (EPS Co., Ltd, Tokyo, Japan).

## results

### patient characteristics

From October 2000 to June 2002, a total of 602 patients were registered by 44 hospitals in Japan. All patients had been followed up for >2 years, and 447 patients had died as of June 2004. Of the 602 patients registered, 151 were allocated to the reference treatment, IP, and 150, 151, and 150 patients were allocated to TC, GP, and NP, respectively. Since 10 patients did not receive chemotherapy and 11 patients were subsequently found to be ineligible, 592 patients were assessable for toxicity and 581 patients were assessable for efficacy. Four patients did not receive chemotherapy due to electrolytic disorder, fever, symptomatic brain metastases, and rapid tumor progression in IP, two patients due to refusal and pneumonia in TC, four patients due to lower WBC counts (two patients), rapid tumor progression, and nephritic syndrome in NP. Two patients were ineligible due to wrong stage in IP, two patients were wrong stage and one patient had double cancer in TC, two patients were wrong diagnosis, one patient had massive pleural effusion, one patient received prior chemotherapy in GP, one patient had no target lesions in NP. Age, gender, PS, stage, and LDH and albumin values were well balanced in each arm (Table 1). Fewer patients with adenocarcinoma and more patients with squamous cell carcinoma were, however, entered in three experimental arms than in IP.

### objective tumor response and response duration

Objective tumor response is shown in Table 2. Forty-five partial responses occurred in the 145 assessable patients in the reference arm, IP, for an objective response rate of 31.0% with a median response duration of 4.8 months. The response rate and median response duration were 32.4% and 4.0 months in TC, 30.1% and 3.5 months in GP, and 33.1% and 3.4 months in NP. The response rates in TC, GP, and NP were not statistically different from the rate in IP according to the results of the  $\chi^2$  test.

Table 1. Patient characteristics and treatment delivery

	Cisplatin + irinotecan	Carboplatin + paclitaxel	Cisplatin + gemcitabine	Cisplatin + vinorelbine
Assessable patients	145	145	146	145
Gender (male/female)	97/48	99/46	101/45	101/44
Age, median (range)	62 (30–74)	63 (33–74)	61 (34–74)	61 (28–74)
PS (0/1)	44/101	44/101	45/101	45/100
Histology				
Adenocarcinoma	121	104	108	109
Squamous cell carcinoma	16	31	29	29
Others	8	10	9	7
Stage (IIIB/IV)	31/114	28/117	30/116	26/119
No. of cycles				
Mean $\pm$ SD	3.0 $\pm$ 1.3	3.5 $\pm$ 1.5	3.2 $\pm$ 1.2	3.1 $\pm$ 1.3
Median	3	3	3	3
Range	1–7	1–10	1–7	1–8

PS, performance status; SD, standard deviation.

Table 2. Survival, TTP, TTF, response rate, and response duration

	N	Median survival, months	1-year survival (%)	Difference in 1-year survival from IP	2-year survival (%)	TTP (median) months	TTF (median) months	Response rate (%)	Response duration (median) months
Cisplatin + irinotecan	145	13.9	59.2	–	26.5	4.7	3.3	31.0	4.8 (n = 45)
Carboplatin + paclitaxel	145	12.3	51.0	–8.2% (95% CI –19.6% to 3.3%)	25.5	4.5 (P = 0.355) <sup>a</sup>	3.2 (P = 0.282) <sup>a</sup>	32.4 (P = 0.801) <sup>b</sup>	4.0 (n = 47)
Cisplatin + gemcitabine	146	14.0	59.6	0.4% (95% CI –10.9% to 11.7%)	31.5	4.0 (P = 0.170) <sup>a</sup>	3.2 (P = 0.567) <sup>a</sup>	30.1 (P = 0.868) <sup>b</sup>	3.5 (n = 44)
Cisplatin + vinorelbine	145	11.4	48.3	–10.9% (95% CI –22.3% to 0.5%)	21.4	4.1 (P = 0.133) <sup>a</sup>	3.0 (P = 0.091) <sup>a</sup>	33.1 (P = 0.706) <sup>b</sup>	3.4 (n = 48)

<sup>a</sup>Compared with IP by the generalized Wilcoxon test.

<sup>b</sup>Compared with IP by the  $\chi^2$  test.

CI, confidence interval; IP, cisplatin plus irinotecan; TTP, time to progressive disease; TTF, time to treatment failure.

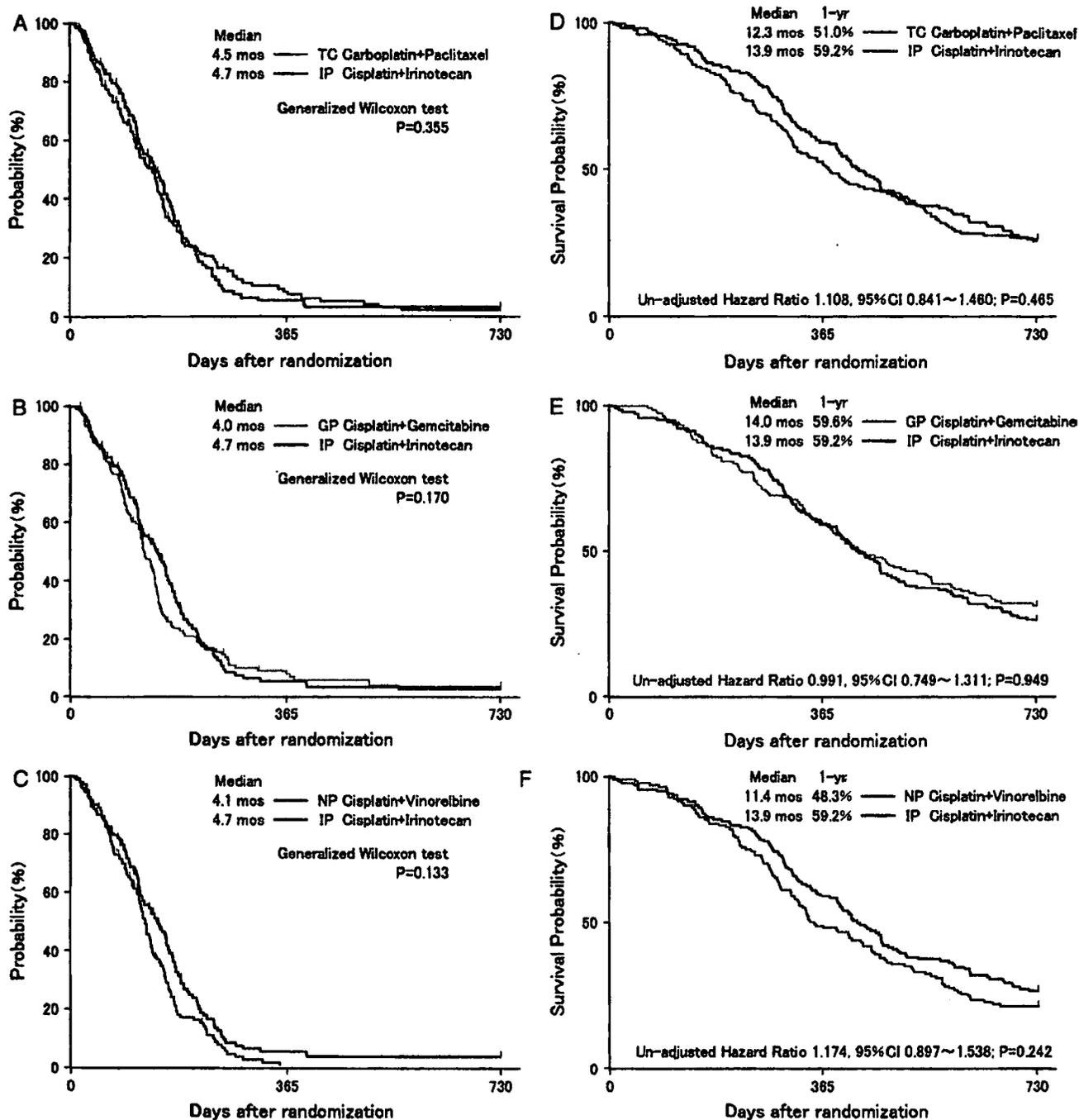
## OS, TTP disease, and TTF

OS and TTP are shown in Figure 1. Median survival time (MST), the 1-year, and 2-year survival rate in IP were 13.9 months, 59.2%, and 26.5%, respectively. The MSTs, 1-year, and 2-year survival rates were, respectively, 12.3 months, 51.0%, and 25.5% in TC; 14.0 months, 59.6%, and 31.5% in GP; and 11.4 months, 48.3%, and 21.4% in NP. The lower limits of the 95% CI of the difference in 1-year survival rate between IP and TC (–19.6%), GP (–10.9%), and NP (–22.3%) were below –10%, which was considered the lower equivalence limit (Table 2). Thus, the results did not show non-inferiority in three experimental regimens compared with reference treatment. Median TTP and median TTF were 4.7 and 3.3 months, respectively in IP. Median TTP and TTF were, respectively, 4.5 and 3.2 months in TC, 4.0 and 3.2 months in GP, and 4.1 and 3.0 months in NP. There were no statistical differences in either TTP or TTF in TC, GP, or NP, compared with IP according to the results of the generalized Wilcoxon test (Table 2).

## hematologic and non-hematologic toxicity

In IP, 47.6% and 83.7% of patients developed grade 3 or worse leukopenia and neutropenia, respectively (Table 3). The incidences of grade 3 or worse leukopenia (33.1%,  $P = 0.010$ ) and neutropenia (62.9%,  $P < 0.001$ ) were significantly lower in GP than in IP. The incidence of grade 3 or worse leukopenia (67.1%,  $P < 0.001$ ) was significantly higher in NP than in IP. Grade 3 or worse thrombocytopenia developed in 5.4% of the patients in IP, and the incidence was significantly higher in GP (35.1%,  $P < 0.001$ ). The incidence of febrile neutropenia in IP was 14.3%, and was significantly lower in GP (2.0%,  $P < 0.001$ ).

Grade 2 or worse nausea, vomiting, anorexia, and fatigue occurred in 60.5%, 51.0%, 65.3%, and 38.8%, respectively, of the patients in IP. The incidences of grade 2 or worse nausea (TC: 25.0%,  $P < 0.001$ , NP: 47.3%,  $P = 0.022$ ), vomiting (TC: 22.3%,  $P < 0.001$ , NP: 36.3%,  $P = 0.011$ ), and anorexia (TC: 32.4%,  $P < 0.001$ , NP: 49.3%,  $P = 0.005$ ) were significantly lower in TC and NP than in IP. Grade 2 or worse diarrhea was



**Figure 1.** Overall survival (OS) and time to progressive (TTP) disease. TTP and OS in the carboplatin plus paclitaxel (TC) (A, D), cisplatin plus gemcitabine (GP) (B, E), and cisplatin plus vinorelbine (NP) (C, F) were not statistically significantly different from the values in the cisplatin plus irinotecan.

significantly less frequent in TC (6.8%), GP (8.6%), and NP (11.6%) than in IP (48.3%,  $P < 0.001$ ). The incidences of grade 2 or worse sensory neuropathy (16.9%,  $P < 0.001$ ), arthralgia (21.6%,  $P < 0.001$ ), and myalgia (17.6%,  $P < 0.001$ ) were significantly higher in TC than in IP. Grade 2 alopecia occurred in 30.6% of the patients in IP, and its incidence was significantly higher in TC (44.6%,  $P = 0.013$ ) and significantly lower in GP (15.2%,  $P = 0.001$ ) and NP (8.9%,  $P < 0.001$ ). Grade 2 injection site reactions were more frequent in NP (26.7%) than in IP (4.8%,  $P < 0.001$ ).

A total of five patients died of treatment-related toxicity: three in IP (cerebral hemorrhage, interstitial pneumonia, acute circulatory failure/disseminated intravascular coagulation: 2.0%), one in TC (acute renal failure: 0.7%), and one in NP (pulmonary embolism: 0.7%).

#### second-line treatment

Data on second-line treatment, but not third-line or later treatment, was available in this study, and they showed that

Table 3. Toxicity

	IP (n = 147)			TC (n = 148)			GP (n = 151)			NP (n = 146)		
	Grade (%)			Grade (%)			Grade (%)			Grade (%)		
	2	3	4	2	3	4	2	3	4	2	3	4
Leukocytes	42	43	5	39	42	3	40	31 <sup>a</sup>	2 <sup>a</sup>	25	51 <sup>b</sup>	16 <sup>b</sup>
Neutrophils	11	39	45	5	19	69	21	40	23 <sup>a</sup>	5	16	72
Hemoglobin	42	24	7	42	13 <sup>a</sup>	2 <sup>a</sup>	44	22	5	43	25	5
Platelets	6	5	1	9	11	0	22	35 <sup>b</sup>	0 <sup>b</sup>	3	1 <sup>a</sup>	0 <sup>a</sup>
Febrile neutropenia	–	14	0	–	18	0	–	2 <sup>a</sup>	0 <sup>a</sup>	–	18	0
Nausea	32	29	–	14 <sup>c</sup>	11 <sup>c</sup>	–	35	23	–	33 <sup>c</sup>	14 <sup>c</sup>	–
Vomiting	38	13	0	17 <sup>c</sup>	5 <sup>c</sup>	0 <sup>c</sup>	34	14	0	29 <sup>c</sup>	7 <sup>c</sup>	0 <sup>c</sup>
Anorexia	30	33	2	15 <sup>c</sup>	17 <sup>c</sup>	1 <sup>c</sup>	31	26	1	29 <sup>c</sup>	20 <sup>c</sup>	1 <sup>c</sup>
Fatigue	27	12	1	26	2	1	17 <sup>c</sup>	3 <sup>c</sup>	0 <sup>c</sup>	23 <sup>c</sup>	3 <sup>c</sup>	0 <sup>c</sup>
Diarrhea	33	15	1	4 <sup>c</sup>	3 <sup>c</sup>	0 <sup>c</sup>	7 <sup>c</sup>	2 <sup>c</sup>	0 <sup>c</sup>	8 <sup>c</sup>	4 <sup>c</sup>	0 <sup>c</sup>
Constipation	27	7	0	30	8	0	33	9	0	40 <sup>d</sup>	14 <sup>d</sup>	0 <sup>d</sup>
Neuropathy, motor	1	0	0	1	1	1	0	0	0	0	0	0
Neuropathy, sensory	1	0	0	14 <sup>d</sup>	3 <sup>d</sup>	0 <sup>d</sup>	0	0	0	0	0	0
Alopecia	31	–	–	45 <sup>d</sup>	–	–	15 <sup>c</sup>	–	–	9 <sup>c</sup>	–	–
Arthralgia	2	0	0	20 <sup>d</sup>	2 <sup>d</sup>	0 <sup>d</sup>	0	0	0	1	0	0
Myalgia	1	0	0	16 <sup>d</sup>	2 <sup>d</sup>	0 <sup>d</sup>	0	0	0	1	1	0
Injection site reaction	5	0	–	5	0	–	5	0	–	27 <sup>d</sup>	0 <sup>d</sup>	–
Pneumonitis	0	1	1	0	1	0	0	0	0	0	1	0
Creatinine	8	1	0	2 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	7	0	0	8	1	0
AST	7	1	1	5	1	0	6	3	0	1	3	0
Fever	2	0	0	5	1	0	1	0	0	1	0	0
Treatment-related death	3 (2.0%)			1 (0.7%)			0			1 (0.7%)		

<sup>a</sup>Incidence of grade 3 or 4 toxicity significantly ( $P < 0.05$ ) lower than that with IP.

<sup>b</sup>Incidence of grade 3 or 4 toxicity significantly ( $P < 0.05$ ) higher than that with IP.

<sup>c</sup>Incidence of grade 2 or worse toxicity is significantly ( $P < 0.05$ ) lower than that with IP.

<sup>d</sup>Incidence of grade 2 or worse toxicity significantly ( $P < 0.05$ ) higher than that with IP.

GP, cisplatin plus gemcitabine; IP, cisplatin plus irinotecan; NP, cisplatin plus vinorelbine; TC, carboplatin plus paclitaxel.

AST, aspartate aminotransferase; –, no category in the criteria.

60%–74% of the patients received chemotherapy and 6%–9% received thoracic irradiation as second-line treatment (Table 4). The percentages of patients in each treatment group who received second-line chemotherapy were not significantly different ( $P = 0.081$ ).

### quality of life

The details of the QoL analysis will be reported elsewhere. No statistically significant difference in global QoL was observed among the four treatment groups based on either the FACT-L Japanese version or the QoL-ACD. Only the physical domain evaluated by QoL-ACD was significantly better in TC, GP, and NP than in IP.

### discussion

Many randomized phase III studies have compared platinum-plus-new-agent doublets in NSCLC, but, this is the first to evaluate the efficacy of an irinotecan-containing regimen in comparison with other platinum-plus-new-agent doublets in NSCLC [14–17]. Although non-platinum-containing chemotherapy regimens are used as alternatives, doublets of platinum and a new-generation anticancer agent, such as TC, GP, and NP, are considered standard chemotherapy regimens for advanced NSCLC worldwide [13–17, 25]. Although the non-

inferiority of none of the three experimental regimens could be confirmed in this study, no statistically significant differences in response rate, OS, TTP, or TTF were observed between the reference regimen and the experimental regimens. All four platinum-based doublets have similar efficacy against advanced NSCLC but different toxicity profiles. Nevertheless, IP was still regarded as the reference regimen in this study because the non-inferiority of none of the three experimental regimens could be confirmed.

OS in this study was relatively longer than previously reported. The estimated 1-year survival rate in the reference arm was 43%, but the actual 1-year survival rate was 59.2%, much higher than expected. The MSTs reported for patients treated with TC, GP, and NP in recent phase III studies have ranged from 8 to 10 months, and in the present study they were 12.3, 14.0, and 11.4 months, respectively [14–17]. One reason for the good OS in this study was the difference in patient selection criteria, for example exclusion of PS2 patients. Ethnic differences in pharmacogenomics have also been indicated as a possible reason for the good OS in this study [26]. The OS in IP in this study, however, was better than in previous Japanese studies [18, 19]. TTP in this study ranged from 4.0 to 4.7 months, and was similar to the TTP of 3.1–5.5 months reported in the literature [15, 16]. OS not TTP was longer in this study

Table 4. Second-line treatment

	Cisplatin + irinotecan	Carboplatin + paclitaxel	Cisplatin + gemcitabine	Cisplatin + vinorelbine	
Number of patients	145	145	146	145	
Chemotherapy	107 (74%)	87 (60%)	101 (69%)	95 (66%)	<i>P</i> = 0.081
Docetaxel	39	25	50	51	
Gefitinib	11	9	18	12	
Paclitaxel	15	14	7	11	
Gemcitabine	24	28	17	28	
Vinorelbine	9	12	2	9	
Irinotecan	15	4	3	3	
Thoracic irradiation	8	10	13	10	

than previously reported, and higher 2-year survival rates, 21.4%–31.5%, were observed in the minimum 2-year follow-up in this study. Second-line or later treatments may affect survival, because docetaxel has been established as standard second-line chemotherapy for advanced NSCLC [27, 28]. Gefitinib is also effective as second-line or later chemotherapy for advanced NSCLC, especially in Asian patients, never smokers and patients with adenocarcinoma [29–32].

The toxicity profile of each treatment differed and the toxicity of all four regimens was well tolerated. Overall QoL was similar in the four platinum-based doublets. Only physical domain QoL evaluated by the QoL-ACD was statistically better in TC, GP, and NP than in IP. This finding is presumably attributable to the fact that diarrhea is a statistically less frequent adverse effect of TC, GP, and NP than of IP.

In conclusion, all four platinum-based doublets had similar efficacy for advanced NSCLC but different toxicity profiles. All the four regimens can be used to treat advanced NSCLC patients in clinical practice.

## appendix

Institutions of the FACS Cooperative Group: National Hospital Organization (NHO) Hokkaido Cancer Center, Tohoku University Hospital, Yamagata Prefectural Central Hospital, Niigata Cancer Center Hospital, Tochigi Cancer Center, NHO Nishigunma National Hospital, Saitama Cancer Center, National Cancer Center Hospital East, Chiba University Hospital, National Cancer Center Hospital, Tokyo Medical University Hospital, Japanese Foundation for Cancer Research, Kanagawa Cancer Center, Yokohama Municipal Citizen's Hospital, Kanagawa Cardiovascular and Respiratory Center, Aichi Cancer Center Hospital, Prefectural Aichi Hospital, Nagoya City University Hospital, NHO Nagoya Medical Center, Nagoya University Hospital, Gifu Municipal Hospital, NHO Kyoto Medical Center, Osaka City General Hospital, Osaka City University Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, NHO Toneyama Hospital, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Kinki University School of Medicine, Rinku General Medical Center Izumisano Municipal Hospital, Kobe Central General Hospital, The Hospital of Hyogo College of Medicine, Hyogo Medical Center for Adults, Tokushima University Hospital, Kagawa Prefectural Central Hospital, NHO Shikoku Cancer Center Hospital, Hiroshima University Medical Hospital, NHO

Kyushu Cancer Center Hospital, Kyushu University Hospital, National Nagasaki Medical Center, Nagasaki Municipal Hospital, Nagasaki University Hospital of Medicine and Dentistry, Kumamoto Chuo Hospital, Kumamoto Regional Medical Center, NTT West Osaka Hospital.

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## Short Communication

## Randomised phase II trial of irinotecan plus cisplatin vs irinotecan, cisplatin plus etoposide repeated every 3 weeks in patients with extensive-disease small-cell lung cancer

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Patients with previously untreated extensive-disease small-cell lung cancer were treated with irinotecan 60 mg m<sup>-2</sup> on days 1 and 8 and cisplatin 60 mg m<sup>-2</sup> on day 1 with (n=55) or without (n=54) etoposide 50 mg m<sup>-2</sup> on days 1–3 with granulocyte colony-stimulating factor support repeated every 3 weeks for four cycles. The triplet regimen was too toxic to be considered for further studies.

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**Keywords:** small-cell lung cancer; chemotherapy; irinotecan; etoposide; three drug combination

Small-cell lung cancer (SCLC), which accounts for approximately 14% of all malignant pulmonary tumours, is an aggressive malignancy with a propensity for rapid growth and early widespread metastases (Jackman and Johnson, 2005). A combination of cisplatin and etoposide (PE) has been the standard treatment, with response rates ranging from 60 to 90% and median survival times (MSTs) from 8 to 11 months in patients with extensive disease (ED)-SCLC (Fukuoka *et al*, 1991; Roth *et al*, 1992). A combination of irinotecan and cisplatin (IP) showed a significant survival benefit over the PE regimen (MST: 12.8 vs 9.4 months,  $P=0.002$ ) in a Japanese phase III trial for ED-SCLC (Noda *et al*, 2002), although another phase III trial comparing these regimens failed to show such a benefit (Hanna *et al*, 2006). Thus, irinotecan, cisplatin and etoposide are the current key agents in the treatment of SCLC. A phase II trial of the three agents, IPE combination, in patients with ED-SCLC showed a promising antitumour activity with a response rate of 77%, complete response (CR) rate of 17% and MST of 12.9 months (Sekine *et al*, 2003).

We have developed these IP and IPE regimens in a 4-week schedule where irinotecan was given on days 1, 8 and 15. The dose of irinotecan on day 15, however, was frequently omitted because of toxicity in both regimens (Noda *et al*, 2002; Sekine *et al*, 2003).

The objectives of this study were to evaluate the toxicities and antitumour effects of IP and IPE regimens in the 3-week schedule in patients with ED-SCLC and to select the right arm for subsequent phase III trials.

## PATIENTS AND METHODS

## Patient selection

Patients were enrolled in this study if they met the following criteria: (1) a histological or cytological diagnosis of SCLC; (2) no prior treatment; (3) measurable disease; (4) ED, defined as having distant metastasis or contralateral hilar lymph node metastasis; (5) performance status of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale; (6) predicted life expectancy of 3 months or longer; (7) age between 20 and 70 years; (8) adequate organ function as documented by a white blood cell (WBC) count  $\geq 4.0 \times 10^3 \mu\text{l}^{-1}$ , neutrophil count  $\geq 2.0 \times 10^3 \mu\text{l}^{-1}$ , haemoglobin  $\geq 9.5 \text{ g dl}^{-1}$ , platelet count  $\geq 100 \times 10^3 \mu\text{l}^{-1}$ , total serum bilirubin  $\leq 1.5 \text{ mg dl}^{-1}$ , hepatic transaminases  $\leq 100 \text{ IU l}^{-1}$ , serum creatinine  $\leq 1.2 \text{ mg dl}^{-1}$ , creatinine clearance  $\geq 60 \text{ ml min}^{-1}$ , and  $\text{PaO}_2 \geq 60 \text{ torr}$ ; and (9) providing written informed consent.

Patients were not eligible for the study if they had any of the following: (1) uncontrollable pleural, pericardial effusion or ascites; (2) symptomatic brain metastasis; (3) active infection; (4) contraindications for the use of irinotecan, including diarrhoea, ileus, interstitial pneumonitis and lung fibrosis; (5) synchronous active malignancies; (6) serious concomitant medical

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illness, including severe heart disease, uncontrollable diabetes mellitus or hypertension; or (7) pregnancy or breast feeding.

### Treatment schedule

In the IP arm, cisplatin, 60 mg m<sup>-2</sup>, was administered intravenously over 60 min on day 1 and irinotecan, 60 mg m<sup>-2</sup>, was administered intravenously over 90 min on days 1 and 8. Prophylactic granulocyte colony-stimulating factor (G-CSF) was not administered in this arm. In the IPE arm, cisplatin and irinotecan were administered at the same dose and schedule as the IP arm. In addition, etoposide, 50 mg m<sup>-2</sup>, was administered intravenously over 60 min on days 1–3. Filgrastim 50 µg m<sup>-2</sup> or lenograstim 2 µg kg<sup>-1</sup> was subcutaneously injected prophylactically from day 5 to the day when the WBC count exceeded 10.0 × 10<sup>3</sup> µl<sup>-1</sup>. Hydration (2500 ml) and a 5HT<sub>3</sub> antagonist were given on day 1, followed by an additional infusion if indicated in both arms. These treatments were repeated every 3 weeks for a total of four cycles.

### Toxicity assessment, treatment modification and response evaluation

Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0.

Doses of anticancer agents in the following cycles were modified according to toxicity in the same manner in both arms. Objective tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse *et al*, 2000).

### Study design, data management and statistical considerations

This study was designed as a multi-institutional, prospective randomised phase II trial. This study was registered on 6 September 2005 in the University hospital Medical Information Network (UMIN) Clinical Trials Registry in Japan (<http://www.umin.ac.jp/ctr/index.htm>), which is acceptable to the International Committee of Medical Journal Editors (ICMJE) (<http://www.icmje.org/faq.pdf>). The protocol and consent form were approved by the Institutional Review Board of each institution. Patient registration and randomisation were conducted at the Registration Center. No stratification for randomisation was performed in this study. The sample size was calculated according to the selection design for pilot studies based on survival (Liu *et al*, 1993). Assuming that (1) the survival curve was exponential for survivals; (2) the MST of the worse arm was 12 months and that of the better arm was 12 months × 1.4; (3) the correct selection probability was 90%; and (4) additional follow-up in years after the end of accrual was 1 year, the estimated required number of patients was 51 for each arm. Accordingly, 55 patients for each arm and their accrual period of 24 months were planned for this study.

The dose intensity of each drug was calculated for each patient using the following formula as previously described:

$$\text{The dose intensity (mg m}^{-2} \text{ week}^{-1}) = \frac{\text{Total milligrams of a drug in all cycles per body surface area}}{\text{Total days of therapy}/7}$$

where total days of therapy is the number of days from day 1 of cycle 1 to day 1 of the last cycle plus 21 days for both arms (Hryniuk and Goodyear, 1990).

Differences in the reason for termination of the treatment and the frequencies of grade 3–4 toxicities were assessed by  $\chi^2$  tests. Survival was measured as the date of randomisation to the date of death from any cause or the date of the most recent follow-up for overall survival and to the date of disease progression or the date

of death for progression-free survival (PFS). The survival of the arms was estimated by the Kaplan–Meier method and compared in an exploratory manner with log-rank tests (Armitage *et al*, 2002).

## RESULTS

### Patient characteristics

From March 2003 to May 2005, 55 patients were randomised to IP and 55 patients to IPE. One patient in the IP arm was excluded because the patient was ineligible and did not receive the study treatment. The remaining 109 patients were included in the analyses of toxicity, tumour response and patient survival. There were no differences between the two arms in any demographic characteristics listed (Table 1).

### Treatment delivery

Treatment was well tolerated with respect to the number of cycles delivered in both arms (Table 2). Among reasons for termination of the treatment, disease progression was noted in nine (17%)

**Table 1** Patient characteristics

	IP (n = 54)	IPE (n = 55)
Sex		
Female	11	8
Male	43	47
Age (years)		
Median (range)	63 (42–70)	62 (48–70)
PS		
0	11	12
1	42	41
2	1	2
Weight loss		
0–1%	38	43
5–9%	10	10
≥ 10%	6	2

**Table 2** Treatment delivery

	IP (n = 54) No. (%)	IPE (n = 55) No. (%)
Number of cycles delivered		
6 <sup>a</sup>	—	1 (2)
4	41 (76)	36 (65)
3	6 (11)	6 (11)
2	3 (6)	6 (11)
1	4 (7)	6 (11)
Reasons for termination of the treatment <sup>b</sup>		
Completion	40 (74)	35 (64)
Disease progression	9 (17)	12 (22)
Toxicity	3 (6)	13 (24)
Patient refusal	2 (4)	4 (7)
Others	0 (0)	1 (2)
Total number of cycles delivered	192 (100)	186 (100)
Total number of omission on day 8	35 (18)	37 (17)
Total number of cycles with dose reduction	28 (15)	31 (17)

<sup>a</sup>P = 0.013 by  $\chi^2$  test. <sup>b</sup>Protocol violation.

patients in the IP arm and in two (4%) patients in the IPE arm, whereas toxicity was noted in three (6%) patients in the IP arm and 13 (24%) patients in the IPE arm ( $P = 0.013$ ) (Table 2). The dose of irinotecan on day 8 was omitted in 35 (18%) cycles in the IP arm and 37 (17%) cycles in the IPE arm (Table 2). The total dose and dose intensity of cisplatin and etoposide were similar between the IP and IPE arms in the present study (Table 3).

### Toxicity

The myelotoxicity was more severe in the IPE arm (Table 4). Grade 3 febrile neutropaenia was noted in 5 (9%) patients in the IP arm and 17 (31%) patients in the IPE arm ( $P = 0.005$ ). Packed red blood

cells were transfused in 4 (7%) patients in the IP regimen and 14 (26%) patients in the IPE regimen ( $P = 0.011$ ). Platelet concentrates were needed in none in the IP regimen and 2 (4%) patients in the IPE regimen ( $P = 0.16$ ). Grade 3–4 diarrhoea was observed in 8 (15%) patients in the IP arm and 13 (24%) patients in the IPE arm ( $P = 0.262$ ). Grade 3–4 fatigue was more common in the IPE arm with marginal significance (2 vs 11%,  $P = 0.054$ ). The severity of other non-haematological toxicities did not differ significantly between the arms. No treatment-related death was observed in this study.

### Response, treatment after recurrence and survival

Four CRs and 37 partial responses (PRs) were obtained in the IP arm, resulting in the overall response rate of 76 with 95% confidence interval (CI) of 65–87%, whereas six CRs and 42 PRs were obtained in the IPE arm, and the overall response rate was 87% with a 95% CI of 79–96% ( $P = 0.126$ ). Median PFS was 4.8 months (95% CI, 4.0–5.6) in the IP and 5.4 months (95% CI, 4.8–6.0) in the IPE arm ( $P = 0.049$ ) (Figure 1A). After recurrence, 22 (44%) patients in the IP arm and 8 (16%) patients in the IPE arm received etoposide-containing chemotherapy. The MST and 1-year survival rate were 12.4 months (95% CI, 9.7–15.1) and 54.8% (95% CI, 41.4–68.2%) in the IP and 13.7 months (95% CI, 11.9–15.5) and 61.5% (95% CI, 48.6–74.4%) in the IPE arm ( $P = 0.52$ ), respectively (Figure 1B).

**Table 3** Total dose and dose intensity

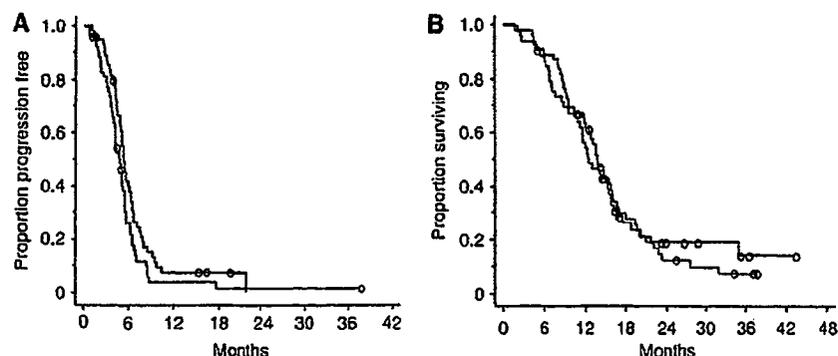
	3-week regimens in this study		4-week regimen <sup>a</sup>
	IP (n = 54) Median (range)	IPE (n = 55) Median (range)	IPE (n = 30) Median (range)
<b>Total dose (mg m<sup>-2</sup>)</b>			
Cisplatin	240 (60–240)	240 (60–360)	240 (60–240)
Irinotecan	420 (60–480)	390 (60–720)	563 (60–720)
Etoposide	0	600 (150–900)	600 (150–600)
<b>Dose intensity (mg m<sup>-2</sup> week<sup>-1</sup>)</b>			
Cisplatin	19 (14–25)	20 (16–34)	15 (12–15)
Irinotecan	33 (14–40)	35 (15–55)	35 (19–45)
Etoposide	0	48 (34–68)	37 (28–38)

<sup>a</sup>From our previous study (Sekine *et al.* 2003).

**Table 4** Grade 3–4 toxicities

	IP (n = 54)			IPE (n = 55)		
	Grade 3	4	3+4 (%)	Grade 3	4	3+4 (%)
Leukocytopenia	9	1	10 (19)	18	11	29 (53) <sup>a</sup>
Neutropaenia	17	11	28 (52)	24	28	52 (95) <sup>a</sup>
Anaemia	18	0	18 (25)	16	9	25 (45)
Thrombocytopenia	2	0	2 (4)	13	0	13 (13) <sup>f</sup>
Febrile neutropaenia	5	0	5 (9)	17	0	7 (13)
Diarrhoea	8	0	8 (15)	11	2	13 (24)
Vomiting	4	0	4 (7)	3	0	3 (5)
Fatigue	1	0	1 (2)	5	1	6 (11) <sup>f</sup>
Hyponatraemia	9	3	12 (22)	11	2	13 (24)
AST elevation	0	0	0 (0)	3	0	3 (5)
CRN elevation	1	0	1 (2)	0	0	0 (0)

<sup>a</sup> $P < 0.001$ ; <sup>f</sup> $P < 0.01$ ; and <sup>f</sup> $P = 0.054$  by  $\chi^2$  test.



**Figure 1** Progression-free survival (A) and overall survival (B). Thick line indicates the IPE regimen and thin line indicates the IP regimen.

for the PE regimen was 10.2 months and that for the IP regimen was 9.3 months (Hanna *et al*, 2006). The discrepancy between the Japanese and American trials may be explained by the different cisplatin dose schedules; cisplatin was delivered at a dose of  $60 \text{ mg m}^{-2}$  on day 1 every 3 or 4 weeks in the Japanese trials, whereas cisplatin was delivered at a dose of  $30 \text{ mg m}^{-2}$  on days 1 and 8 every 3 weeks in the American one. A platinum agent administered at divided doses was associated with poor survival in patients with ED-SCLC in our previous randomised phase II study (Sekine *et al*, 2003).

The issue of adding further agents to the standard doublet regimen has been investigated in patients with ED-SCLC. The addition of ifosfamide or cyclophosphamide and epirubicin to the cisplatin and etoposide combination produced a slight survival benefit, but at the expense of greater toxicity (Loehrer *et al*, 1995; Pujol *et al*, 2001). Phase III trials of cisplatin and etoposide with or without paclitaxel showed unacceptable toxicity with 6–13% toxic deaths in the paclitaxel-containing arm (Mavroudis *et al*, 2001; Niell *et al*, 2005). The results in these studies and the current study are consistent in the increased toxicity despite the G-CSF support and no definite survival benefit in the three or four drug combinations over the standard doublet in patients with ED-SCLC.

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In conclusion, the IPE regimen was marginally more effective than the IP regimen, but was too toxic despite the administration of prophylactic G-CSF.

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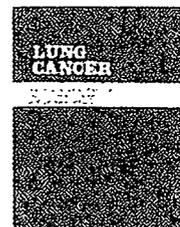


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## Randomized phase II trial of three intrapleural therapy regimens for the management of malignant pleural effusion in previously untreated non-small cell lung cancer: JCOG 9515

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### KEYWORDS

Non-small cell lung cancer;  
Malignant pleural effusion;  
Intrapleural therapy;  
Management of malignant pleural effusion;  
Bleomycin;  
OK-432;  
Cisplatin plus etoposide

**Summary** To evaluate the efficacy and toxicity of three intrapleural therapy regimens consisting of bleomycin (BLM), OK-432 (a pulverized product of heat-killed *Streptococcus pyogenes*) or cisplatin plus etoposide (PE) for the management of malignant pleural effusion (MPE) in previously untreated non-small cell lung cancer. Eligible patients were randomized to the BLM arm: BLM 1 mg/kg (maximum 60 mg/body), the OK-432 arm: OK-432 0.2 Klinische Einheit units (KE)/kg (maximum 10 KE/body), or the PE arm: cisplatin (80 mg/m<sup>2</sup>) and etoposide (80 mg/m<sup>2</sup>). Pleural response was evaluated every 4 weeks according to the study-specific criteria. All responders received systemic chemotherapy consisting of PE every 3–4 weeks for two or more courses. Pleural progression-free survival (PPFS) was defined as the time from randomization to the first observation of pleural progression or death due to any cause. The primary endpoint was the 4-week PPFS rate. Of 105 patients enrolled, 102 were assessed for response. The 4-week PPFS rate for the BLM arm was 68.6%, 75.8% for the OK-432 arm, and 70.6% for PE arm. Median survival time (MST) for the BLM arm was 32.1 weeks, 48.1 weeks for the OK-432 arm, and 45.7 weeks

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for the PE arm. However, the outcomes did not differ significantly between groups. Toxicity was tolerable in all arms except for one treatment-related death due to interstitial pneumonia induced by BLM. We will select intrapleural treatment using OK-432 in the management of MPE in NSCLC for further investigation because it had the highest 4-week PPFs rate.

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## 1. Introduction

Malignant pleural effusion (MPE) is a significant problem in the treatment of patients with advanced malignancies and is a major cause of poor prognosis [1]. The most widely used therapy for MPE is tube drainage with intrapleural instillation of sclerosing agents to prevent fluid reaccumulation [2].

Despite many reported trials of chemical pleurodesis, there has been no agreement as to the optimal treatment protocol for MPE [3–5]. The variety of response rates of individual agents among those studies has resulted from heterogeneous patient populations and differences in treatment procedures and response criteria [2,3,6]. To resolve these problems, we conducted a randomized phase II trial in which patient selection was limited to previously untreated patients with MPE due to non-small cell lung cancer (NSCLC) and, in view of adequate estimation of the efficacy of each intrapleural therapy regimen, single instillation of chemical agents and uncomplicated study-specific response criteria were applied. In this study, to select the most promising regimen for intrapleural therapy consisting of sclerosing or chemotherapeutic agents, we chose three regimens—BLM, OK-432 and cisplatin plus etoposide (PE). BLM was chosen because it is one of the most frequently used agents and is considered to have high efficacy, low toxicity and high availability [3,5,7,8]. OK-432 (a preparation of *Streptococcus pyogenes*, type A3, Chugai Pharmaceutical Co., Tokyo) has been used as an anti-tumor immunomodulator for lung cancer [9,10] and is reported to give superior responses for MPE compared to mitomycin C [11] and BLM [12]. At the beginning of this study, PE regimens were considered one of the standard combination chemotherapy regimens for NSCLC, and a phase II trial using this regimen for intrapleural therapy suggested potential survival benefit as well as local control effects [13].

## 2. Methods

### 2.1. Patient selection

The eligibility criteria were as follows: cytologically or histologically proven malignant pleural effusion associated with newly diagnosed NSCLC; no prior chemotherapy, thoracic radiotherapy or thoracic surgery; age of 75 years or less; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2 after tube thoracostomy; full lung reexpansion after tube thoracostomy; adequate bone marrow reserve (WBC count  $\geq 4000 \mu\text{L}^{-1}$ , hemoglobin  $\geq 9.5 \text{ g/dL}$ , and platelet count  $\geq 100,000 \mu\text{L}^{-1}$ ), and liver (total bilirubin  $\leq 1.5 \text{ mg/dL}$  and transaminase levels  $\leq$  twice the upper limit of the normal value) and renal (BUN  $\leq 25 \text{ mg/dL}$ , serum creatinine  $\leq 1.2 \text{ mg/dL}$ , and creatinine clearance  $\geq 50 \text{ mL/min}$ ) functions. All patients gave written, informed consent, and the protocol and the consent form were approved by the

Clinical Trial Review Committee of the Japan Clinical Oncology Group (JCOG) and by the institutional review boards of all participating institutions.

The exclusion criteria were bilateral pleural effusion or pericardial effusion, symptomatic brain metastases requiring whole-brain irradiation or administration of corticosteroids, an active synchronous cancer, interstitial pneumonitis, pulmonary fibrosis, uncontrolled angina pectoris or myocardial infarction within the preceding 3 months, uncontrolled diabetes mellitus or hypertension, pregnancy or breast-feeding, and penicillin allergy.

### 2.2. Treatment and monitoring

All patients were required to have either large-bore chest tubes or small-bore catheters placed, with radiographic evidence of reexpansion of the affected lung following suction or gravity drainage. Patients were stratified by institution and PS after tube drainage and then randomly assigned to the three treatment groups (Fig. 1). Intrapleural therapy was performed as follows. In the BLM and OK-432 arms, following instillation of either BLM (1 mg/kg, maximum 60 mg/body) or OK-432 (0.2 Klinische Einheit units (KE)/kg, maximum 10 KE/body), diluted in 100 ml of physiologic saline, the tube was clamped and the patient's position rotated for 3 h. Then the tube was unclamped and allowed to drain. In the PE arm, cisplatin (80 mg/m<sup>2</sup>) and etoposide (80 mg/m<sup>2</sup>) diluted in 100 ml of physiologic saline were simultaneously administered into the pleural cavity, the tube was clamped and the patient's position rotated for 3 h. Seventy-two hours later, the tube was unclamped and allowed to drain.

The tube was removed when the pleural effusion decreased to 100 ml or less per day. If more than 100 ml of drained fluid continued for 7 days or the pleural effusion increase by chest radiographs within 4 weeks, the patient was taken off the protocol and considered as a treatment failure.

### 2.3. Response criteria

The response criteria used were (i) response—disappearance or residual effusion with no need of local treatment (no greater than one quarter of the treated lung field nor remarkable increase compared to baseline chest radiographs) and (ii) pleural progression—a greater than one quarter of the treated lung field increase in pleural effusion compared to baseline chest radiographs.

### 2.4. Response evaluation and systemic chemotherapy

Pleural response was evaluated at the 4th, 8th, 12th and 24th week according to the study-specific criteria (see

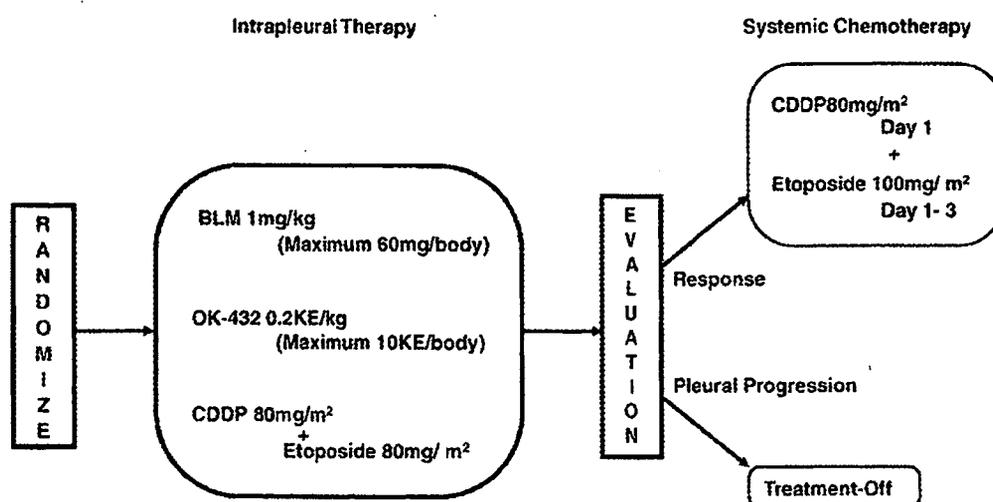


Fig. 1 Treatment schema.

above). A responder identified within 2 weeks after the first (4-week) evaluation received systemic chemotherapy consisting of cisplatin (80 mg/m<sup>2</sup>) on day 1 and etoposide (100 mg/m<sup>2</sup>) on days 1–3, which was repeated every 3–4 weeks for two or more courses.

## 2.5. Toxicity criteria and dose modification

Adverse reactions were graded according to the JCOG Toxicity Criteria [14], which are modifications of the National Cancer Institute's common toxicity criteria issued in 1991. The second or subsequent cycles of systemic chemotherapy were delayed if on day 1 the WBC count was less than 3000  $\mu\text{L}^{-1}$  or the platelet count was less than 75,000  $\mu\text{L}^{-1}$ . If grade 4 hematological toxicity occurred during the previous course, the dose of etoposide was reduced to 75%. Cisplatin was permanently discontinued at any time when the serum creatinine level was greater than 2.0 mg/dL. If the serum creatinine level was 1.5–2.0 mg/dL, the next cycle was delayed until it was 1.2 mg/dL or less, and the dose of cisplatin was then reduced to 75%.

## 2.6. Data management and statistical analysis

This study was designed as a multicenter randomized phase II trial among 21 participating centers in the Lung Cancer Study Group in the JCOG. Pleural progression-free survival (PPFS) was defined as the time from randomization to the first observation of pleural progression or death due to any cause. The primary endpoint of this study was 4-week PPFS rate. Assuming that the 4-week PPFS rate was at least 50% for these arms, the required number for each arm was 30 to select the better arm correctly with 90% probability if the better arm's 4-week PPFS rate was 70% or higher [15]. Planned accrual was set at 35 per arm. Secondary end-points were 8-, 12- and 24-week PPFS rates, overall survival (OS) and toxicity. The duration for OS was measured from the date of randomization to the date of death due to any cause or last follow-up. The mandated time to start treatment

following randomization was within a week. Survival distribution was estimated by the Kaplan–Meier method, and confidence intervals were based on Greenwood's formula [16].

Patient randomization and data management were performed by the JCOG Data Center (JCOG DC). In-house interim monitoring was performed by the JCOG Data and Safety Monitoring Committee semiannually. Central review of chest X-rays for all responses in all eligible cases was performed at regular study group meetings by an extramural panel. Statistical analysis was performed by the JCOG DC with SAS software version 6.12 for Windows (SAS Institute Inc., Cary NC).

## 3. Results

### 3.1. Patients

From May 1996 to August 1999, 105 patients were enrolled onto this study from the 21 participating institutions. The clinical characteristics of the patients are listed in Table 1. Three patients were later found to be ineligible (one patient per group): one had malignant pleural effusion secondary to colon cancer; one had no reexpansion of the affected lung after tube drainage; and one had poor renal function. Thus, 102 patients were assessable for response and survival. Four patients did not receive intrapleural therapy because of one self-removal of the drain, one obstruction of the drain, and two cases of intrapleural sclerosis. These four patients were excluded from the analysis of toxicity. The three treatment arms were well balanced for age, sex, and PS.

### 3.2. Treatment compliance and toxicity

Table 2 outlines the compliance with treatment. Fifty-one (50.0%) of the eligible patients completed intrapleural therapy and systemic chemotherapy as defined by the protocol. Forty-one (40.1%) of the eligible patients did not receive systemic chemotherapy because of disease progression. Two

**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) <sup>a</sup>			
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

<sup>a</sup> 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

**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

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.

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	5	0	0	ND	10	13	4	ND

Abbreviation: ND, not defined.

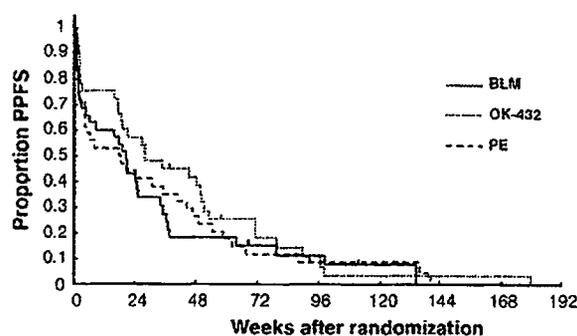


Fig. 2 Pleural progression-free survival (PPFS) in all eligible patients ( $n=102$ ).

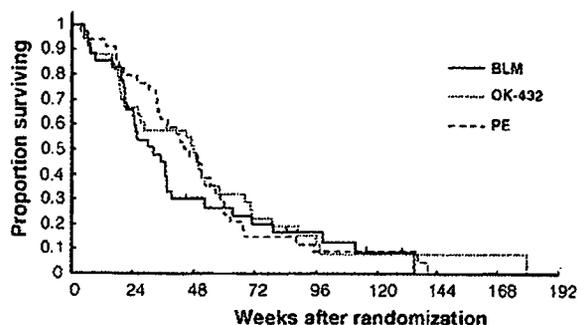


Fig. 3 Overall survival in all eligible patients ( $n=102$ ).

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- $\alpha$ , interferon- $\gamma$ , 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- $\gamma$  [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 ( $\beta$ -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 irinotecan, 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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