Foundation for the Promotion of Cancer Research and by 2nd Term Comprehensive 10-year Strategy for Cancer Control

REFERENCES

1. Martini N, Ginsberg R. Treatment of stage I and II disease, in Aisner J, Arriagada R, Green M, et al, eds. *Comprehensive Textbook of Thoracic Oncology*. Baltimore: Williams & Wilkins; 1996:339–350.
2. Tomizawa Y, Nakajima T, Kohno T, et al. Clinicopathological significance of Fhit protein expression in stage I non-small cell lung carcinoma. *Cancer Res.* 1998;58:5478–5483.
3. Ohta Y, Tomita Y, Oda M, et al. Tumor angiogenesis and recurrence in stage I non-small cell lung cancer. *Ann Thorac Surg.* 1999;68:1034–1038.
4. Herbst RS, Yano S, Kuniyasu H, et al. Differential expression of E-cadherin and type IV collagenase genes predicts outcome in patients with stage I non-small cell lung carcinoma. *Clin Cancer Res.* 2000;6: 790–797.
5. Khuri FR, Lotan R, Kemp BL, et al. Retinoic acid receptor-β as a prognostic indicator in stage I non-small-cell lung cancer. *J Clin Oncol.* 2000;18:2798–2804.
6. Hashimoto T, Tokuchi Y, Hayashi M, et al. p53 null mutations undetected by immunohistochemical staining predict a poor outcome with early-stage non-small cell lung carcinomas. *Cancer Res.* 1999;59:5572–5577.
7. Nelson HH, Christiani DC, Mark EJ, et al. Implications and prognostic value of K-ras mutation for early-stage lung cancer in women. *J Natl Cancer Inst.* 1999;91:2032–2038.
8. Betticher DC, Heighway J, Hasleton PS, et al. Prognostic significance of CCND (cyclin D1) overexpression in primary resected non-small-cell lung cancer. *Br J Cancer.* 1996;73:294–300.
9. Ito H, Oshita F, Kameda Y, et al. Expression of vascular endothelial growth factor and basic fibroblast growth factor in small adenocarcinoma. *Oncol Rep.* 2002;9:119–123.
10. Oshita F, Kameda Y, Ikehara M, et al. Increased expression of integrin β1 is a poor prognostic factor in small-cell lung cancer. *Anticancer Res.* 2002;22:1065–1070.
11. Ikehara M, Oshita F, Kameda Y, et al. Expression of survivin correlated with vessel invasion is a poor prognostic factor in small adenocarcinoma of the lung. *Oncol Rep.* 2002;9:835–838.
12. Van Zandwijk N, Smit EF, Krame GWP, et al. Gemcitabine and cisplatin as induction regimen for patients with biopsy-proven stage IIIA N2 non-small-cell lung cancer: A phase II study of the European Organization for Research and Treatment of Cancer Lung Cancer Cooperative Group (EORTC 08955). *J Clin Oncol.* 2000;18:2658–2664.
13. Oshita F, Nomura I, Yamada K, et al. Detection of K-ras mutation of bronchoalveolar lavage fluid cells aids the diagnosis of lung cancer in small pulmonary lesions. *Clin Cancer Res.* 1999;5:617–620.

TABLE 2. Multivariate Regression Analysis of Variables in Predicting Overall Survival

Variable	Assigned Score	Hazards Ratio	95% CI	*P* Value
Lymphatic invasion		0.488	0.094–2.549	0.400
Negative	0			
Positive	1			
Vessel invasion		0.493	0.098–2.473	0.390
Negative	0			
Positive	1			
Bronchioalveolar carcinoma		1.062	0.102–11.053	0.960
yes	0			
no	1			
Combination of gene expression		0.087	0.009–0.801	0.031
Positive 0–1	0			
Positive 2–4	1			

By multivariate analysis controlling for each gene expression, no gene expression was an independent marker of poor prognosis. When we examined whether the number of positive gene expression in the tumors influence the prognosis, the overall survival of complex gene expression (2 or more gene-positive) group (n = 35) was significantly worse than that of the 0 or 1 gene-positive group (n = 37; log-rank test, $P = 0.0011$; Wilcoxon test, $P = 0.0011$, Fig. 1 and Table 1). When the association between survival and pathologic factors, including lymphatic invasion, venous invasion, type of bronchioalveolar carcinoma, and complex gene expression was analyzed, only complex gene expression was found to be a significant independent factor (hazard ratio = 0.085, $P = 0.0299$, Table 2). It can be concluded that multiple but not single increased expression oncogene is a poor prognostic factor in patients with small adenocarcinoma of the lung.

DISCUSSION

Changes in gene expression are at the basis of many crucial physiological and pathologic processes. Tumorigenesis involves a loss of balance between regulators of cell proliferation and apoptosis. A previous study showed positive expression of survivin was a poor prognostic factor in small adenocarcinomas <2 cm in diameter.[11] However, the present study showed that not only survivin but also cyclin D1 and integrin β1 were poor prognostic factors. The present study demonstrated that 49%, 64%, 8%, and 22% of resected tumors <2 cm in diameter of pathologic stage I showed positive expression of survivin, cyclin D1, integrin β1, and VEGF, respectively. Only 9 patients (12.5%) had no expression of every 4 genes in resected small adenocarcinoma but many others had single or multiple gene expression in this study. This fact may explain that small adenocarcinoma <2 cm in diameter of pathologic stage I is in a transition from early to advanced stage. After all, multiple regression analysis demonstrated that no gene expression was an independent marker of poor prognosis, but complex gene expression show poor prognosis in small adenocarcinoma of the lung.

Lung cancer has a high potential of distant metastasis, and induction therapy followed by surgery and/or radiotherapy has become standard therapy for stage III disease.[12] Several gene expression analyzed in the present study is an important predictive factor for recurrence after curative resection in early stage lung cancer. The information obtained by this analysis is a powerful prognostic discriminator for patients with stage I disease and may be useful for decisions concerning which patients should and should not receive systemic treatment in addition to surgical resection. Furthermore, new strategies may be also considered with reference to multiple oncogene expression to improve treatment of locally advanced NSCLC. Targeted chemotherapy against positive expressed gene, such as using monoclonal antibodies, may be an ideal approach to treating multiple oncogene expressed tumors. When adjuvant chemotherapy after surgical resection is considered, not only single target therapy but also multitarget therapy such as combination of antiapoptotic, anticell cycle, antiadhesion and others, should be required, because multiple gene expressions in resected tumor is a poor prognostic factor presented in this study.

Lung cancer appears as small nodules in the peripheral part of the lung, and pathologic or cytologic diagnosis is essential. Patients suspected of having lung cancer often undergo fiberscopic examination, with a tumor biopsy examination or a cytologic approach. When a lesion is inaccessible to bronchoscopic biopsy, or when the biopsy specimen is nondiagnostic, a diagnosis of cancer may be possible by cytologic examination of bronchoalveolar lavage fluid (BALF). In a previous report, we demonstrated that detection of the K-*ras* mutation in BALF cells, by PCR-PIREMA, aids the diagnosis of lung cancer in patients with small pulmonary lesions with negative cytologic findings.[13] BALF from patients with small adenocarcinoma may contain survivin, cyclinD1, integrin β1, and VEGF, and it is possible that the gene expressions can be detected as a diagnostic marker.

In conclusion, multiple but not single oncogene expressions in tumor cells is a poor prognostic factor in patients with small adenocarcinoma of the lung. Detection of the gene expressions appears to be not only a useful diagnostic marker but also a potential new target for anticancer therapy for early stage NSCLC.

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TABLE 1. Characteristics of Gene Expression and Overall Survival*

			P Value	
		No. of Patients	Log-rank	Wilcoxon
Survivin	Negative	37	0.014	0.014
	Positive	35		
Cyclin D1	Negative	26	0.049	0.052
	Positive	46		
Integrin β1	Negative	66	0.021	0.01
	Positive	6		
VEGF	Negative	46	0.68	0.65
	Positive	26		
Combination	0–1	37	0.0011	0.0011
	2–4	35		

*Combination; survivin, cyclin D1, integrin β1, and VEGF were included.

Thirty-seven patients had tumor with positive expression of 0 or 1 gene and 35 patients had tumor with positive expression of 2 to 4 genes.

In an attempt to better understand tumor progression in NSCLC, expression of survivin, cyclin D1, integrin β1, and vascular endothelial growth factor (VEGF), which have different mechanisms in tumor progression, were investigated prognosis in adenocarcinoma <2 cm in diameter of stage I in the present study.

PATIENTS AND METHODS

Patients with lung adenocarcinoma <2 cm in diameter of pathologic stage I, resected between January 1992 and December 1999, were enrolled in the present study.

The tumor specimens obtained by resection were subjected to immunostaining for survivin, cyclin D1, integrin β1, and VEGF. Formalin-fixed, paraffin-embedded, 5-im-thick tumor sections were mounted on charged glass slides, deparaffinized and rehydrated in a graded alcohol series. Immunohistochemical staining was performed using an automated processor. Details of immunostaining were shown in previous reports.[8–11] Each factors immunostaining levels were classified as positive (>10% of cells stained for survivin, integrin β1, and VEGF, and >20% of cells stained for cyclin D1) or negative (≤10% of cells stained for survivin, integrin β1, and VEGF, and ≤20% of cells stained for cyclin D1).

Two pathologists examined the staining patterns of each factor independently, and recorded the percentage of positive cells in each specimen. At least 20 high-power fields were chosen randomly and 2000 cells were counted. The ratio of each gene-positive cell was calculated by dividing the number of positive cells by the total number of cells, and was expressed as a percentage.

Kaplan-Meier survival curves were constructed and analyzed for statistical significance by means of the log-rank and generalized Wilcoxon tests. The influence of each variable on survival was examined by the Cox proportional hazards model in multivariate regression analyses. Differences at $P < 0.05$ were considered to be statistically significant.

RESULTS

Seventy-two patients with resected tumors <2 cm in diameter of pathologic stage I were entered into the study. There were 29 males and 43 females, with a median age of 64 years (range 26–83 years). Each patient underwent curative surgical resection for lung cancer between July 1992 and November 1999. The resected tumors were subjected to immunostaining for each gene. Thirty-five, 26, 6, and 16 patients had tumors with >10% survivin-, >20% cyclin D1-, >10% integrin β1-, and >10% VEGF-positive cells, respectively.

When the survival of 72 patients was compared according to each gene expression, the overall survival of patients with positive expression of survivin, cyclin D1, and integrin β1 was significantly worse than that of individuals whose tumors had negative expression of each gene (Table 1). We analyzed how many of the 4 genes expressed positively in each resected tumor, 9, 28, 24, and 11 patients had tumors with positive expression of 0, 1, 2, and 3 genes, respectively. There were no patients with tumor expressed every 4 genes.

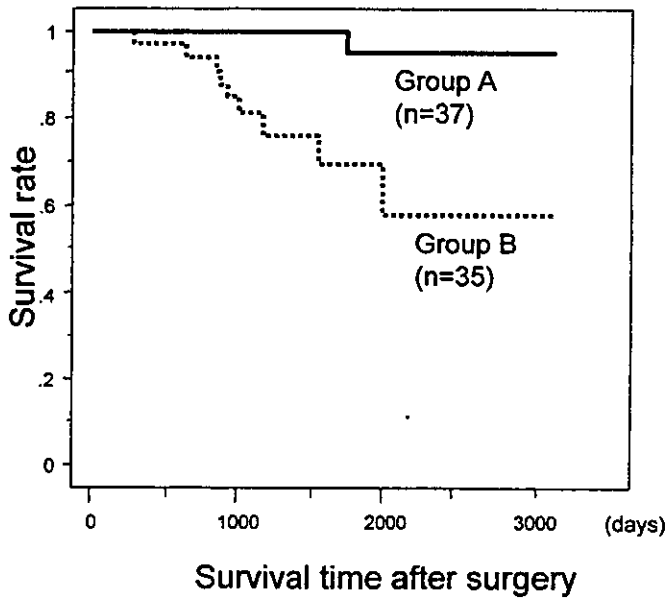

FIGURE 1. Survival curves according to gene immunostaining, constructed using the Kaplan-Meier method. Survival after surgery of patients with 2 or more positive expression of genes in tumor was worse than that of those with 0 or 1-positive expression of gene in tumor (log-rank $P = 0.0011$, Wilcoxon $P = 0.0011$).

13. Obara N, Imagawa S, Nakano Y et al. Hematological aspects of a novel 9-aminoanthracycline, amrubicin. Cancer Sci 2003; 94: 1104–1106.
14. Noda T, Watanabe T, Kohda A et al. Chronic effect of a novel synthetic anthracycline derivative (SM-5887) on normal heart and doxorubicin-induced cardiomyopathy in beagle dogs. Invest New Drugs 1998; 16: 121–128.
15. Yana T, Negoro S, Takada Y et al. Phase II study of amrubicin (SM-5887), a 9-amino-anthracycline, in previously untreated patients with extensive stage small-cell lung cancer (ES-SCLC): a West Japan Lung Cancer Group trial. Proc Am Soc Clin Oncol 1998; 17: 450a.
16. Stahel RA, Ginsberg R, Havemann K et al. Staging and prognostic factors in small cell lung cancer: a consensus report. Lung Cancer 1989; 5: 119–126.
17. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 200; 92: 205–216.
18. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53: 457–481.
19. Grant SC, Gralla RJ, Kris MG et al. Single-agent chemotherapy trials in small-cell lung cancer, 1970 to 1990: The case for studies in previously treated patients. J Clin Oncol 1992; 10: 484–494.
20. Blackstein M, Eisenhauer EA, Weirzbicki R et al. Epirubicin in extensive small-cell lung cancer: A phase II study in previously untreated patients: A National Cancer Institute of Canada Clinical Trials Group study. J Clin Oncol 1990; 8: 385–389.
21. Eckhardt S, Kolaric K, Vukas D et al. Phase II study of 4′-epi-doxorubicin in patients with untreated, extensive small cell lung cancer. Med Oncol Tumor Pharmacother 1990; 7: 19–23.
22. Masuda N, Matsui K, Negoro S et al. Combination of irinotecan and etoposide for treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 1998; 16: 3329–3334.
23. Ohe Y, Saijo N. Results of recent Japanese clinical trials in lung cancer. Clin Lung Cancer 2002; 3: 243–248.

assess the efficacy and safety of the drugs delivered at their RD in chemonaive ED-SCLC, and to examine pharmacokinetics.

The topoisomerase I inhibitor, irinotecan, is also very effective for SCLC [6]. Combinations of topoisomerase I and topoisomerase II inhibitors, such as irinotecan plus etoposide, have been reported as active combination chemotherapy for SCLC [22]. Thus, combination of irinotecan and amrubicin is another candidate for new combination chemotherapy for SCLC. A phase I study of irinotecan and amrubicin for chemonaive non-SCLC was performed in National Cancer Center Hospital (unpublished data). However, the MTD was less than irinotecan 60 mg/m^2 on days 1 and 8 and amrubicin 35 mg/m^2 on days 2–4, due to relatively severe myelotoxicity. We considered that amrubicin <35 mg/m^2 on days 2–4 with irinotecan 60 mg/m^2 on days 1 and 8 was insufficient to treat SCLC.

In this study, we determined the RD to be amrubicin 40 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1 every 3 weeks, and 41 patients were treated at the RD. Main toxicities of this combination chemotherapy were myelosuppression, especially leukopenia and neutropenia, and gastrointestinal toxicities including anorexia, nausea, vomiting, constipation, diarrhea, stomatitis and gastric ulcer. Of 41 patients, 32 (78%) patients received four or more courses of chemotherapy, and 22 (54%) patients completed four courses of chemotherapy without dose modification. One patient developed myocardial infarction; however, other cardiac toxicity, including decrease in left ventricle ejection fraction, was not observed in up to six courses of chemotherapy. The total dose of amrubicin was 720 mg/m^2. Grade 3 or 4 hyponatremia occurred in nine (22%) patients; however, most of the patients were asymptomatic. No unexpected toxicities and no treatment-related deaths were observed in this study. Toxicities observed in this study were manageable.

Four CRs and 32 PRs occurred, for an objective response rate of 87.8% (95% CI 73.8% to 95.9%) in 41 patients treated at the RD. In most patients, ProGRP levels changed in parallel with tumor responses. The MST of the 41 patients was 13.6 months, and the 1-year survival rate was 56.1%. These results were better than recently reported results for irinotecan and cisplatin in chemonaive ED-SCLC: an objective response rate of 84% and MST of 12.8 months [6]. The combination of amrubicin and cisplatin has demonstrated an impressive response rate and MST in patients with previously untreated ED-SCLC. A possible reason for the better results is overselection of patients, because we used unusual exclusion criteria such as non-steroidal anti-inflammatory drug or adrenal cortical steroid use for >50 days, and gastric and/or duodenal ulcer. However, in a phase II study, this kind of bias is not uncommon.

Combination chemotherapy with etoposide plus cisplatin or etoposide plus cisplatin, alternating with cyclophosphamide, doxorubicin and vincristine, had been considered as standard chemotherapy for SCLC in North America and Japan. A Japanese phase III trial (JCOG 9511) demonstrated that treatment with four cycles of irinotecan plus cisplatin every 4 weeks yielded a highly significant improvement in survival in ED-SCLC patients over standard etoposide plus cisplatin, with less myelosuppression [6]. Based on the results of the JCOG 9511 trial, irinotecan plus cisplatin is considered to be the reference chemotherapy arm for ED-SCLC in future trials in Japan [23]. The JCOG are preparing a phase III clinical trial of amrubicin and cisplatin for previously untreated ED-SCLC to compare combination therapy of irinotecan with cisplatin.

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References

1. Turrisi A, Kim K, Blum R et al. Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. N Engl J Med 1999; 340: 265–271.
2. Armitage JO. Bone marrow transplantation. N Engl J Med 1994; 330: 827–838.
3. Furuse K, Fukuoka M, Nishiwaki Y et al. Phase III study of intensive weekly chemotherapy with recombinant human granulocyte colony-stimulating factor versus standard chemotherapy in extensive-disease small-cell lung cancer. J Clin Oncol 1998; 16: 2126–2132.
4. Murray N, Livingston RB, Shepherd FA et al. Randomized study of CODE versus alternating CAV/EP for extensive-stage small-cell lung cancer: an Intergroup Study of the National Cancer Institute of Canada Clinical Trials Group and the Southwest Oncology Group. J Clin Oncol 1999; 17: 2300–2308.
5. Fukuoka M, Furuse K, Saijo N et al. Randomized trial of cyclophosphamide, doxorubicin, and vincristine versus cisplatin and etoposide versus alternation of these regimens in small-cell lung cancer. J Natl Cancer Inst 1991; 83: 855–861.
6. Noda K, Nishiwaki Y, Kawahara M et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 2002; 346: 85–91.
7. Ohe Y, Nakagawa K, Fujiwara Y et al. In vitro evaluation of the new anticancer agents KT6149, MX-2, SM5887, menogaril, and liblomycin using cisplatin- or adriamycin-resistant human cancer cell lines. Cancer Res 1949; 49: 4098–4102.
8. Noguchi T, Ichii S, Morisada S et al. In vivo efficacy and tumor-selective metabolism of amrubicin to its active metabolite. Jpn J Cancer Res 1998; 89: 1055–1060.
9. Noguchi T, Ichii S, Morisada S et al. Tumor-selective distribution of an active metabolite of the 9-aminoanthracycline amrubicin. Jpn J Cancer Res 1998; 89: 1061–1066.
10. Yamaoka T, Hanada M, Ichii S et al. Cytotoxicity of amrubicin, a novel 9-aminoanthracycline, and its active metabolite amrubicinol on human tumor cells. Jpn J Cancer Res 1998; 89: 1067–1073.
11. Hanada M, Mizuno S, Fukushima A et al. A new antitumor agent amrubicin induces cell growth inhibition by stabilizing topoisomerase II-DNA complex. Jpn J Cancer Res 1998; 89: 1229–1238.
12. Yamaoka T, Hanada M, Ichii S et al. Uptake and intracellular distribution of amrubicin, a novel 9-amino-anthracycline, and its active metabolite amrubicinol in P388 murine leukemia cells. Jpn J Cancer Res 1999; 90: 685–690.

Table 6. Response rates

	n	CR	PR	SD	PD	NE	Response rate (%) (95% CI)
All	44	4	35	3	0	2	88.6 (75.4–96.2)
Treated at RD	41	4	32	3	0	2	87.8 (73.8–95.9)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; 95% CI, 95% confidence interval; RD, recommended dose.

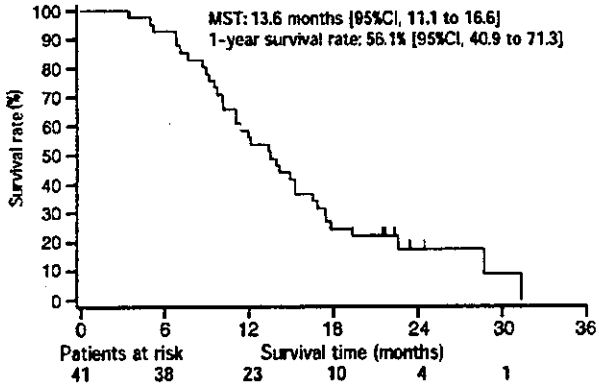

Figure 1. Overall survival of patients with extensive-stage small-cell lung cancer who were treated with amrubicin and cisplatin at the recommended dose. MST, median survival time; 95% CI, 95% confidence interval.

Toxicity in patients treated at the RD

The worst grades of hematological and non-hematological toxicities experienced by each patient are listed in Table 7. Hematological toxicity, especially leukopenia and neutropenia, was common and relatively severe. Grade 3 or worse leukopenia and neutropenia occurred in 65.9% and 95.1% of patients, respectively. Febrile neutropenia was observed in two patients at level 2. Grade 3 or worse anemia and thrombocytopenia occurred in 53.7% and 24.4% of patients, respectively. Four patients received platelet transfusions. Common non-hematological toxicities were gastrointestinal toxicity, such as anorexia, nausea, vomiting, constipation, diarrhea and stomatitis. Gastric ulcers developed in three patients. Hepatic and renal toxicity were not common in this study. Grade 3 or worse hyponatremia and hypokalemia occurred in 22% and 9.8% of patients, respectively. One patient developed myocardial infarction; however, cardiac toxicity was not common. No treatment-related deaths were observed.

Discussion

Doxorubicin and epirubicin are classified as active agents for SCLC, for which single-agent activity is a >20% response rate [19]. Doxorubicin has been used as a constituent of combination therapy for SCLC in the CAV (cyclophospamide, doxorubicin and vincristine) and CAP (cyclophosphamide, doxorubicin and cisplatin) regimens. Epirubicin has shown

Table 7. Toxicity in patients treated at the recommended dose ($n=41$)

	Grade (NCI CTC)					Grade 3/4 (%)
	0	1	2	3	4	
Leukopenia	1	0	13	20	7	65.9
Neutropenia	0	1	1	7	32	95.1
Febrile neutropenia	41	–	–	0	0	0.0
Hemoglobin	1	8	10	17	5	53.7
Thrombocytopenia	9	14	8	10	0	24.4
Stomatitis	22	13	5	1	0	2.4
Anorexia	1	14	13	13	0	31.7
Nausea	3	15	14	9	0	22.0
Vomiting	20	8	11	2	0	4.9
Constipation	24	1	13	3	0	7.3
Diarrhea	26	12	1	2	0	4.9
Gastric ulcer	38	0	1	2	0	4.9
Bilirubin	24	12	4	1	0	2.4
Hyponatremia	18	14	–	7	2	22.0
Hypokalemia	31	6	–	4	0	9.8
Hyperkalemia	33	3	4	1	0	2.4
Hypocalcemia	31	5	4	0	1	2.4

NCI CTC, National Cancer Institute Common Toxicity Criteria.

50% and 48% response rates in two clinical studies in 41 and 80 previously untreated patients, respectively, with ED-SCLC [20, 21]. However, currently, combination modalities containing doxorubicin or epirubicin are not being used in the therapy of SCLC, in preference to combination therapy with cisplatin and etoposide. Since amrubicin has shown excellent single-agent activity [15], it can be expected to be superior to other anthracyclines in the treatment of SCLC. Additionally, the present results of combination therapy with cisplatin support the view that amrubicin may be a promising agent that overcomes the therapeutic plateau of SCLC.

Amrubicin is one of the most promising new agents for the treatment of SCLC. In a previous phase II study of amrubicin 45 mg/m^2 on days 1–3 every 3 weeks as a monotherapy for chemonaive ED-SCLC, a 76% overall response rate and 11.7 month MST were observed [15]. The overall response rate and MST were comparable to those achieved with standard combination chemotherapy, such as etoposide plus cisplatin [5, 6]. Moreover, only a few patients treated in the phase II study received salvage chemotherapy consisting of cisplatin and etoposide [15]. The major toxicity of amrubicin as a monotherapy was hematological toxicity: grade 4 leukopenia and neutropenia were seen in 12.1% and 39.4% of patients, respectively, and thrombocytopenia and anemia of grade 3 or worse in 21.2%. Hepatic, renal and cardiac toxicities with amrubicin were not common. Cisplatin is a key drug for the treatment of SCLC and its hematological toxicity, such as leukopenia and neutropenia, is not severe. Thus, we conducted a phase I–II study of amrubicin and cisplatin treatment for chemonaive ED-SCLC to determine the MTD of this combination therapy, to

Table 2. Toxicities during the first course in the phase I study

	Level 1 ($n=4$)					Level 2 ($n=3$)				
Amrubicin	40 mg/m^2 days 1–3					45 mg/m^2 days 1–3				
Cisplatin	60 mg/m^2 day 1					60 mg/m^2 day 1				
	Grade (NCI CTC)					Grade (NCI CTC)				
	0	1	2	3	4	0	1	2	3	4
Leukopenia	0	1	1	2	0	0	0	1	1	1
Neutropenia	0	0	0	2	2	0	0	0	0	3
Febrile neutropenia	4	–	–	0	0	1	–	–	2	0
Hemoglobin	1	1	2	0	0	2	1	0	0	0
Thrombocytopenia	1	2	0	1	0	0	2	0	1	0
Stomatitis	3	0	1	0	0	3	0	0	0	0
Nausea	1	1	2	0	–	1	1	0	1	–
Constipation	3	0	1	0	0	1	0	1	1	0
Hyponatremia	2	1	0	0	1	1	2	0	0	0
Hypocalcemia	3	0	1	0	0	3	0	0	0	0

Dose limiting toxicity at level 2: febrile neutropenia, two patients; grade 4 neutropenia ≥4 days, one patient; grade 3 constipation, one patient.
NCI CTC, National Cancer Institute Common Toxicity Criteria.

Table 3. Pharmacokinetics of amrubicin in plasma

Dose	n	Day	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	V_d (l)	CL (l/h)	AUC_{0-24h} (ng h/ml)
40 mg/m^2	4	1	0.11 ± 0.04	2.29 ± 0.31	46.6 ± 11.0	13.6 ± 1.8	2995 ± 434
	4	3	0.08 ± 0.01	2.89 ± 0.34	50.0 ± 10.6	11.6 ± 1.9	3511 ± 514
45 mg/m^2	3	1	0.13 ± 0.05	2.39 ± 0.34	56.3 ± 10.6	14.9 ± 1.8	3052 ± 402
	3	3	0.09 ± 0.03	2.27 ± 0.18	51.9 ± 3.7	14.2 ± 2.3	3217 ± 479

$T_{1/2\alpha}$, half-life at distribution phase; $T_{1/2\beta}$, half-life at elimination phase; V_d, volume of distribution; CL, clearance; AUC, area under the concentration–time curve.

courses of chemotherapy, and 10 (31%) of these 32 patients needed dose reduction of amrubicin at the fourth course (Table 5). Of 41 patients, 22 (54%) patients completed four courses of chemotherapy without dose modification. The main cause of dose reduction was myelosuppression, especially leukopenia and neutropenia.

Objective tumor response and overall survival

The objective tumor responses are given in Table 6. Four CRs and 32 PRs occurred, for an objective response rate of 87.8% [95% confidence interval (CI) 73.8% to 95.9%] in 41 patients treated at the RD. The objective response rate for all 44 patients was 88.6% (95% CI 75.4% to 96.2%). The overall survival times of the 41 patients treated at the RD are shown in Figure 1. The MST of the 41 patients was 13.6 months (95% CI 11.1–16.6), with a median follow-up time for eight censored patients of 16.4 months (95% CI 14.2–18.8). The 1- and 2-year survival rates were 56.1% and 17.6%, respectively. The MST of all 44 patients was 13.8 months (95% CI 11.1–16.6). The 1- and 2-year survival rates of all 44 patients were 56.8% and 21.4%, respectively.

Table 4. Pharmacokinetics of amrubicinol in red blood cells

Dose	n	Day	$T_{1/2}$ (h)	AUC_{0-24h} (ng·h/ml)
40 mg/m^2	4	1	21.0 ± 3.1	1412 ± 314
	4	3	20.7 ± 4.8	2159 ± 622
45 mg/m^2	3	1	19.6 ± 6.1	1098 ± 277
	3	3	18.1 ± 5.7	2027 ± 332

$T_{1/2}$, elimination half-life; AUC, area under the concentration–time curve.

Table 5. Treatment received in patients treated at the recommended dose

Cycle	n	Amrubicin (mg/m^2)			Cisplatin (mg/m^2)	
		40	35	30	60	45
1	41	41			41	
2	36	30	6		36	
3	33	26	5	2	33	
4	32	22	8	2	32	
5	18	9	5	4	18	
6	13	6	3	4	12	1

and/or unequivocal progression of existing non-target lesions and/or appearance of new lesions. The evaluation of objective tumor response for all patients was performed by an external review committee.

Toxicity grading criteria of the National Cancer Institute Common Toxicity Criteria (version 2.0) was used for evaluation of toxicity.

Statistical analysis

This study was designed to reject response rates of 70% (P0) at a significance level of 0.05 (one-tailed) with a statistical power of 80% to assess the activity of the regimen as a 85% response rate (P1) at the recommended dose. The upper limit of rejection was 29 responses (CR+PR) among 37 evaluable patients. Overall survival was defined as the interval between the first administration of the drugs in this study and death or the last follow-up visit. Median overall survival was estimated using the Kaplan–Meier method [18].

Table 1. Characteristics of treated patients

	Phase I	Phase II	Total
Number of patients	7	37	44
Gender			
Male	5	31	36
Female	2	6	8
Age (years)			
Median	65	64	64.5
Range	54–73	50–74	50–74
ECOG PS			
0	0	5	5
1	7	32	39
2	0	0	0
Stage			
IIIB	0	2	2
IV	7	35	42
Prior therapy			
Yes	0	1	1
No	7	36	43
Serum ALP			
Normal	7	29	36
Elevated	0	7	7
Serum LDH			
Normal	3	14	17
Elevated	4	23	27
Na			
Normal	6	35	41
Decreased	1	2	3
Number of metastases			
0	0	2	2
1	4	27	31
2	3	6	9
3	0	1	1
4 or more	0	1	1

In one patient, serum ALP level could not be measured.
ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

Pharmacokinetic analysis

Pharmocokinetic analysis was performed in patients entering the phase I section of this study. One milliliter of the blood was taken from the patients before administration of amrubicin, and at 0 min, 15 min, 1, 2, 3, 4, 8 and 24 h after administration on days 1 and 3 in the first course of chemotherapy. Concentrations of amrubicin and its active metabolite, amrubicinol, in plasma and red blood cells were measured as reported elsewhere [9].

Results

Patient characteristics

Between April 2001 and December 2002, 45 patients with ED-SCLC were enrolled and 44 were treated in this study (Table 1). One patient did not receive the protocol treatment because atrial fibrillation was observed just before administration on day 1 of the first course. All treated patients were assessed for response, survival and toxicity. The median age of the treated patients was 64.5 years (range 50–74). There were 36 males and eight females. Five patients had an ECOG PS 0 and 39 patients had PS 1. Only one patient received surgery for brain metastasis as a prior therapy.

MTD and DLT in the phase I study

Four patients were enrolled at dose level 1 (amrubicin 40 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1) and three patients at level 2 (amrubicin 45 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1). Toxicities in the phase I study are listed in Table 2. No DLT were observed during the first course of level 1. At level 2, grade 4 neutropenia for ≥4 days and febrile neutropenia occurred in one patient, and febrile neutropenia and grade 3 constipation occurred in another patient. Consequently, the MTD and RD were determined to be level 2 and level 1, respectively.

Pharmacokinetics of amrubicin and its active metabolite, amrubicinol

Pharmacokinetic parameters of amrubicin in plasma were almost identical on days 1 and 3 at the two dose levels (Table 3). No clear dose relationship in the area under the concentration–time curve (AUC) of amrubicin in the plasma was observed. The AUC of amrubicinol in red blood cells tended to increase on day 3 at both doses (Table 4). No clear dose relationship in the AUC of amrubicinol in red blood cells was observed. Combination with cisplatin did not alter the pharmacokinetics of amrubicin and amrubicinol (data not shown).

Treatment received in patients treated at the RD

Forty-one patients were treated at the RD: amrubicin 40 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1. Of 41 patients, 32 (78%) patients received more than three

xenografts. Amrubicin and its 13-hydroxy metabolite, amrubicinol, inhibit purified human DNA topoisomerase II [11]. Amrubicinol is 10–100 times more cytotoxic than amrubicin [9]. The potent therapeutic activity of amrubicin is caused by the selective distribution of its highly active metabolite, amrubicinol, in tumors [9]. In an experimental animal model, amrubicin did not exhibit any chronic cardiotoxicity potential, and no deleterious effects on doxorubicin-induced cardiotoxicity in dogs was observed [14]. In a phase II study of amrubicin using a schedule of 45 mg/m^2 on days 1–3 every 3 weeks, in 33 previously untreated ED-SCLC patients, an overall response rate of 76% and a complete response (CR) rate of 9% were reported [15]. Moreover, median survival time (MST) was 11.7 months in the single-agent phase II study of amrubicin. Amrubicin is one of the most active new agents for SCLC. Thus, we conducted a phase I/II study of amrubicin plus cisplatin for untreated ED-SCLC, because cisplatin is considered as one of the most important drugs in the treatment of SCLC. The aims of this trial were to determine the maximum-tolerated doses (MTD) of combination therapy of amrubicin with cisplatin, to assess the efficacy and safety for ED-SCLC at their recommended doses (RD), and to examine the pharmacokinetics of the drug combination.

Patients and methods

Patient selection

Patients with histologically and/or cytologically documented SCLC were eligible for this study. Each patient was required to meet the following criteria: extensive-stage disease [16]; no prior therapy for primary lesion; measurable lesion; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2; expected survival time >2 months; age 20–74 years; adequate hematological function [white blood cell (WBC) count 4000–12 000/mm^3, neutrophils ≥2000/mm^3, platelets ≥100 000/mm^3, hemoglobin ≥10 g/dl]; adequate hepatic function [total bilirubin within 1.5× the upper limit of normal; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within 2.5× the upper limit of normal]; adequate renal function (creatinine within the upper limit of normal); partial pressure of arterial oxygen 60 torr; no abnormality requiring treatment on electrocardiogram; left ventricle ejection fraction >60%; written informed consent. Patients with symptomatic brain metastasis, pleural effusion that required drainage, non-steroidal anti-inflammatory drug or glucocorticoid use for >50 days, pericarditis carcinomatous, active infection, varicella, superior vena cava syndrome, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), gastric and/or duodenal ulcer, severe heart disease, severe renal disease, active concomitant malignancy, symptomatic pneumonitis and/or pulmonary fibrosis and pregnant/nursing women were excluded. This study was approved by the Institutional Review Board at each hospital.

Patient evaluation

Pretreatment evaluation consisted of complete blood cell counts, differential, routine chemistry measurements, progastrin-releasing peptide (ProGRP), neuron-specific enolase, electrocardiogram, echocardiography, chest radiograph, chest and abdominal computed tomography (CT) scan, whole-brain magnetic resonance imaging (MRI) or CT scan, and isotope bone scan. Complete blood cell counts, differential and routine chemistry measurements were performed at least once a week during the chemotherapy.

Treatment schedule

At level 1, chemotherapy consisted of cisplatin 60 mg/m^2 on day 1 and amrubicin 40 mg/m^2 on days 1–3. Amrubicin was administered as an intravenous injection over 5 min and cisplatin was administered as a drip infusion over 60–120 min with adequate hydration. At level 2 the dose of amrubicin was increased to 45 mg/m^2 on days 1–3. Level 3 was planned with cisplatin 80 mg/m^2 on day 1 and amrubicin 45 mg/m^2 on days 1–3. The chemotherapy was repeated every 3 weeks for four to six courses. Intrapatient dose escalation was not allowed. Administration of granulocyte colony-stimulating factor (G-CSF) was permitted prophylactically for patients expected to experience grade 3 neutropenia during the first course. Prophylactic administration of G-CSF was only permitted at second or later courses.

The administrations of both cisplatin and amrubicin were postponed if patients met the following criteria: WBC <3000/mm^3; neutrophils <1500/mm^3; platelets <100 000/mm^3; AST and ALT >5× the upper limit of normal; total bilirubin >1.5× the upper limit of normal; creatinine >1.3× the upper limit of normal; ECOG PS 3 or 4; active infection; grade 2 or worse non-hematological toxicity, except for alopecia, anorexia, nausea, vomiting or fatigue.

The administrations of both cisplatin and amrubicin were withdrawn if patients met the following criteria: tumor regression <15% after first course or <30% after second course; WBC <3000/mm^3; neutrophils <1500/mm^3; platelets <100 000/mm^3; no recovery from grade 3 or 4 non-hematological toxicity at 6 weeks after the start of previous chemotherapy; abnormality of electrocardiogram requiring treatment for more than 6 weeks; left ventricle ejection fraction <48%; treatment delay of >4 weeks.

The dose of amrubicin was decreased 5 mg/m^2/day if patients met the following criteria: grade 4 leukopenia or neutropenia for ≥4 days; grade 3 neutropenia with fever; platelets <20 000/mm^3 during the previous course. The dose of cisplatin was decreased to 75% if creatinine increased to >1.5× the upper limit of normal during the previous course.

The dose-limiting toxicity (DLT) was defined as follows: grade 4 leukopenia or neutropenia for ≥4 days; grade 3 febrile neutropenia; platelets <20 000/mm^3; grade 3 or worse non-hematological toxicity except for nausea, vomiting, anorexia, fatigue, hyponatremia and infection. Initially, three patients were treated at each dose level. If DLT was not observed in any of the three patients, dose escalation was carried out. If DLT was observed in one of three patients, an additional three patients were entered at the same dose level. If DLT was observed in three or more of six patients, or two or three of the initial three patients, we considered that dose to be the MTD. If DLT was observed in one or two of six patients, dose escalation was also carried out. Dose escalation was determined based only on the data from the first course of chemotherapy.

Response and toxicity evaluation

Response was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) and tumor markers were excluded from the criteria [17]. CR was defined as the complete disappearance of all clinically detectable tumors for at least 4 weeks and no new lesions. Partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameters of target lesion, taking as reference the baseline sum longest diameter, the required non-progression in non-target lesions and no new lesions for at least 4 weeks. Stable disease (SD) included: regression of target lesions insufficient to meet the criteria for PR, a <20% increase in the sum of the longest diameter of target lesion, taking as reference the smallest sum longest diameters recorded since the treatment started, the required non-progression in non-target lesions and no new lesions for at least 6 weeks. Progressive disease (PD) indicated a >20% increase in the sum of the longest diameters of target lesion, taking as reference the smallest sum longest diameter recorded since the treatment started

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Phase I–II study of amrubicin and cisplatin in previously untreated patients with extensive-stage small-cell lung cancer

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Background: Amrubicin, a totally synthetic 9-amino-anthracycline, demonstrated excellent single-agent activity for extensive-stage small-cell lung cancer (ED-SCLC). The aims of this trial were to determine the maximum-tolerated doses (MTD) of combination therapy with amrubicin and cisplatin, and to assess the efficacy and safety at their recommended doses (RD).

Patients and methods: Eligibility criteria were patients having histologically or cytologically proven measurable ED-SCLC, no previous systemic therapy, an Eastern Cooperative Oncology Group performance status of 0–2 and adequate organ function. Amrubicin was administered on days 1–3 and cisplatin on day 1, every 3 weeks.

Results: Four patients were enrolled at dose level 1 (amrubicin 40 mg/m^2/day and cisplatin 60 mg/m^2) and three patients at level 2 (amrubicin 45 mg/m^2/day and cisplatin 60 mg/m^2). Consequently, the MTD and RD were determined to be at level 2 and level 1, respectively. The response rate at the RD was 87.8% (36/41). The median survival time (MST) was 13.6 months and the 1-year survival rate was 56.1%. Grade 3/4 neutropenia and leukopenia occurred in 95.1% and 65.9% of patients, respectively.

Conclusions: The combination of amrubicin and cisplatin has demonstrated an impressive response rate and MST in patients with previously untreated ED-SCLC.

Key words: anthracycline, cisplatin, phase I–II, small-cell lung cancer

Introduction

Small-cell lung cancer (SCLC) is one of the most chemosensitive solid tumors, and the outcome of SCLC patients is slowly but surely improving. Combination chemotherapy consisting of cisplatin plus etoposide and concurrent twice-daily thoracic radiotherapy has yielded a 26% 5-year survival rate in limited-stage (LD) patients [1]. Despite the high response rate to combination chemotherapy, however, local and distant failure is very common, especially in extensive-stage (ED) patients. Moreover, resistance to chemotherapeutic agents develops easily after failure of initial treatment. Thus, long-term survivors are still very rare among patients with ED-SCLC. To improve the outcome of SCLC patients, several strategies, such as high-dose chemotherapy, dose-intensive chemotherapy, alternating chemotherapy and introduction of new drugs, have been investigated [2–6]. However, only the introduction of new agents has improved the outcome of SCLC patients. Combination chemotherapy with etoposide plus cisplatin or etoposide plus cisplatin alternating cyclophosphamide, doxorubicin and vincristine had been mainly used for SCLC in North America. Recently, a Japanese trial [Japan Clinical Oncology Group (JCOG) 9511] demonstrated the superiority of the combination of irinotecan and cisplatin for ED-SCLC patients over the combination of etoposide and cisplatin [6]. The development of more active chemotherapy, and especially the introduction of effective new drugs, is therefore essential to improve the survival of SCLC patients.

Amrubicin (SM-5887) is a totally synthetic anthracycline and a potent topoisomerase II inhibitor [7–14]. It has antitumor activity, and is more potent than doxorubicin against various mouse experimental tumors and human tumor

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[13] Ranson M, Hammond L, Ferry D, et al. ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of phase I trial. J Clin Oncol 2002;20:2240–50.
[14] Herbst R, Maddox AM, Rothenberg M, et al. Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumors: results of a phase I trial. J Clin Oncol 2002;120:3815–25.
[15] Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with non-small-cell lung cancer. J Clin Oncol 2003;21:2237–46.
[16] Kris M, Natale R, Herbst R, et al. A phase II trial of ZD 1839 ('Iressa') in advanced non-small-cell lung cancer (NSCLC) patients who had failed platinum- and docetaxel-based regimen (IDEAL 2). Proc Am Soc Clin Oncol 2002;21:292a.
[17] Giaccone G, Johnson DH, Manegold C, et al. A phase III clinical trial of ZD 1839 ('Iressa') in combination with gemcitabine and cisplatin in chemotherapy-naive patients with advanced non-small-cell lung cancer (INTACT 1). Ann Oncol 2002;113(Suppl 5):A-4.
[18] Johnson DH, Herbst R, Giaccone G, et al. ZD1839 ('Iressa') in combination with paclitaxel and carboplatin in chemotherapy naive patients with advanced non-small-cell lung cancer (NSCLC): results from a phase III clinical trial (INTACT 2). Ann Oncol 2002;13(Suppl 5):A-468.
[19] Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
[20] Santoro A, Cavina R, Latteri F, et al. Activity of a specific inhibitor, gefitinib (Iressa™, ZD1839), of epidermal growth factor receptor in refractory non-small-cell lung cancer. Ann Oncol 2004;15:33–7.
[21] Shimomura Y, Matsuo H, Samoto T, Maruo T. Up-regulation by progesterone of proliferating cell nuclear antigen and epidermal growth factor expression in human uterine leiomyoma. J Clin Endocrinol Metab 1998;83:2192–8.
[22] Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127–37.
[23] Fujiwara K, Kiura K, Ueoka H, et al. Dramatic effect of ZD 1839 ('Iressa') in a patient with advanced non-small-cell lung cancer and poor performance status. Lung Cancer 2003;40:73–6.
[24] Ranson M, Thatcher N. Commentary on ZD1839 (Iressa) in non small cell lung cancer. Lung Cancer 2003;40:77–8.
[25] Cappuzzo F, Ardizzoni A, Soto-Parra H, et al. Epidermal growth factor receptor targeted therapy by ZD1839 (Iressa) in patients with brain metastases from non-small-cell lung cancer (NSCLC). Lung Cancer 2003;41:227–31.
[26] Cappuzzo F, Bartolini S, Ceresoli GL, et al. Efficacy and tolerability of gefitinib in pretreated elderly patients with advanced non-small-cell lung cancer (NSCLC). Br J Cancer 2004;90:82–6.
[27] Hidalgo M, Siu LL, Nemunaitis J, et al. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. J Clin Oncol 2001;19:3267–79.
[28] Miller VA, Patel J, Shah N, et al. The epidermal growth factor receptor tyrosine kinase inhibitor Erlotinib (OSI-774), shows promising activity in patients with bronchioalveolar cell carcinoma (BAC): Preliminary results of a phase II trial. Proc Am Soc Clin Oncol 2003;22:A-2491.
[29] Wang SL, Milles M, Wu-Wang CY, et al. Effect of cigarette smoking on salivary epidermal growth factor (EGF) and EGF receptor in human buccal mucosa. Toxicology 1992;75:145–57.
[30] Cappuzzo F, Gregorc V, Rossi E, et al. Gefitinib in pretreated non-small-cell lung cancer (NSCLC): analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. J Clin Oncol 2003;21:2658–63.
[31] Subramaniam S, Whitsett JA, Hull W, Gairola CG. Alteration of pulmonary surfactant proteins in rats chronically exposed to cigarette smoke. Toxicol Appl Pharmacol 1996;140:274–80.
[32] Foster DJ, Yan X, Bellotto DJ, et al. Expression of epidermal growth factor and surfactant proteins during postnatal and compensatory lung growth. Am J Physiol Lung Cell Mol Physiol 2002;283:981–90.
[33] Zhang F, Pao W, Umphress SM, Jakowlew SB, et al. Serum levels of surfactant protein D are increased in mice with lung tumors. Cancer Res 2003;63:5889–94.
[34] Tamura K, Yamamoto N, Takeda K, et al. An epidemiological survey for interstitial lung disease induced by gefitinib in patients with advanced non-small-cell lung cancer. West Japan Thoracic Oncology Group (WJTOG). Proc Eur Cancer Soc 2003;1(Suppl 5):A-56.

Estrogen and progesterone may up-regulate EGFR in normal tissues [21], and activation of steroid hormones might impact on EGFR function in NSCLC [22]. Another explanation may be that the steroid hormone receptor might interact with EGFR and influence the response of an EGFR inhibitor.

Multivariate analysis in IDEAL-1 showed that PS was not a significant prognostic factor, however, the population of the study was restricted with regards to good PS. Although gefitinib was considered as an effector of symptom improvement in the phase II trial, the indication for patients with poor PS is controversial. Several authors described the case reports about the efficacy of gefitinib in NSCLC patients with poor PS [23,24] or with brain metastases [25]. Although 'good PS' were significant prognostic factor in this trial, gefitinib still might be a candidate drug for patients with poor PS, because of restriction of the use of other anti-cancer drug by their toxicities.

Elderly patients exhibited an equivalent response to young patients in this study. Recent data suggested, gefitinib is safe and well tolerated in elderly pretreated NSCLC patients [26]. A phase II study of gefitinib for elderly patients in NSCLC is needed.

A low smoking index was revealed as a predictive prognostic factor following a single regimen of gefitinib. Erlotinib is also administered orally and is a highly selective EGFR tyrosine kinase inhibitor [27] with a quinazolinamine-based structure similar to that of gefitinib. In the phase II study of erlotinib in NSCLC or bronchial alveolar carcinoma [28], a non-smoking history was also a prognostic factor. Chronic exposure to nicotine increases the expression level and phosphorylation status of EGFR and impairs its function [29]. Moreover, smoking produces overexpression of Her2/neu that binds to EGFR as a hetero-dimer in the tissue of normal bronchus. Expression of EGFR or Her2/neu or both in tissue samples by immunohistochemistry has not correlated in the response of gefitinib [30], however the different type of dimers formed between EGFR families might influence the response to gefitinib.

Four patients (4% of the patients) developed interstitial lung disease (ILD). Continuous smoking disrupted surfactant protein A or D [31,32], and the serum levels of the proteins were increased [33]. As 'smoking history' and 'male' are significant risk factors of ILD and also in treatment with gefitinib [34], a serum level of the surfactant protein A or D might be a predictive marker of ILD. Patients who are female and non-smokers are most likely to receive a high benefit and low risk with gefitinib treatment.

Although more basic biological research is needed to find the mechanism of action, we have found several predictive prognostic factors associated with the practical use of gefitinib. This is necessary clinical information which is important in order to set eligibility criteria for future clinical trials with gefitinib.

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References

[1] Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest 1997;111:1710–7.
[2] Non-Small-Cell Lung Cancer Collaborative Group. Chemotherapy in non-small-cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Br Med J 311 (1995) 899–909.
[3] Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 2000;18:2095–103.
[4] Fossella FV, DeVore R, Kerr RN, et al. Randomized phase II trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum containing chemotherapy regimens. J Clin Oncol 2000;18:2354–62.
[5] Salomon D, Brandt R, Ciardiello F, et al. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995;19:183–232.
[6] Rusch V, Baselga J, Cordon-Cardo C, et al. Differential expression of the epidermal growth factor receptor and its ligands in primary non-small-cell lung cancers and adjacent benign lung. Cancer Res 1993;53:2379–85.
[7] Fujino S, Enokibori T, Tezuka N, et al. A comparison of epidermal growth factor receptor levels and other prognostic parameters in non-small-cell lung cancer. Eur J Cancer 1996;32A:2070–4.
[8] Pavelic K, Banjac Z, Pavelic J, et al. Evidence for a role of EGF receptor in the progression of human lung carcinoma. Anticancer Res 1993;13:1133–7.
[9] Volm M, Rittgen W, Drings P. Prognostic value of ERBB-1, VEGF, cyclin A, FOS, JUN and MYC in patients with squamous cell lung carcinomas. Br J Cancer 1998;77:663–9.
[10] Baselga J, Pfister D, Cooper M, et al. Phase I study of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. J Clin Oncol 2000;18:904–14.
[11] Baselga J, Averbuch S. ZD1839 ('Iressa') as an anticancer agent. Drugs 2000;60(Suppl 1):33–40.
[12] Nakagawa K, Tamura T, Negoro S, et al. Phase I pharmacokinetic trial of the selective oral epidermal growth factor receptor tyrosine kinase inhibitor gefitinib ('Iressa', ZD1839) in Japanese patients with solid malignant tumors. Ann Oncol 2003;14:922–30.

Table 6 Patients with drug-related adverse events (NCI-CTC)

Adverse event	Number of patients ($N = 101$)				
	Grade 1	Grade 2	Grade 3	Grade 4/5	Total
Rash	33 (32.6%)	21 (20.8%)	3 (3.0%)	0	57 (56.4%)
Dry skin	24 (23.7%)	3 (3.0%)	0	0	27 (26.7%)
Pruritis	9 (9.0%)	7 (7.0%)	0	0	16 (16.0%)
Diarrhea	19 (18.8%)	4 (4.0%)	0	0	23 (22.8%)
Nausea	6 (6.0%)	1 (1.0%)	0	0	7 (7.0%)
Vomiting	3 (3.0%)	0	0	0	3 (3.0%)
Anorexia	7 (7.0%)	0	0	0	7 (7.0%)
ALT increased	5 (5.0%)	2 (2.0%)	5 (5.0%)	0	12(13.0%)
AST increased	8 (8.0%)	2 (2.0%)	3 (3.0%)	0	13 (13.0%)
Pneumonitis	0	0	2 (2.0%)	2[a] (2.0%)	4 (4.0%)

[a] Treatment-related death (Grade 5).

tors in a practical setting, we retrospectively analysed the patients who received a single regimen of gefitinib at our institute. Multivariate analysis demonstrated that the predictive factors which were associated with a response were 'female', 'good PS' and 'never-smoker'. In survival analyses, the factors 'female', 'good PS', and a low smoking index also significantly prolonged survival.

The mechanism by which these factors produced better prognosis has not been clarified.

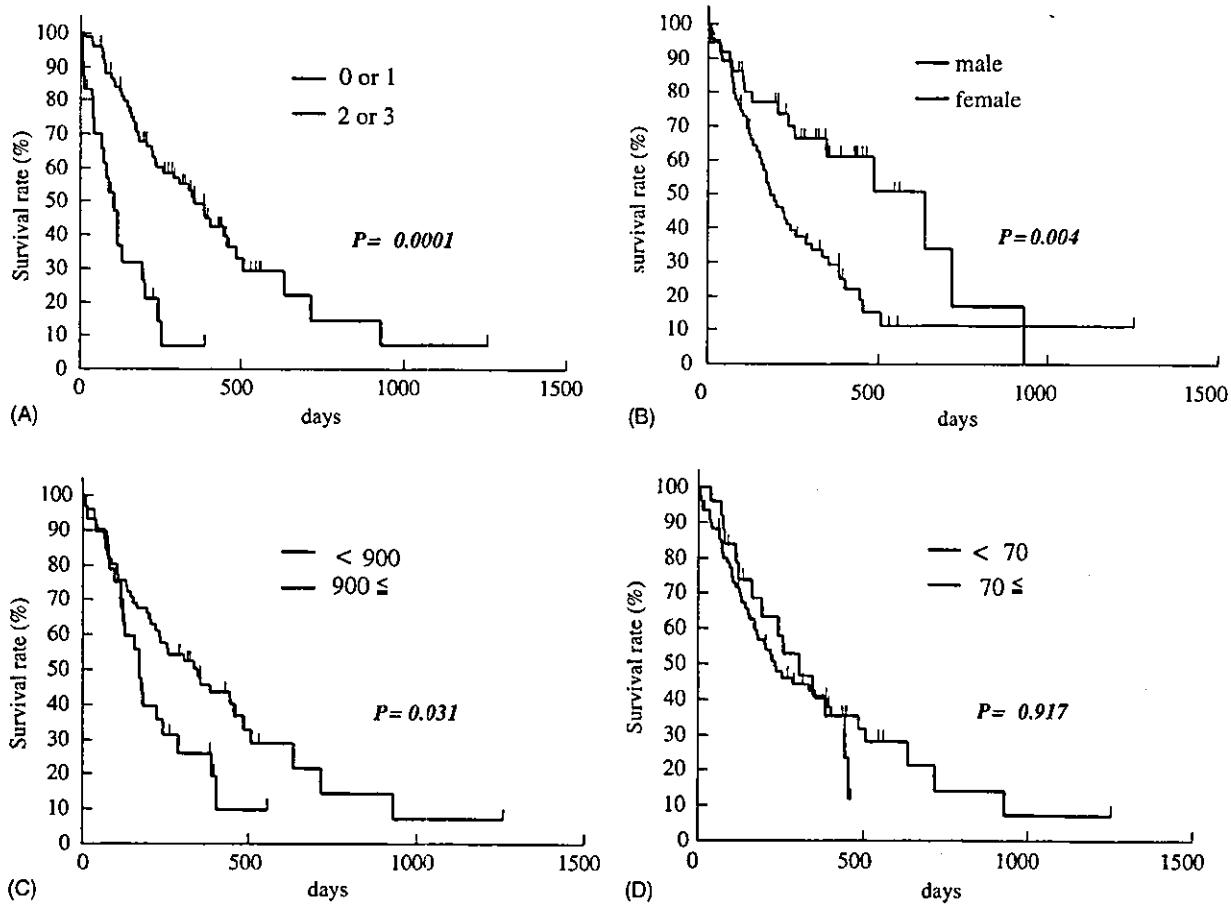

Fig. 2 A comparison of survival of: (A) PS 0, 1 vs. PS 2, 3; (B) gender: male vs. female; (C) smoking index: <900 vs. ≥900; and (D) age: <70 vs. ≥70.

Table 4 Predictive factors associated with an objective response by univariate analysis

Parameter	*N*	Responder	RR (%)	*P*-value
Smoking index				
Non-smoker	55	15	32.6	
Smoker	46	5	9.1	0.0025
Gender				
Female	37	14	37.8	
Male	64	6	9.4	0.0006
Histology				
Adenocarcinoma	81	20	24.7	
Others	20	0	0.0	0.0104
PS				
0–1	77	20	26.0	
≥2	24	0	0.0	0.0028
Pre-treatment				
≤2 regimens	58	13	22.4	
≥3 regimens	43	7	16.3	N.S.
Age (years)				
≤70	74	13	17.6	
≥71	27	7	25.9	N.S.
Stage				
IIIB	18	4	22.2	
IV	83	16	19.3	N.S.

Abbreviations: N.S., not significant.

3.3. Toxicity

Drug-related AEs of all patients are shown in (Table 6). A total of 101 patients were evaluated for toxicity. The most frequent drug-related AEs were a rash, dry skin and diarrhea. Most of these AEs were mild (Grade 1 or Grade 2) and were controllable. Of all the drug-related AEs evaluated, Grade 3 or Grade 4 AEs were seen in less than 5%, and Grade 4 drug-related AEs were only pneumonitis. Grade 3 or 4 AEs required a treatment interruption, but recovered after discontinuation of gefitinib, except with pneumonitis. Four patients developed greater than Grade 3 pneumonitis requiring hospitalization. All patients had a fever and severe hypoxemia on admission. As soon as possible, all patients were administered steroid therapy. While two patients recovered with the steroid therapy, two patients died within 40 days after the administration of gefitinib. Hematological toxicities were not observed.

3.4. Survival

The median survival time of the patients who were 'good PS' (0 or 1) and 'poor PS' (2 or 3) was 353 and 97 days, respectively, and this difference was significant ($P = 0.0001$, log-rank test) (Fig. 2A). The MST of females was significantly longer than that of males (596 days versus 178 days, $P = 0.004$) (Fig. 2B). Furthermore, a low smoking index (<900) significantly prolonged survival (MST: 301 days versus 149 days, $P = 0.031$) (Fig. 2C). Age did not influence the survival benefit of the patients treated with gefitinib (Fig. 2D).

4. Discussion

Gefitinib is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways, and is one of the promising molecular targeted drugs used in the treatment of advanced NSCLC [16,17,20]. Although the large scale of the phase II study (IDEAL-1) [15] has already confirmed that there were statistically significant differences in efficacy for 'adenocarcinoma' and 'female' by multivariate analysis, the population was essentially biased towards young people with good performance status who had conserved, good organ functions. To clarify the predictive prognostic fac-

Table 5 Predictive factors associated with an objective response by multivariate analysis

Parameter	Odds ratio	95% CI	*P*-value
Extraction of smoking			
Gender (female vs. male)	0.163	0.040–0.585	0.0032
Performance status (1 vs. 2)	0.061	0.000–0.415	0.0018
Histology (Adeno[a] vs. others)	3.326	0.435–infinity	N.S.
Extraction of gender			
Non-smoking (non vs. ≥1)	0.297	0.063–0.959	0.0417
Performance status (1 vs. 2)	0.096	0.000–0.628	0.0101
Histology (Adeno vs. others)	4.385	0.588–infinity	N.S.

Abbreviations: N.S., not significant; CI, confidence interval.
[a] Adenocarcinoma.

Table 3 Overall objective response

	Number	%
Number of patients evaluated	101	
Complete response (CR)	1	1.0
Partial response (PR)	19	18.8
Stable disease (SD)	52	51.5
Progressive disease (PD)	25	24.8
Not evaluable	4	4.0
Response rate % (95% CI)	19.8 (12.0–27.6)	
Disease control rate[a] % (95% CI)	71.3 (62.5–80.1)	

[a] CR + PR + S.D.

who had failed several previous chemotherapy regimens, and patients with an ECOG PS score of 3.

3.2. Response to treatment

Table 3 shows an objective response observed in this study. Twenty responders were evaluated and the overall response rate was 19.8%. One patient achieved a complete response, 19 patients exhibited a partial response and 52 patients had stable disease, resulting in a disease control rate (objective responses plus stable disease) of 71.3%. When evaluated using patient characteristics, we determined the response rate detailed in Fig. 1. All patients that responded had adenocarcinoma of the lung as the histological subtype. In addition, for the factors 'female' and 'never-smoker', there were higher response rates than in 'male' and 'smoker' respectively, while RR was similar for age, stage and pre-treatment. The response rate of 'female' and 'never-smoker' were 37.8 and 32.6%, respectively. Using the Fisher's exact test, the predictive factors which were associated with a response were 'female' (37.8% versus 9.4%; $P = 0.0006$), 'adenocarcinoma' (24.7% versus 0%; $P = 0.0104$), 'good PS' (0–1) (26.0% versus 0%; $P = 0.0028$), and never-smoker (32.6% versus 9.1%; $P = 0.0025$). There were no significant differences for age, stage and pre-treatment (Table 4). A multivariate analysis was performed against the four significant predictive factors in univariate analysis (Table 5). Because the incidence of the female factor is very strongly correlated to the never-smoker factor, the statistical assay was rather unstable if the two factors were analyzed simultaneously. We then investigated two patterns of multivariate analysis. One analysis excluded smoking and the other excluded gender. If smoking status was extracted, then female and good performance status were statistically significant. If gender was extracted, then non-smoking and good performance were statistically significant. The odds of a response were over three times higher for patients with adenocarcinoma than for patients with other histologies, however, this is not considered to be statistically significant because the group in this study was of a small size and included a high percentage of adenocarcinoma.

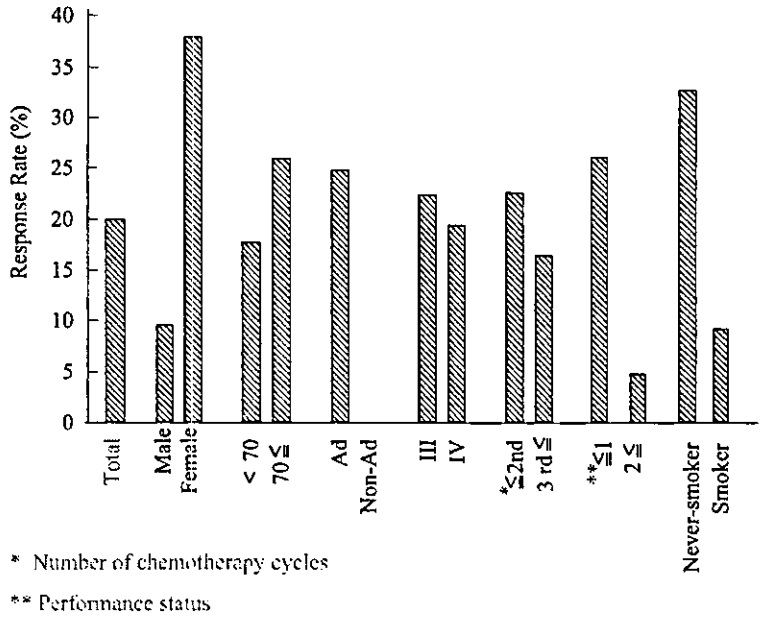

Fig. 1 Tumor response rate of the subgroups.

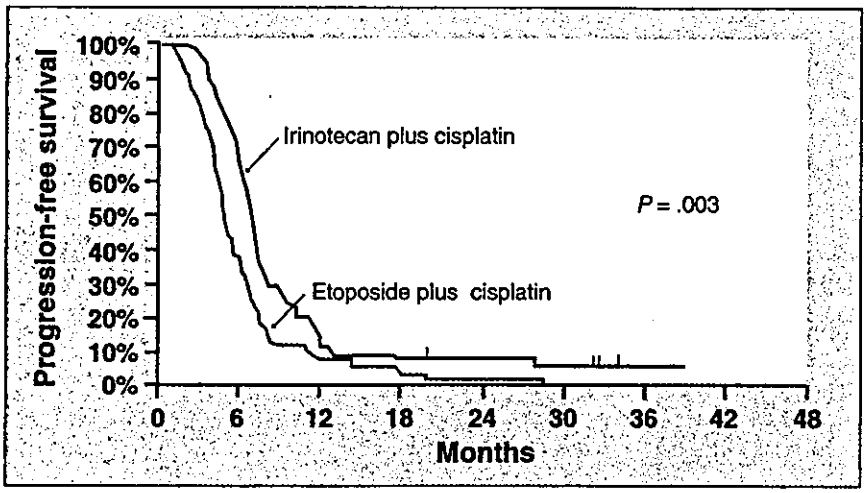

Figure 1: Progression-Free Survival—Progression-free survival of patients with extensive small-cell lung cancer who were assigned to treatment with irinotecan plus cisplatin or etoposide plus cisplatin (JCOG 9511).

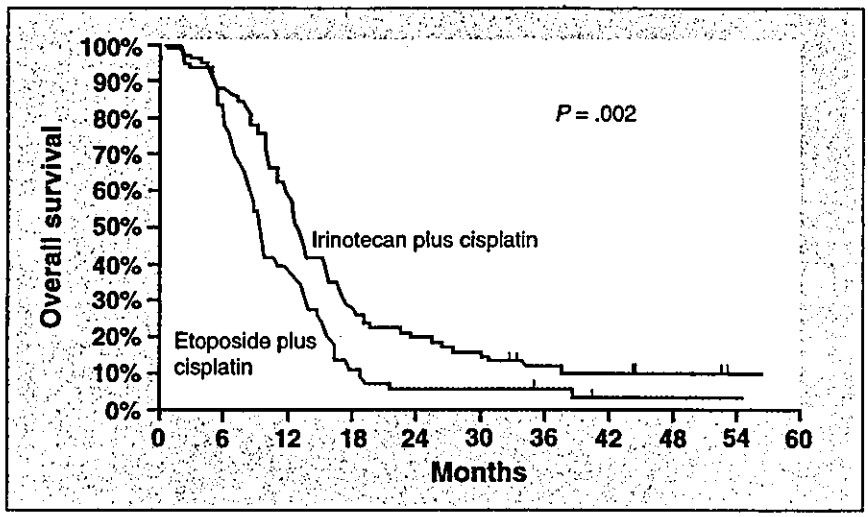

Figure 2: Overall Survival—Overall survival of patients with extensive small-cell lung cancer who were assigned to treatment with irinotecan plus cisplatin or etoposide plus cisplatin (JCOG 9511).

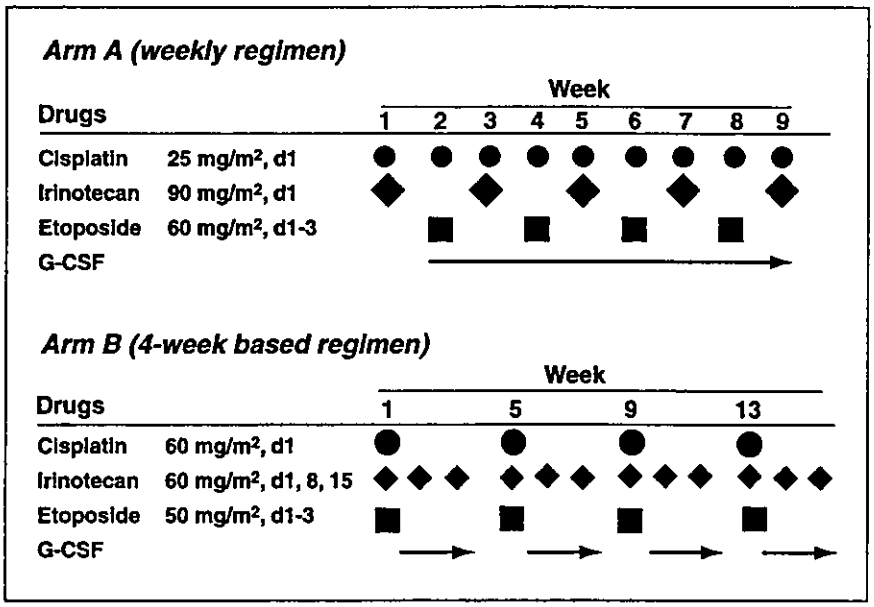

Figure 3: Study JCOG 9902-DI—Treatment schema of arm A (weekly regimen) and arm B (4-week regimen).

files also showed a minimal overlap.[11-16] In a phase II trial of irinotecan and cisplatin, the response rate was 86%.[17] In these trials, the principal toxicities were neutropenia and diarrhea.

Phase III Trial Comparing Irinotecan and Cisplatin With Cisplatin and Etoposide

Based on the results of the phase II trial, the Japan Clinical Oncology Group (JCOG) conducted a multiinstitutional randomized phase III trial (JCOG-9511) comparing irinotecan and cisplatin (IP) with cisplatin and etoposide (EP) in patients with previously untreated ED-SCLC.[18] The patient characteristics in this trial included an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 and age ≤ 70 years. Patients with symptomatic central nervous system metastases requiring radiation or corticosteroid treatment were excluded from the trial. The experimental arm consisted of irinotecan at 60 mg/m^2 administered on days 1, 8, and 15 of each 4-week cycle, along with cisplatin at 60 mg/m^2 administered on day 1 for a total of four 4-week cycles (IP). This treatment regimen was compared with a regimen of etoposide at 100 mg/m^2 administered on the first 3 days of each 3-week cycle along with cisplatin at 80 mg/m^2 administered on day 1 for a total of four 3-week cycles (EP).

The principal end point was overall survival. The projected accrual for this trial was 230 patients (115 patients per arm). An interim analysis conducted after 77 patients had been accrued in each arm showed a significant survival advantage for the IP arm. Therefore, further enrollment in the trial was discontinued.

The response rate was significantly higher in the IP arm than in the EP arm (84% vs 68%; P = .02). Additionally, the IP arm showed a statistically significant improvement in both progression-free survival (6.9 vs 4.8 months; P = .003) (Figure 1) and median overall survival (12.8 vs 9.4 months; P = .002) (Figure 2).

The results of this trial were the most exciting to be seen in patients with previously untreated SCLC. The IP regimen is thus another platinum-based combination that should be considered for the treatment of ED-SCLC. Appropriately, the combination of cisplatin and irinotecan has become the new standard treatment for patients with ED-SCLC in Japan. However, several points must be examined before the IP regimen can be fully established as the new standard treatment for ED-SCLC. Three randomized controlled trials comparing the EP regimen with the IP regimen are presently under way in Europe and the United States.

Table 1

Patient Characteristics in JCOG 9902-DI

	Arm A (n = 30)		Arm B (n = 30)	
	Number of Patients	Percentage	Number of Patients	Percentage
Sex				
Female	3	10%	3	10%
Male	27	90%	27	90%
Median age (range)	64 yr	(47–70 yr)	63 yr	(46–68 yr)
Performance status				
0	2	7%	3	10%
1	25	83%	25	83%
2	3	10%	2	7%
Body weight loss				
< 5%	23	77%	21	70%
5%–10%	6	20%	8	27%
> 10%	1	3%	1	3%

Table 2

Number of Chemotherapy Cycles Delivered in JCOG 9902-DI

Arm A			Arm B		
Number of Cycles	Number of Patients	Percentage	Number of Cycles	Number of Patients	Percentage
9	22	73%	4	21	70%
8	4	13%	3	5	17%
5	1	3%	2	2	7%
4	1	3%	1	2	7%
2	1	3%			
1	1	3%			

Table 3

Total Administered Dosage and Dose Intensity Delivered in JCOG 9902-DI

	Arm A	Arm B
	Median (Range) Total Dosage	
Cisplatin	225 mg/m² (25–225 mg/m²)	240 mg/m² (60–240 mg/m²)
Irinotecan	450 mg/m² (90–450 mg/m²)	563 mg/m² (60–720 mg/m²)
Etoposide	720 mg/m² (0–720 mg/m²)	600 mg/m² (150–600 mg/m²)
	Median (Range) Dose Intensity	
Cisplatin	21 mg/m²/wk (13–25 mg/m² wk)	15 mg/m²/wk (12–15 mg/m²/wk)
Irinotecan	40 mg/m²/wk (21–90 mg/m²/wk)	35 mg/m²/wk (15–45 mg/m²/wk)
Etoposide	68 mg/m²/wk (0–80 mg/m²/wk)	37 mg/m²/wk (28–38 mg/m²/wk)

Table 4

Toxicity in JCOG 9902-DI

	Arm A (n = 30)		Arm B (n = 30)	
Toxicity (Grade 3/4)	**Number of Patients**	**Percentage**	**Number of Patients**	**Percentage**
Leukocytopenia	15	50%	16	53%
Neutropenia	17	57%	26	87%
Anemia	13	43%	14	47%
Thrombocytopenia	8	27%	3	10%
Infection	2	7%	4	13%
Diarrhea	2	7%	3	10%
Hyponatremia	4	13%	6	20%
CRN elevation	1	3%	1	3%
Treatment-related death	1	3%	0	0%

CRN = creatinine.

Phase II Trial of Cisplatin, Irinotecan, and Etoposide Administered Weekly or Every 4 Weeks

JCOG 9511 showed that the IP regimen was significantly better than the EP regimen. However, because etoposide was still considered to be a key drug in the treatment of SCLC, a combination of these three drugs—irinotecan, cisplatin, and etoposide (IPE)—seemed to be a promising strategy for the treatment of ED-SCLC. The recommended weekly doses (JCOG 9507) and the dosages for each 4-week cycle (JCOG 9512) for IPE were decided using dose-escalation trials. For these reasons, a phase II trial of irinotecan, cisplatin, and etoposide administered weekly or every 4 weeks for ED-SCLC (JCOG 9902-DI) was performed.[19]

The purpose of this trial was to evaluate the toxicity and antitumor effect of the combination of irinotecan, cisplatin, and etoposide administered according to two schedules, weekly (arm A) and every 4 weeks (arm B), for the treatment of previously untreated ED-SCLC, and to select the appropriate arm for use in phase III trials. Patients were enrolled in this trial if they met the following criteria: (1) a histologic or cytological diagnosis of SCLC; (2) no prior treatment; (3) measurable disease; (4) extensive disease, defined as distant metastasis or contralateral hilar lymph node metastasis; (5) performance status of 0 to 2 on the ECOG scale; (6) a life expectancy of 3 months or longer; (7) age between 20 and 70 years; (8) adequate organ function; and (9) written informed consent.

The treatment schedule is shown in Figure 3. In arm A, cisplatin at 25 mg/m² was administered intravenously (IV) over 60 minutes on day 1 and at 1-week intervals for 9 weeks; irinotecan at 90 mg/m² was administered IV over 90 minutes on day 1 on weeks 1, 3, 5, 7, and 9; and etoposide at 60 mg/m² was administered by IV over 60 minutes on days 1 to 3 of weeks 2, 4, 6, and 8. Granulocyte colony-stimulating factor (G-CSF) was administered prophylactically on the days when a cytotoxic drug was not given, unless the white blood cell (WBC) count exceeded 10.0×10^9/L.

In arm B, cisplatin at 60 mg/m² was administered by IV over 60 minutes on day 1; irinotecan at 60 mg/m² was administered by IV over 90 minutes on days 1, 8, and 15; and etoposide at 50 mg/m² was administered by IV over 60 minutes on days 1 to 3. G-CSF was injected subcutaneously from day 5 until the day when the WBC count exceeded 10.0×10^9/L. This treatment was repeated every 4 weeks for a total of four cycles.

Patient characteristics are listed in Table 1. Between August 1999 and October 2000, 30 patients were entered in each arm. The last follow-up

examination was performed in February 2002. All enrolled patients were included in the toxicity, tumor response, and patient survival analyses. No differences in any of the listed characteristics were observed between the two arms.

Treatment delivery is listed in Table 2. Of the 30 patients in each arm, 22 (73%) and 21 (70%) patients in arms A and B, respectively, received full cycles of chemotherapy (nine cycles in arm A and four cycles in arm B). Therapy was stopped because of toxicity in four (13%) patients in arm A and in six (20%) patients in arm B. Therapy was stopped because of tumor progression in three (10%) patients in each arm. The need for treatment delay in arm A and treatment skipping in arm B, however, was significant. Only eight (27%) patients in arm A completed the treatment without delay, and only seven (23%) patients in arm B received all the planned doses. A total of 105 chemotherapy cycles were administered to 30 patients in arm B, but eight (8%) doses of irinotecan on day 8, and 33 (31%) doses of irinotecan on day 15 were omitted because of toxicity, according to criteria in the protocol.

The median total dosages of cisplatin and etoposide administered per patient were maintained at the planned dosage levels in both arms (Table 3). The median total dosage of irinotecan as a percentage of the scheduled dosage (the relative total dosage) was 100% in arm A, but only 78% in arm B, reflecting the doses of irinotecan that were skipped on days 8 and 15.

Dose intensity was evaluated in 29 patients in arm A and 28 patients in arm B (Table 3). The median relative dosage intensity was well maintained at a level of 80% or higher, except that of irinotecan in arm B (77%). The median actual dosage intensity of etoposide was 70 mg/m²/wk in arm A and 37 mg/m²/wk in arm B.

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Table 5

Antitumor Responses In JCOG 9902-DI

	Arm A (n = 30)		Arm B (n = 30)	
Responses	Number of Patients	Percentage	Number of Patients	Percentage
Complete	2	7%	5	17%
Partial	23	77%	18	60%
No change	1	3%	0	0%
Progressive disease	3	10%	4	13%
No effect	1	3%	3	10%
Response rate	83% (95% CI = 65%–94%)		77% (95% CI = 58%–90%)	

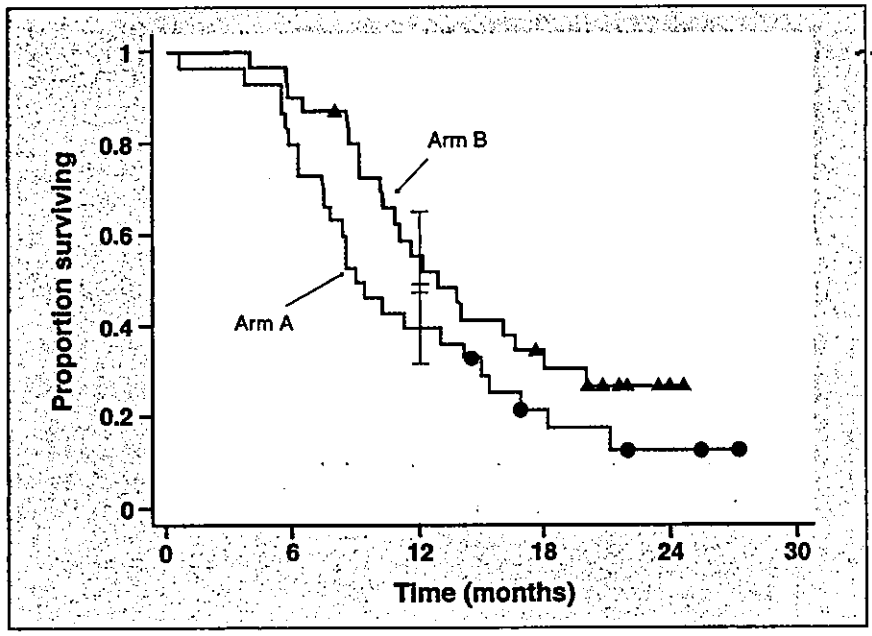

Figure 4: Study JCOG 9902-D1—Survival in treatment arms.

Toxicity was evaluated in all patients. The incidences of grade 3/4 neutropenia, anemia, thrombocytopenia, infection, and diarrhea in arm A were 57%, 43%, 27%, 7%, and 7%, respectively, and 87%, 47%, 10%, 13%, and 10%, respectively, in arm B. A treatment-related death occurred in one patient in arm A (Table 4).

Two complete responses (CRs) and 23 partial responses (PRs) were obtained in arm A, resulting in an overall clinical response rate of 83%, whereas five CRs and 18 PRs were obtained in arm B, resulting in an overall response rate of 77% (Table 5). The median time to survival and 1-year survival rate in arm A were 8.9

months and 40%, respectively, and 12.9 months and 57%, respectively, in arm B (Figure 4).

In this trial, the two IPE schedules were both effective against ED-SCLC and had an acceptable toxicity level. Arm B was adopted as the investigational arm in phase III trials.

Conclusion

The combination of cisplatin and irinotecan has become the new standard treatment for patients with ED-SCLC in Japan. However, SCLC is rarely cured, although the response rate has been improved and the survival time extended through the use of chemotherapy. Based on the results of JCOG 9511 and JCOG 9902-DI, a randomized trial comparing IP with IPE administered every 3 weeks in patients with previously untreated ED-SCLC is now being performed in Japan.

References

1. Kristjansen PE, Hansen HH: Management of small cell lung cancer: A Summary of the Third International Association for the Study of Lung Cancer Workshop on Small Cell Lung Cancer. *J Natl Cancer Inst* 82:263-266, 1990.

2. Pujol JL, Carestia L, Daures JP: Is there a case for cisplatin in the treatment of small-cell lung cancer?: A meta-analysis of randomized trials of a cisplatin-containing regimen vs a regimen without this alkylating agent. *Br J Cancer* 83:8-15, 2000.

3. Berghmans T, Paesmans M, Mascaux C, et al: A meta-analysis of the role of etoposide (VP-16) and cisplatin (CDDP) in small cell lung cancer (SCLC) with a methodology assessment (abstract). *Eur J Cancer* 35(suppl):S248, 1999.

4. Kunimoto T, Nitta K, Tanaka T, et al: Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. Cancer Res 47:5944-5947, 1987.

5. Matsuzaki T, Yokokura T, Mutai M, et al: Inhibition of spontaneous and experimental metastasis by a new derivative of camptothecin, CPT-11, in mice. *Cancer Chemother Pharmacol* 21:308-312, 1988.

6. Tsuruo T, Matsuzaki T, Matsushita M, et al: Antitumor effect of CPT-11, a new derivative of camptothecin, against pleiotropic drug-resistant tumors in vitro and in vivo. *Cancer Chemother Pharmacol* 21:71-74, 1988.

7. Taguchi T, Wakui A, Hasegawa K, et al: Phase I clinical study of CPT-11. *Gan To Kagaku Ryoho* 17:115-120, 1990. (in Japanese)

8. Ohno R, Okada K, Masaoka T, et al: An early phase II study of CPT-11: A new derivative of camptothecin, for the treatment of leukemia and lymphoma. *J Clin Oncol* 8:1907-1912, 1990.

9. Negoro S, Fukuoka M, Niitani H, et al: Phase II study of CPT-11, new camptothecin derivative, in small cell lung cancer (SCLC) (abstract). *Proc Am Soc Clin Oncol* 10:241, 1991.

10. Masuda N, Fukuoka M, Kusunoki Y, et al: CPT-11: A new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. *J Clin Oncol* 10:1225-1229, 1992.

11. Kudoh S, Takada M, Masuda N, et al: Enhanced anti-tumor efficacy of a combination of CPT-11, a new derivative of camptothecin, and cisplatin against human lung tumor xenografts. *Jpn J Cancer Res* 84:203-207, 1993.

12. Masumoto N, Nakano S, Esaki T, et al: Inhibition of cis-diamminedichloroplatinum (II)-induced DNA interstrand cross-link removal by 7-ethyl-10-hydroxy-camptothecin in HST-1 human squamous-carcinoma cells. *Int J Cancer* 62:70-75, 1995.

13. Pei XH, Nakanishi Y, Takayama K, et al: Effect of CPT-11 in combination with other anticancer agents in lung cancer cells. *Anticancer Drugs* 8:231-237, 1997.

14. Masuda N, Fukuoka M, Kudoh S, et al: Phase I and pharmacologic study of irinotecan in combination with cisplatin for advanced lung cancer. *Br J Cancer* 68:777-782, 1993.

15. Masuda N, Fukuoka M, Kudoh S, et al: Phase I study of irinotecan and cisplatin with granulocyte colony-stimulating factor support for advanced non-small-cell lung cancer. *J Clin Oncol* 12:90-96, 1994.

16. Nakagawa K, Fukuoka M, Niitani H, et al: Phase II study of irinotecan (CPT-11) and cisplatin in patients with advanced non-small-cell lung cancer (NSCLC) (abstract). *Proc Am Soc Clin Oncol* 12:332, 1993.

17. Kudoh S, Fujiwara Y, Takada Y, et al: Phase II study of irinotecan combined with cisplatin in patients with previously untreated small-cell lung cancer. West Japan Lung Cancer Group. *J Clin Oncol* 16:1068-1074, 1998.

18. Noda K, Nishiwaki Y, Kawahara M, et al: Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* 346:85-91, 2002.

19. Sekine I, Nishiwaki Y, Noda K, et al: Randomized phase II study of cisplatin, irinotecan and etoposide combinations administered weekly or every 4 weeks for extensive small-cell lung cancer (JCOG9902-DI). *Ann Oncol* 14:709-714, 2003.