version 2.0. Changes in physical and laboratory findings were assessed at least every 2 weeks.

Serum Sample Collection and DNA Extraction

Blood samples from patients were collected before and 14 days after the initiation of gefitinib administration. Separated serum was stocked at −80°C until use. DNA extraction from the serum samples was performed using a nonorganic method (Oncor, Gaithersburg, MD). Serum DNA was purified using Qiamp Blood Kit (Qiagen, Hilden, Germany), with the following protocol modifications. One column was used repeatedly until the whole sample had been processed. The extracted DNA was stocked at −20°C until use.

Tissue Sample Collection and DNA Extraction

Tumor specimens were obtained on protocols approved by the institutional review board. Twenty paraffin blocks of tumor material, obtained from 15 patients for diagnosis before treatment, were collected retrospectively. Eleven tumor samples were collected from primary cancer by means of transbronchial lung biopsy, one was resected by operation, and nine were from metastatic sites (four from bone, three from lymph nodes, one from the brain, and one from the colon). All specimens underwent histologic examination to confirm the diagnosis of NSCLC. DNA extraction from tumor samples was performed using the TaKaRa DEXPAT kit (TaKaRa Biomedicals, Shiga, Japan).

PCR Amplification

PCR was performed in 25-μl volumes using 15 μl of template DNA, 0.75 units of Ampli Taq Gold DNA polymerase (Perkin-Elmer, Roche Molecular Systems, Inc., Branchburg, NJ), 2.5 μl of PCR buffer, 0.8 mM dNTP, 0.5 μM of each primer, and different concentrations of $MgCl_2$, depending on the polymorphic marker. A set of designed primers was used to amplify exon 19 of *EGFR* (upper primer, 5′-CAGCCCCCAGCAATATCAGCCTTAGGT-3′; lower primer, 5′- CACTAGAGCTAGAAAGGGAAAGACATA-3′). Thirty cycles of amplification were performed using a thermal cycler (Perkin-Elmer, Foster City, CA) (95°C for 45 seconds, 55.5°C for 30 seconds, 72°C for 30 seconds, followed by incubation at 72°C for 10 minutes). The bands were visualized using a 2100 bioanalyzer, DNA 500 Labchip kit (Aglient Technologies, Waldbronn, Germany). If no PCR products were detected by the first PCR, an additional 20 cycles of PCR was carried out and the sample was revisualized. To confirm the deletional mutation in exon 19, and to detect the mutation in exons 18 and 21 of *EGFR*, PCR was performed again using another primer set as described previously.[13]

Sequencing

Amplification and sequencing were performed in duplicate for each sample using an ABI prism 310 (Applied Biosystems). The sequences were compared with the GenBank-archived human sequence for *EGFR* (accession no AY588246).

Trial Design and Statistical Methods

The trial was a two-stage multicenter phase II study. The primary endpoint was response rate, and secondary endpoints were disease control rate, safety, TTP, and OS. As a correlative study, *EGFR* mutations in tumor and serum samples were analyzed. The protocol and consent form were approved by the institutional review board of each participating hospital. Initially, 15 patients were recruited to the study. If one of these patients responded to treatment with gefitinib monotherapy, an additional 10 patients were recruited. If five or more of these 25 patients responded to therapy, treatment with gefitinib was concluded to be effective. According to Simon's minimax design,[21] our study, with a sample size of 25, had an 80% power to support the hypothesis that the true objective response rate was greater than 30% and a 5% significance to deny the hypothesis that the true objective response rate was less than 10%. Assuming a nonevaluability rate of less than 20%, we projected an accrual of 30 patients. In analysis of *EGFR* mutation in serum samples, the categorical variables were compared using the Fisher's exact test. A value of $p < 0.05$ was considered significant. The statistical analyses were performed using the StatView software package, version 5.0 (SAS Institute, Inc., Cary, NC).

RESULTS

Patients

From October of 2002 to August of 2003, 30 patients were enrolled into the study. Patient characteristics are summarized in Table 1. The most common histologic subtype was adenocarcinoma (25 patients [83.3%]). Three patients had undergone surgery and three had received radiotherapy to bone or brain metastases. Twenty patients were current or previous smokers. Twenty-six patients (86.7%) had good PS (0–1) and 86.7% of enrolled patients had stage IV disease. A total of 43 sites of metastatic lesions in 26 patients were diagnosed. Thirteen of the 26 patients had more than one metastatic lesion. All four patients with stage IIIb disease had pleural effusion and were ineligible for radiotherapy.

Efficacy

All patients were assessable for tumor response (Table 2). Complete response was not observed. Ten patients achieved PR, nine had SD as their best response, and 11 patients had progressive disease (PD). The objective response rate was 33.3% (95% confidence interval, 16.2–49.8%) and the disease control rate was 63.3% (95% confidence interval, 46.0–80.5%). All responders had adenocarcinoma. Of the responders, four were male patients and six were female patients. None of the prognostic factors such as gender (male versus female), PS (0–1 versus 2), smoking (never-smoker versus smoker), histology (adenocarcinoma versus nonadenocarcinoma), clinical stage (IIIb versus IV), and prior treatment (yes versus no) was significantly associated with tumor responses (Table 2). Disease control was observed in 19 patients (eight men and 11 women). A significantly higher disease control rate was observed in female patients ($p = 0.018$) and nonsmokers ($p = 0.049$). The other factors did not affect the disease control rate (Table 2).

TABLE 1. Patient Characteristics

Characteristic	Value
No. of patients	30
Age (yr)	
Median	64
Range	44–87
Gender	
Male	18
Female	12
Histology	
Adenocarcinoma	25
Squamous-cell carcinoma	3
Large-cell carcinoma	2
Stage	
IIIB	4
IV	26
Metastatic sites	
Pulmonary	16
Bone	12
Brain	11
Others	4
ECOG performance status	
0	20
1	6
2	4
Prior treatment	
Yes	6
Operation	6
Radiation	3
No	24
Smoking	
Yes	20
Pack-years (mean ± SD)	51 ± 39
No	10

ECOG, Eastern Cooperative Oncology Group.

TABLE 2. Response to Gefitinib Monotherapy and Prognostic Factors*

	No.	PR	SD	PD	RR (%)	p Value	DCR (%)	p Value
Total	30	10	9	11	33.3		63.3	
Prognostic factors								
Gender								
Male	18	4	4	10	22.2	0.14	44.4	0.018
Female	12	6	5	1	50.0		91.7	
Smoking habit								
Smoker	20	5	5	10	25	0.231	50	0.049
Nonsmoker	10	5	4	1	50		90	
Histologic type								
Adenocarcinoma	25	10	8	7	40	0.139	72	0.327
Nonadenocarcinoma	5	0	2	3	0		40	
PS								
0, 1	26	8	8	10	30.8	0.584	61.5	0.999
2	4	2	1	1	50		75	
Clinical stage								
IIIb	4	2	1	1	50	0.584	75	0.999
IV	26	8	8	19	31		62	
Prior treatment								
Yes	24	9	5	10	37.5	0.999	58.3	0.215
No	6	1	4	1	16.7		83.3	

*RR and DCR were compared between prognostic factors using Fisher's exact test.
†PR, partial response; SD, stable disease; PD, progressive disease; RR, response rate; DCR, disease control rate.

TTP and OS

At a median follow-up of 12 months, 20 patients had died and 26 patients were refractory or had become resistant to gefitinib monotherapy. Median TTP was 3.3 months (range, 0.3–19.6 months) and median OS was 10 months (range, 1.7–21.4 months) (Figure 1. Duration of response for patients with partial response was 5.8 months. OS and TTP were not affected by histologic type, smoking, PS, stage, or prior treatment. However, there was a significant difference in survival in gender (median survival time, >12 months in female patients versus 7.7 months in male patients; log-rank test, $p < 0.04$; Wilcoxon test, $p < 0.04$).

Tolerability

Table 3 shows drug-related adverse events. Twenty-six patients (86.7%) experienced drug-related adverse events, most of which were mild. Frequent adverse events included diarrhea, skin rash, and elevated transaminases. Twenty-two patients experienced skin toxicities, such as acne, pruritus, and rash. Grade 3 skin toxicities were observed in two patients, but these resolved spontaneously during treatment. Diarrhea was observed in 12 patients (40.0%) and was controlled with antidiarrheal agents such as loperamide. One patient developed grade 3 diarrhea, which required temporal interruption of therapy. Two patients developed drug-related pneumonitis; both were treated with steroid therapy, antibiotics, and oxygen inhalation and recovered within a few weeks. These patients were smokers and had not received thoracic radiotherapy. No patients experienced hematologic toxicities.

Postgefitinib Treatment

Twenty-five patients became resistant or were refractory to gefitinib monotherapy. Eight of these patients received neither chemotherapy nor radiotherapy because of deterioration of PS in four patients and withdrawal of informed consent to chemotherapy in three patients. One patient underwent palliative surgery and two received radiotherapy for symptomatic brain metastases. Fifteen patients received chemotherapy as postgefitinib treatment (platinum-based chemotherapy in 14 patients and vinorelbine monotherapy in one patient). Five patients achieved PR and four showed SD by the second-line chemotherapy.

EGFR Mutations in Tumor Samples

Twenty tumor samples were obtained from 15 patients retrospectively. Sequencing of exons 18, 19, and 21 in *EGFR* was performed in 12 of 20 samples under the same PCR conditions. *EGFR* mutations were detected in four tumor

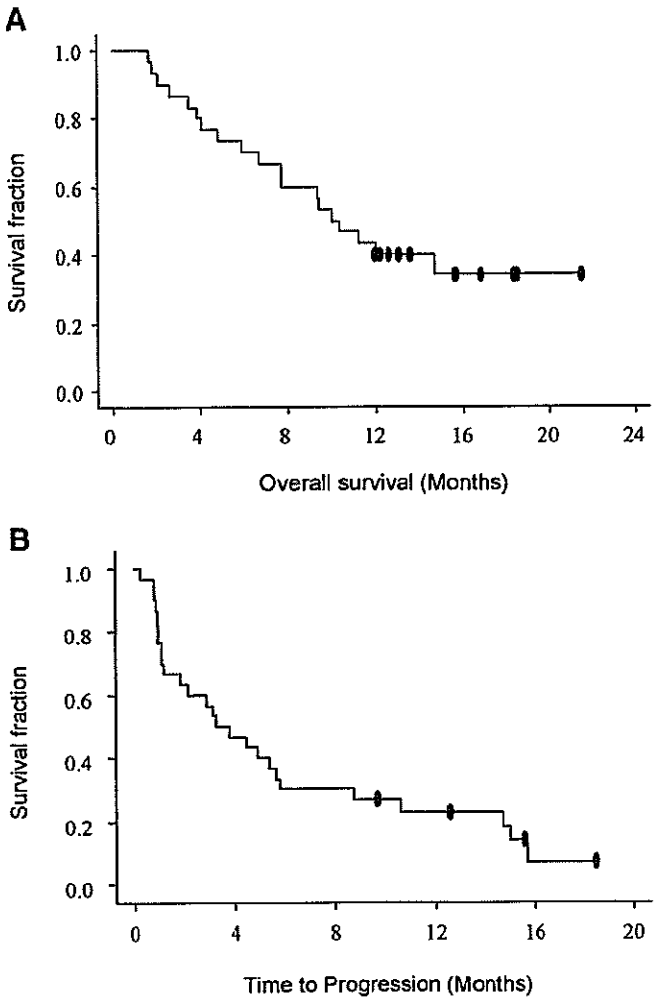

FIGURE 1. Kaplan-Meier curve showing (*A*) overall survival and (*B*) time to progression in all patients.

samples (33.3%). Three of them had a 15 base pair deletion (E746 A750del) in exon 19. Another of them had L858R in exon 21. The histologic types in patients with *EGFR* mutations were adenocarcinoma in three and large-cell carcinoma in one. All patients with E746 A750del in tumor samples had adenocarcinoma. The responses to gefitinib in these four patients were PR in two, SD in one, and PD in one. There were no responders among nine patients without an *EGFR* gene mutation.

EGFR Mutations in Serum Samples

The serum DNA in serum samples from 27 NSCLC patients was examined. Serum DNA was detected in all 54 samples at concentrations of up to 1720 ng/ml.

Exon 19 of *EGFR* in pretreatment serum samples obtained from 21 of 27 patients (77%) was detected (Figure 2 *A*). The lower band was also detected in 10 of 27 (37%) pretreatment serum samples. Sequencing of the PCR products confirmed that the upper and lower bands corresponded to wild-type and E746 A750del, respectively (Figure 2 *B*). No

TABLE 3. Drug-Related Adverse Events

	NCI-CTC Grade	No. of Patients	%
Diarrhea	1	8	26.7
	2	3	10.0
	3	1	3.3
Nausea	1	8	26.7
	2	2	6.7
	3	0	0.0
Vomiting	1	2	6.7
	2	0	0.0
	3	0	0.0
Skin toxicity	1	15	50.0
	2	5	16.7
	3	2	6.7
Elevation of transaminases	1	4	13.3
	2	1	3.3
	3	2	6.7
Pneumonitis	1	0	0.0
	2	0	0.0
	3	2	6.7

NCI-CTC, National Cancer Institute Common Toxicity Criteria.

point mutation in exon 18, 19, or 21 was detected in the PCR products from serum samples. Wild-type *EGFR* was detected in all 10 of the deletion-positive cases. The pattern of bands was reproducible when using another primer set.[13]

When compared according to histologic type, E746 A750del was detected in eight of 25 (32%) cases of adenocarcinoma, in zero of three cases of squamous carcinoma, and in two of two cases of large-cell carcinoma (Table 4). In contrast, the serum *EGFR* status was not correlated statistically with either the clinical response, the gender, or the recorded adverse effects (Table 5).

In serum samples obtained after the initiation of gefitinib treatment, 19 of 27 (70%) cases were wild-type positive and 14 of 27 (52%) cases were deletion-positive (Figure 2 *C*). In the posttreatment serum samples, E746 A750del was more frequently observed. Furthermore, the deletional mutant of *EGFR* was significantly more frequently observed in samples from patients who showed a PR or SD (12 of 16 cases [75%]) than in samples from patients with PD (two of 11 cases [18%]) ($p = 0.0063$, Fisher's exact test) (Table 6). The deletional mutant *EGFR* was more frequently detected in female patients (six of nine cases [67%]) than in male patients (eight of 18 cases [44%]), but this difference was not significant (Table 6). No correlations were seen statistically between the presence of mutation and the adverse effects.

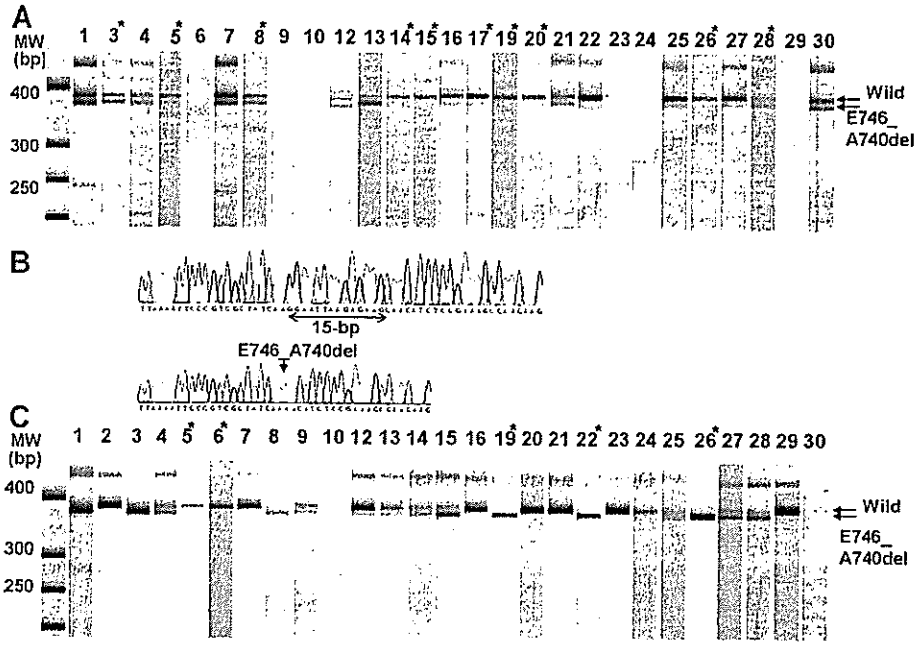

FIGURE 2. (*A*) Detection of genomic *EGFR* in the serum of pretreatment patients. (*B*) The sequences of the PCR products from patient 19 (days 0 and 14) are shown. (C) PCR of the serum samples obtained on day 14. Serum-derived genomic DNA PCR was performed. Exon 19 of *EGFR* in serum obtained from the patients was amplified by PCR, and the products were detected using a Bioanalyzer. A second round of PCR (20 cycles) was performed when no band was detected in the first round of PCR (30 cycles). Row numbers indicate the patient number. *Band detected in the first round of PCR.

TABLE 4. Frequency of Serum *EGFR* in Lung Cancer Patients According to Histology and Response to Gefitinib*

	Pre		Post	
	Wild	Deletion	Wild	Deletion
Adenocarcinoma	18/23	8/23	15/22	13/22
Squamous-cell carcinoma	1/2	0/2	3/3	0/3
Large-cell carcinoma	2/2	2/2	1/2	1/2

*A total of 27 samples were obtained from 28 patients both before and after treatment. A pretreatment sample of patient 2 and a posttreatment sample of patient 17 were lacking.

TABLE 5. Frequency of Serum *EGFR* in Lung Cancer Patients According Response to Gefitinib and Gender: Detection of Deletion-Type Mutation on Day 0*

	+	-	*p* Value
Response			
PR/SD	8	9	
PD	2	8	0.2305
Gender			
Male	5	12	
Female	5	5	0.4153

*A total of 27 samples were obtained from 28 patients both before and after treatment. A pretreatment sample of patient 2 and a posttreatment sample of patient 17 were lacking. SD, stable disease; PD, progressive disease; PR, partial response; +, deletion-positive; -, wild-type.

Comparison of *EGFR* Mutation Status between Tumor Samples and Serum Samples

Pairs of tumor samples and serum samples were obtained from 12 patients retrospectively (Table 7). The *EGFR* mutation status in the tumors was consistent with those in serum of seven of 12 of the paired samples. Among the other five patients, *EGFR* mutation was negative in the tumor and positive in the serum in four patients, and in the other patient it was positive in the tumor and negative in the serum, from whose tumor sample L858R was detected.

TABLE 6. Frequency of Serum *EGFR* in Lung Cancer Patients According to Response to Gefitinib and Gender: Detection of Deletion-Type Mutation on Day 14*

	+	-	*p* Value
Response			
PR/SD	12	4	
PD	2	9	0.0063
Gender			
Male	8	10	
Female	6	3	0.4197

*A total of 27 samples were obtained from 28 patients both before and after treatment. A pretreatment sample of patient 2 and a posttreatment sample of patient 17 were lacking. SD, stable disease; PD, progressive disease; PR, partial response; +, deletion-positive; -, wild-type.

DISCUSSION

The overall response of 33.3% in this phase II study was comparable not only to that achieved in Japanese population enrolled in the IDEAL-1 trial (27.5%)[7] but also to a retrospective analysis conducted of patients in Japan.[22] Gefitinib monotherapy appeared to be equally effective in patients with chemotherapy-naive NSCLC and in patients with pretreated NSCLC.

Drug-related adverse events were generally mild compared with cytotoxic chemotherapy. Grade 3 pulmonary toxicities were observed in two patients. In this study, the

TABLE 7. *EGFR* Mutation Status in Tumor Samples and Serum Samples*

					EGFR Mutation Status			
					Serum Samples			
					Pre		Post	
No.	Gender	Histology	Response	Tumor Sample	Wild	Mutation	Wild	Mutation
43	M	Large	SD	Wild	+	+		+
45	M	SCC	PD	Wild	ND	ND	+	
52	F	SCC	PD	Wild	+		+	
53	M	Adeno	PD	Wild			+	
55	M	Adeno	PR	L858R	+	+		
57	F	Adeno	SD	Wild			+	+
61	M	Large	PD	E746-A750 del	+	+	+	
64	M	Adeno	PD	Wild	+		+	
70	M	Adeno	PD	Wild	+	+	+	
72	M	Adeno	SD	E746-A750 del	+			+
75	F	Adeno	PR	E746-A750 del	+	+	+	+
77	M	Adeno	PD	Wild	+		+	+

*Pairs of both tumor samples and serum samples were obtained from 12 patients. M, male; F, female; SD, stable disease; PD, progressive disease; PR, partial response; SCC, squamous-cell carcinoma; Adeno, adenocarcinoma; Large, large-cell carcinoma; ND, not determined.

incidence of drug-related pneumonitis was 6.7% and was comparable to results of other studies.[23,24] Therefore, gefitinib monotherapy as a first-line treatment appears to be equally tolerable as a second-line treatment.

Thirteen of 22 patients who became resistant or were refractory to gefitinib monotherapy received salvage chemotherapy. The objective response rate was 30.8%, comparable to that of first-line chemotherapy. These results suggest that cancer cell populations that are sensitive to gefitinib might not be identical to those sensitive to chemotherapeutic drugs such as platinum agents or taxanes.

Somatic mutations in the tyrosine kinase domain of the *EGFR* gene were reported, and these mutations induced increased activity of *EGFR* and sensitivity to gefitinib in vitro and the predictive factor of response to gefitinib.[13,14] We evaluated *EGFR* gene status in 13 tumor samples and detected *EGFR* gene mutation in four tumors. Objective responses were achieved in two patients, but one patient showed PD whose tumor had a 15 base pair deletion mutation in exon 19. This suggested that response to gefitinib may not be determined by *EGFR* mutation in exon 19 or 21, and other mechanisms may relate to gefitinib resistance.

The detection of EGFR mutation from serum samples was carried out as a correlative study. These results provided us two major findings: (1) E746_A750del was detectable in serum sample obtained from NSCLC patients; and (2) E746_A750del was frequently observed in posttreatment serum samples obtained from the PR and SD patients.

It may be explained that DNA derived from destructive tumor cells that have responded to gefitinib may be more frequently observed in the circulating blood. Previous reports regarding detection of mutations in serum did not elucidate the changes in mutation status during treatment. We would like to do this in the next experiments to confirm our speculation. Our hypothesis is that serum detection of *EGFR* mutation will be a convenient means of predicting the sensitivity to gefitinib, although we could only demonstrate the feasibility of the *EGFR* mutation in serum in this report. We need to develop a highly sensitive methodology to improve the predictability of this assay.

In comparison of the mutation status of *EGFR* in actual tumors with serum DNA obtained from the same patients before treatment, 70% of patients who had sequence data obtained from both serum and tumor samples were conforming. Esteller et al. reported detection of aberrant promoter hypermethylation of tumor suppressor genes (*p16*, *DAP*, *GSTP1*, and *MGMT*) in serum DNA obtained from NSCLC patients and demonstrated that 73% of serum samples showed abnormal methylated DNA in the patients with the methylated primary tumors.[19] Another report investigating a point mutation of the *p53* gene and hypermethylation of *p16* in plasma DNA from breast cancer patients demonstrated that 66% of the patients with at least one molecular event in tumor DNA had some alteration in plasma DNA.[25] We believe that the sensitivity of our assay is equivalently sensitive to those of these previous reports.

CONCLUSION

In conclusion, 250 mg of oral gefitinib monotherapy as a first-line treatment produces obvious antitumor activity, with acceptable toxicities. Oral gefitinib monotherapy as a first-line treatment merits investigation in further clinical trials. Using serum samples from NSCLC patients, the *EGFR* mutation was detected. The detection of E746_A750del in the serum of untreated patients was not a predictor of gefitinib response in this study. However, further prospective studies using serum samples may be necessary to confirm this con-

clusion. The presence of *EGFR* mutation in serum may be a useful biomarker for monitoring gefitinib response.

ACKNOWLEDGMENTS

We thank the patients who enrolled into the trial and the investigators who enrolled them.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, et al. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001;94:153–156.
2. Roskoski R Jr. The ErbB/HER receptor protein-tyrosine kinases and cancer. *Biochem Biophys Res Commun* 2004;319:1–11.
3. Scagliotti GV, Selvaggi G, Novello S, et al. The biology of epidermal growth factor receptor in lung cancer. *Clin Cancer Res* 2004; 10: 4227s–4232s.
4. 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: 2379–2385.
5. Rusch V, Klimstra D, Venkatraman E, et al. Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor alpha is frequent in resectable non-small cell lung cancer but does not predict tumor progression. *Clin Cancer Res* 1997;3:515–522.
6. Fontanini G, De Laurentiis M, Vignati S, et al. Evaluation of epidermal growth factor-related growth factors and receptors and of neoangiogenesis in completely resected stage I–IIIA non-small-cell lung cancer: amphiregulin and microvessel count are independent prognostic indicators of survival. *Clin Cancer Res* 1998;4:241–249.
7. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2003;21:2237–2246.
8. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;290:2149–2158.
9. Herbst RS, Giaccone G, Schiller JH, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 2. *J Clin Oncol* 2004;22:785–794.
10. Giaccone G, Herbst RS, Manegold C, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 1. *J Clin Oncol* 2004;22:777–784.
11. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2006;366: 1527–1537.
12. Johnson DH. Gefitinib (Iressa) trials in non-small cell lung cancer. *Lung Cancer* 2003; 41 (Suppl 1): S23–S28.
13. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.
14. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304: 1497–1500.
15. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancer from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 2004;101:13306–13311.
16. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2004;97:339–346.
17. Han SW, Kim TY, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2006;23:2493–2501.
18. Sanchez-Cespedes M, Monzo M, Rosell R, et al. Detection of chromosome 3p alterations in serum DNA of non-small-cell lung cancer patients. *Ann Oncol* 1998;9:113–116.
19. Esteller M, Sanchez-Cespedes M, Rosell R, et al. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res* 1999;59:67–70.
20. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–216.
21. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;10:1–10.
22. Takano T, Ohe Y, Kusumoto M, et al. Risk factors for interstitial lung disease and predictive factors for tumor response in patients with advanced non-small cell lung cancer treated with gefitinib. *Lung Cancer* 2004;45:93–104.
23. Takeda K, Yamamoto N. An epidemiological survey for interstitial lung disease induced by gefitinib in patients with advanced non-small cell lung cancer. *Lung Cancer* 2003;41:S250.
24. Hotta K, Harita S, Bessho A, et al. Interstitial lung disease (ILD) during gefitinib treatment in Japanese patients with non-small cell lung cancer (NSCLC): Okayama Lung Cancer Study Group. *Proc Am Soc Clin Oncol* 2004;23:629.
25. Silva JM, Dominguez G, Garcia JM, et al. Presence of tumor DNA in plasma of breast cancer patients: clinicopathological correlations. *Cancer Res* 1999;59:3251–3256.

Phase I pharmacokinetic and pharmacogenomic study of E7070 administered once every 21 days

Yasuhide Yamada,[1,3] Noboru Yamamoto,[1] Tatsu Shimoyama,[1] Atsushi Horiike,[1] Yasuhito Fujisaka,[1] Kyoko Takayama,[1] Terumi Sakamoto,[1] Yuki Nishioka,[2] Sanae Yasuda[2] and Tomohide Tamura[1]

[1]Medical Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045; and [2]Clinical Research Center, Eisai Company, 4-6-10 Koishikawa, Bunkyo-ku, Tokyo 112-8088, Japan

(Received April 14, 2005/Revised July 12, 2005/Accepted July 29, 2005/Online publication October 3, 2005)

E7070 is a novel sulfonamide anticancer agent that disrupts the G1/S phase of the cell cycle. The objectives of this phase I study of E7070 were to estimate the maximal tolerated dose (MTD), to determine the recommended dose for phase II, and to clarify the pharmacokinetic profile of E7070 and its relation to polymorphisms of CYP2C9 (*2, *3) and CYP2C19 (*2, *3) in Japanese patients. Patients received 1–2-h i.v. infusions of E7070 (400, 600, 700, 800 or 900 mg/m^2) on day 1 of a 21-day cycle. Twenty-one patients received between one and eight cycles of E7070. The dose-limiting toxicities (DLT) comprised leukopenia, neutropenia, thrombocytopenia, elevation of aspartate aminotransferase, colitis, and ileus. The mean area under the plasma concentration–time curve (AUC) for successive dose levels increased in a non-dose-proportional manner. Two patients were heterozygous for the CYP2C9 mutation. For CYP2C19, eight patients were wild type and the remainder had heterozygous (n = 8) or homozygous mutations (n = 5). Regarding the CYP2C19 genotype, the AUC of patients with mutant alleles were higher than those of patients with wild type at a dose of 600 mg/m^2 or more. The severity of toxic effects, such as myelosuppression, seemed to depend on the AUC. No partial responses were observed. One patient treated at a dose of 700 mg/m^2 experienced a maximum tumor volume reduction of 22.5%. The MTD was estimated to be 900 mg/m^2. A dose of 800 mg/m^2 is recommended for further phase II studies. The pharmacokinetic/pharmacodynamic properties of E7070 seemed to be influenced by CYP2C19 genotype. The observed safety profile and preliminary evidence of antitumor activity warrant further investigation of this drug in monotherapy or in combination chemotherapy. (*Cancer Sci* 2005; 96: 721–728)

E7070, *N*-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide, has been reported to exhibit a potent antitumor activity by blocking cell cycle progression. *In vitro* studies indicate that the drug disrupts the G_1/S phase, thereby inducing cell cycle arrest and apoptosis.[1] Although E7070 is not a direct inhibitor of cyclin-dependent kinases (CDK), it causes depletion of cyclin E, with a reduction in CDK2 catalytic activity.[2] The exact mechanism of cyclin E/CDK2 inactivation is unclear. Transcriptional repression of cyclin H in a p53-independent manner also occurs in response to E7070.[3] The reduction in G_1 CDK activity induces arrest at the G_1/S boundary, accompanied by hypophosphorylation of retinoblastoma (Rb) protein and decreases in CDK2, cyclin A, and cyclin B proteins.[1] E7070 activity is associated with upregulation of p53 and p21, which may contribute to the reduced Rb phosphorylation, as well as subsequent apoptosis. In preclinical models, E7070 was cytotoxic to human HCT116 colon carcinoma and LX-1. E7070 exhibits more potent *in vivo* antitumor effects than 5-fluorouracil, mitomycin C, and irinotecan.[4]

E7070 has been the subject of four clinical phase I studies. In the first trial, E7070 was administered once every 21 days at doses between 50 and 1000 mg/m^2,[5] and in the second trial E7070 was administered five times per day once every 3 weeks at doses between 10 and 200 mg/m^2 per day.[6] Other schedules used were a weekly infusion given over 4 consecutive weeks repeated every 6 weeks,[7] and a continuous intravenous infusion for 5 days every 3 weeks.[8] In the single-dose every 3 weeks study, neutropenia and thrombocytopenia were dose-limiting at 700 and 800 mg/m^2.[5] In the second study, neutropenia and thrombocytopenia were dose-limiting at 160 and 200 mg/m^2.[6] In the study that used a weekly dose schedule, neutropenia and thrombocytopenia were also dose-limiting toxicities (DLT) and other DLT included stomatitis, diarrhea, nausea, and fatigue.[7] Partial responses were observed in patients with breast and endometrial cancer.[6,7]

During a phase I trial, three patients receiving prophylactic daily oral maintenance therapy with acenocoumarol developed a hemorrhagic tendency and/or a prolonged prothrombin time following treatment with 700 and 800 mg/m^2 of E7070.[5] The major metabolic enzyme for acenocoumarol is cytochrome P450 (CYP)2C9.[9] *In vitro* studies have shown that E7070 has the potential to inhibit CYP2C9 and CYP2C19.[10] The pharmacokinetic drug–drug interaction study indicated that primary interaction of the two drugs could occur via inhibition by E7070 of acenocoumarol metabolism.

Based on these results from the previous phase I and pharmacokinetic trials, the present phase I study was designed to evaluate ascending doses of E7070 administered as a single dose by 1–2-h i.v. infusion every 21 days. The objectives of the study were to determine the maximum tolerated dose (MTD) and the dose to be recommended for use in future phase II studies, as well as to assess the safety, pharmacokinetic profile and preliminary antitumor activity of the drug. We also evaluated the influence of genetic polymorphisms of CYP2C9 and CYP2C19 on the pharmacokinetics of E7070.

[3]To whom correspondence should be addressed. E-mail: yayamada@ncc.go.jp

 doi: 10.1111/j.1349-7006.2005.00109.x

Materials and Methods

Patient selection

Japanese patients with histologically or cytologically confirmed malignant solid tumors refractory to conventional chemotherapy, or tumors for which no effective therapy was available, were candidates for this study. Eligibility criteria included the following: age between 20 and 75 years; World Health Organization (WHO) performance status 0–1, life expectancy ≥ 3 months, absolute leukocyte count ≥ 4000/μL and < 12 000/μL, absolute neutrophil count ≥ 2000/μL, hemoglobin level ≥ 9 g/dL, platelet count ≥ 100 000/μL, serum creatinine level <1.5 mg/dL or creatinine clearance ≥ 50 mL/min, and arterial partial pressure of oxygen of 65 torr or more. Additional entry criteria were serum bilirubin ≤ 1.5 mg/dL, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 100 IU/L. Before study entry, a 6-week interval was required for patients previously treated with mitomycin C or nitrosoureas, a 4-week interval was required for other chemotherapy, endocrinotherapy, surgery, radiation therapy, immunotherapy treatments or other investigational agents, and a 2-week interval after blood transfusion or administration of granulocyte-colony stimulating factor (G-CSF). Patients were ineligible for the study if they had symptomatic central nervous system metastases, active infection, or other non-malignant disease, which was considered to be incompatible with the protocol. Patients who were receiving corticosteroids or coumarin anticoagulants less than 2 weeks prior to administration of E7070 were excluded from the study. The protocol was approved by the institutional review boards of the National Cancer Center, and all patients gave written informed consent prior to study entry.

Dosage and dose escalation

E7070 was provided as a lyophilized powder in 500-mg vials by Eisai Co. (Tokyo, Japan). The starting dose of E7070 was set at 400 mg/m^2 because only mild to moderate grade 1 to grade 2 toxicity was observed at doses of 600 mg/m^2 or lower in the previous phase I study.[5] Subsequent doses were to be escalated to 600, 700, 800 and 900 mg/m^2. E7070 was dissolved in 20 mL of distilled water, then added to 500 mL of normal saline for injection, and this solution was administered by intravenous infusion over 1 h at doses of 400 and 600 mg/m^2. Injection site reaction was observed in one of three patients at 400 mg/m^2 and three of three patients at 600 mg/m^2. Therefore, E7070 was given in 1000 mL of normal saline over 2 h at 700, 800, and 900 mg/m^2. Patients were hospitalized for the first course of E7070 and remained hospitalized for close observation for 21 days thereafter. Subsequent courses could be administered on an outpatient basis with weekly patient evaluations by the investigator.

Patients were enrolled in cohorts of three patients per dose level and observed for 21 days; the observation period was extended to 42 days if a longer recovery period was needed. If one of the three patients experienced DLT, then three additional patients were treated at the same dose. If two or more of three or six patients experienced DLT, that dose level was regarded as the MTD. If none of the first three patients demonstrated DLT, then the next three patients were treated at the next (higher) dose level. Individual patients who did not demonstrate DLT and showed no evidence of disease progression could receive E7070 at the originally assigned dose.

After the MTD had been determined, a dose below the MTD was evaluated in a total of six patients for identification of the proposed recommended dose for phase II study. The recommended dose was the highest dose at which less than 33.3% of treated patients experienced DLT.

Definition of dose-limiting toxicity

The DLT was defined as the occurrence of any of the following events during cycle 1 that were attributable to E7070: National Cancer Institute Common Toxicity Criteria (NCI CTC) (version 2.0) grade 3 or 4 non-hematological toxicity (except nausea, vomiting effectively managed with symptomatic treatment, or alopecia), grade 4 leukopenia, grade 4 neutropenia accompanied by fever of ≥ 38.5°C, or that persisted ≥ 5 days, and platelet count < 25 000/μL. Prophylactic use of colony-stimulating factors was not permitted during the first cycle; however, patients who had neutropenia that had met the criteria for DLT were permitted to receive concomitant treatment with G-CSF.

Evaluation of patients

Safety was evaluated on the basis of physical findings, vital signs, adverse events, and laboratory parameters obtained at baseline and periodically throughout the study. During the first cycle, hematology studies were performed at least twice a week, while vital signs, physical examinations (including evaluation of performance status) and serum biochemistry were measured on days 1, 8 and 15. Toxicity evaluations of subjective and objective findings were performed according to the NCI CTC (version 2.0) on days 1, 8 and 15. For the second and subsequent cycles, vital signs, laboratory tests and toxicity evaluations based on subjective and objective findings were performed on days 1, 8, and 15 of each cycle. Blood glucose was monitored before the dose of E7070 and after the end of infusion. Blood coagulation studies were carried out before each dose for all cycles and also at day 8 of the first cycle. Efficacy was assessed by the physician on the basis of antitumor effect according to the Response Evaluation Criteria in Solid Tumors (RECIST).[11] If an antitumor effect was observed, the disease site was re-evaluated at least 4 weeks later to confirm the response.

Pharmacokinetics

Pharmacokinetic studies were performed during the first cycle of treatment. On day 1, blood samples (4 mL each) were drawn from an indwelling intravenous cannula in the arm contralateral to that bearing the infusion line. Samples were collected in heparinized tubes, preinfusion, at 30 min after the start of the infusion, at the end of the 1- or 2-h infusion, and at 10, 30, and 60 min and 2, 4, 6, 10, 24, 48, 72, 96, 168, and 240 h after the infusion. The samples were centrifuged at 1500×g for 10 min at 5°C, and the resulting plasma samples were stored at −20°C until analysis. Urine samples were collected before the start of E7070 infusion and over three 24-h intervals for 72 h after the start of the infusion. The concentrations of E7070 in plasma and in urine were analyzed at Eisai Co. by means of validated high-performance

 doi: 10.1111/j.1349-7006.2005.00109.x

liquid chromatography methods with UV detection (HPLC-UV). The lower limit of quantification was 20 ng/mL. *N*-(3-Chloro-7-indolyl)-4-(*N*-methylsulfamoyl)benzenesulfonamide (ER-67771)[12] was used as an internal standard.

Plasma, the internal standard and 0.1 mol/L phosphate buffer (pH 6.8) were vortexed. After adding ethyl acetate, the mixture was shaken and centrifuged. The organic layer was collected and transferred into a glass tube, then evaporated under nitrogen flow in a drying block. The residue was dissolved in CH_3CN-6.7 mmol/L phosphate buffer (pH 6.6), and the solution was injected into an HPLC apparatus.

Chromatographic separation was achieved by using a column switching method with Asahipak C8P-50 (Showa Denko, Tokyo, Japan) as a separation column and YMC-pack ODS-AM-312 (YMC) as an analytical column. Mobile phases were CH_3CN: 6.7 mmol/L phosphate buffer (pH 6.6; 360:640 [v:v]) for separation and CH_3CN: 6.7 mmol/L phosphate buffer (pH 7.4; 360:640 [v:v]) for analysis. E7070 was monitored by UV detection at 280 nm.

Pharmacokinetic parameters of E7070 after a single dose administration during the first cycle were determined by non-compartmental analysis using WinNONLIN (Pharsight Corporation, CA, USA). The apparent elimination rate constant at the terminal phase (λ_z) was estimated by linear regression analysis from the terminal log-linear declining phase to the last quantifiable concentration. The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2)/\lambda_z$. The area under the plasma concentration–time curve from zero to the last quantifiable sampling time, $AUC_{(0-tn)}$, was obtained by the log trapezoidal rule. The AUC from zero to infinity was calculated as $AUC_{(0-tn)} + C_n/\lambda_z$, where C_n was the last quantifiable concentration. The clearance was calculated as dose/AUC. The mean residence time (MRT) was estimated from AUMC/AUC, where AUMC is the first moment curve. The volume of distribution was calculated as MRT × clearance.

Genotyping procedures for CYP2C9 and CYP2C19

Genotyping was conducted using the Invader assay (BML, Tokyo, Japan). Genomic DNA was isolated from whole blood with the QIAamp DNA Blood Kit (Qiagen, CA, USA). The primary probes (wild type and mutant probes) were used to detect C430T (*2) and A1075C (*3) mutations of CYP2C9, and G681A (*2) and G636A (*3) of CYP2C19, respectively. The invader assay fluorescent resonance energy transfer (FRET)-detection 96-well plates (Third Wave Technologies, WI, USA) contained Cleavase enzyme, FRET probes, MOPS buffer and polyethylene glycol. Eight microliters of mixtures consisting of an appropriate primary probe, Invader oligonucleotide and $MgCl_2$ was added to the wells, followed by addition of 7 μL of the heat-denatured genomic DNA, and this was overlaid with 15 μL of mineral oil. For only CYP2C9*2 (C430T) detection, a dilution of the CYP2C9-specific polymerase chain reaction (PCR) product was used instead of genomic DNA, because the CYP2C9*2 (C430T) detection point has the same sequence on CYP2C19. The plates were incubated at 63°C for 4 h for genomic DNA or 1 h for the PCR product. The fluorescence intensities were measured on a Cytofluor 4000 fluorescence plate reader (Applied Biosystems, CA, USA) with excitation at 485/20 nm (wavelength/bandwidth) and emission at 530/25 nm for FAM dye detection, and excitation at 560/20 nm and emission at 620/40 nm for RED dye detection.

Subjects having either the *2 or *3 allele (*1/*2 or *1/*3) were defined as hetero extensive metabolizers (hetero EM), those with two mutated alleles (*2/*2, *2/*3 or *3/*3) as poor metabolizers (PM), and those with no mutated alleles (*1/*1) as homo EM.

Table 1. Patients' characteristics

No. entered	21
Age (years)	
Median	57
Range	35–70
Male:female (no. patients)	15:6
WHO performance status (no. patients)	
0	7
1	14
Tumor type (no. of patients)	
Colorectal	15
SCLC	2
Gastric	1
NSCLC	1
Liposarcoma	1
Mesothelioma	1
Prior treatment	
Chemotherapy	
No. prior regimens (no. patients)	
0	0
1	1
2	5
>3	15
Surgery (no. patients)	18
Radiation therapy (no. patients)	7

SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; WHO, World Health Organization.

Results

Patients' characteristics

Twenty-one patients (15 male and six female) were enrolled into the study (Table 1). All patients had a good performance status and had received previous chemotherapy regimens. The colon/rectum was the most commonly noted primary disease site. All patients were evaluable for toxicity during the first cycle, and for efficacy. Twenty-one patients received 42 cycles of treatment. The median number of cycles administered per patient was one (range, 1–8).

Dose escalation and identification of DLT, MTD, and the recommended phase II dose

The DLT in this study were leukopenia, neutropenia, thrombocytopenia, elevation of AST, colitis, and ileus. None of the patients treated at any dose of less than 800 mg/m^2 experienced DLT. At a dose of 900 mg/m^2, two of three patients experienced dose-limiting leukopenia, neutropenia, and thrombocytopenia, identifying this dose level as the MTD. At the same dose, grade 3 colitis and grade 3 AST elevation were observed in one patient. Therefore, three additional patients were enrolled at 800 mg/m^2. One of the additional three patients evaluated for safety at 800 mg/m^2 experienced

Table 2. Hematological toxicities during the first cycle of treatment with E7070

Toxicity	Grade	Dose (mg/m²)				
		400 (*n* = 3)	600 (*n* = 3)	700 (*n* = 6)	800 (*n* = 6)	900 (*n* = 3)
Neutropenia	1/2	0	0	3	1	0
	3/4	0	2	0	3	3
Leukopenia	1/2	1	2	3	3	1
	3/4	0	0	0	2	2
Thrombocytopenia	1/2	0	2	1	2	0
	3/4	0	0	0	1	3
Anemia	1/2	1	2	4	2	2
	3/4	0	0	0	1	1

DLT of grade 3 ileus. This patient had previously undergone intestinal surgery for colon cancer. On the basis of these findings, a total of six patients were treated at the dose of 800 mg/m² and one of the six patients experienced DLT. Thus, based on protocol-defined criteria, the MTD was estimated to be 900 mg/m². Therefore, a dose of 800 mg/m² is the recommended dose for single-agent phase II studies.

Hematological toxicity

Neutropenia, leukopenia, and thrombocytopenia were the hematological toxicities observed most commonly during the first cycle (Table 2). Neutropenia was the principal hematological toxicity in this study and was dose-limiting at 900 mg/m². Eight patients treated at 600, 800 and 900 mg/m² experienced grade 3 or more neutropenia. In these patients the median times to nadir neutrophil counts were 12.5 (8–25) days in the first cycle and 15.5 (8–25) days in all cycles, and the median times to recovery from nadir to grade 1 were 5.0 (3–15) days in the first cycle and 6.0 (3–15) days in all cycles. Neutrophil counts recovered to grade 1 within 21 days after E7070 infusion in all patients treated with 400 mg/m², but had not recovered by day 22 in two, one and two patients at 600, 700 and 800 mg/m², respectively. Neutrophil counts recovered by day 29 after E7070 infusion in all patients. G-CSF support was provided during cycle 1 in two of three patients treated at 900 mg/m². One patient treated at 800 mg/m² and three patients treated at 900 mg/m² experienced grade 3 thrombocytopenia. In patients treated at 800 and 900 mg/m², the median time to nadir platelet counts was 10.0 (7–12) days, and the median time to recovery from nadir to grade 1 was 5.0 (2–9) days in the first cycle. Anemia, reported in 13 (62%) patients, did not exceed grade 1–2 severity except in two patients at 800 and 900 mg/m². The numbers of patients with blood cell count toxicity did not tend to increase with increasing number of courses of treatment, suggesting that the hematological toxicity of E7070 is not cumulative.

Non-hematological toxicity

The non-hematological toxicities reported commonly during the first cycle were rash, fatigue, stomatitis, alopecia, injection site reaction, diarrhea and constipation (Table 3). These toxicities were generally mild. Grade 3 and grade 4 toxicities were reported in patients treated with 800 or 900 mg/m². Grade 3 ileus and grade 4 constipation associated with the ileus developed in one patient at 800 mg/m². Grade 3 AST elevation, grade 3 colitis and grade 3 diarrhea accompanying the colitis were observed in one patient at 900 mg/m². The toxicities reported most commonly in subsequent cycles were similar in terms of number of patients affected and severity to those reported during the first cycle of treatment.

Gastrointestinal toxicity, usually mild, was the most common non-hematological toxicity associated with E7070. Diarrhea (grades 1–3) was noted in eight (38%) patients during the first cycle, and the incidence was greater at the 800 mg/m² (5/6) and 900 mg/m² (3/3) doses than at the 400–600 mg/m² doses (none). Severe diarrhea (grade 3) was observed in only one patient, who received 900 mg/m² and had previously undergone surgery for primary colorectal cancer. In almost all cases, nausea and vomiting responded well to antiemetic therapies and patients were able to maintain good oral intake. Mild constipation (grades 1–2) was noted at 600–900 mg/m², except for one patient with grade 4 constipation associated with grade 3 ileus at 800 mg/m². Alopecia was observed in nine (43%) patients. Grades 1–2 injection site reaction, including irritation, pain, or phlebitis, developed in one patient at 400 mg/m² and three patients at 600 mg/m². Therefore, E7070 in 1000 mL of normal saline was given over 2 h at 700, 800, and 900 mg/m². However, three patients at 700 mg/m² and two patients at 800 mg/m² showed injection site reaction, and thus E7070 was given to patients at 900 mg/m² through a central vein. There were no deaths within 28 days of E7070 administration, and none of the deaths that occurred after the study was considered to have been treatment-related.

Pharmacokinetics

Complete pharmacokinetic data sets were obtained in 21 patients. The mean (+SD) plasma concentration-time curves of E7070 are shown in Figure 1. The mean (± SD) pharmacokinetic parameters derived from the plasma concentration are listed in Table 4. After the end of the infusion, plasma concentration of E7070 decreased rapidly for several hours, followed by a slower elimination phase (Fig. 1). During the elimination phase, the E7070 plasma concentration-time profile was convex, which is characteristic of non-linear pharmacokinetics. Maximum plasma concentrations (C_{max}) of E7070 at the 700–900 mg/m² doses were lower than that at 600 mg/m² (Table 4). This is probably related to the change of the infusion time of E7070 from 1 h to 2 h at doses over

 doi: 10.1111/j.1349-7006.2005.00109.x

Table 3. Non-hematological toxicities during the first cycle of treatment with E7070

Toxicity	Grade	Dose (mg/m²)				
		400 (n = 3)	600 (n = 3)	700 (n = 6)	800 (n = 6)	900 (n = 3)
Diarrhea	1/2	0	0	0	5	2
	3	0	0	0	0	1
Constipation	1/2	0	1	2	3	1
	3/4	0	0	0	1	0
Nausea	1/2	0	1	3	1	1
	3	0	0	0	1	0
Vomiting	1/2	0	1	0	0	0
	3	0	0	0	1	0
Anorexia	1/2	0	1	2	1	1
	3	0	0	0	1	0
Stomatitis	1/2	1	1	2	3	3
	3	0	0	0	0	0
Injection site reaction	1/2	1	3	3	2	0*
	3	0	0	0	0	0*
Rash	1/2	1	1	5	2	3
	3	0	0	0	0	0
Fatigue	1/2	1	2	2	3	3
	3	0	0	0	0	0
Headache	1/2	1	1	4	2	0
	3	0	0	0	0	0
Alopecia	1/2	0	1	2	3	3

*E7070 was administered through a central vein at a dose of 900 mg/m².

Table 4. Pharmacokinetic parameters of E7070

Dose (mg/m²)	No. patients	C_{max} (μg/mL)	AUC (μg·h/mL)	CL (mL/min per m²)	MRT (h)	$t_{1/2}$ (h)	V_{ss} (L/m²)	Urinary excretion (%)
400	3	82.2 ± 15.4	1066 ± 140	6.3 ± 0.9	26 ± 8	20 ± 5	9.8 ± 1.6	0.82 ± 0.22
600	3	142.8 ± 12.3	4204 ± 1353	2.6 ± 1.0	53 ± 17	32 ± 11	7.6 ± 0.0	1.67 ± 0.13
700	6	116.1 ± 11.3	3300 ± 1058	3.9 ± 1.3	41 ± 12	21 ± 7	8.7 ± 0.9	1.57 ± 0.39
800	6	117.7 ± 8.6	3943 ± 1243	3.6 ± 1.0	45 ± 11	22 ± 4	9.2 ± 0.8	1.77 ± 0.80
900	3	133.8 ± 0.7	6095 ± 1009	2.5 ± 0.4	59 ± 10	27 ± 8	8.7 ± 0.7	2.47 ± 1.33

C_{max}, maximum plasma concentration; AUC, area under the plasma concentration–time curve; CL, clearance; MRT, mean residence time; $t_{1/2}$, terminal elimination half-life; V_{ss}, distribution volume at steady state; urinary excretion, cumulative excreted amount of E7070 in urine.

600 mg/m² because of injection site reaction. The AUC increased more than expected with increasing dose. The clearance decreased between 400 and 900 mg/m², with mean values of 6.3 mL/min per m² to 2.5 mL/min per m². The mean plasma half-life ($t_{1/2}$) was between 20 and 32 h at the examined doses. Mean 72-h urinary excretion was 0.82% to 2.47% of the administered dose of E7070 in the five cohorts.

Pharmacodynamics

The pharmacodynamic analysis was performed by focusing on leukopenia, neutropenia and thrombocytopenia, because these were the DLT of E7070. Figure 2 shows that the nadirs of white blood cells (WBC), neutrophils, and platelets were related to the AUC of E7070. The percentage decrease rate from the value before dosing to the nadir of WBC, neutrophil or platelet count also showed a good correlation with the AUC of E7070.

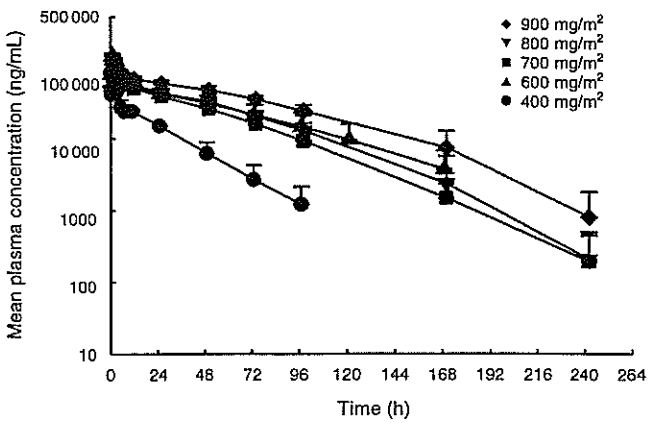

Fig. 1. Mean plasma concentrations of E7070 after single intravenous infusion at each dose level. Circles, 400 mg/m²; triangles, 600 mg/m²; squares, 700 mg/m²; inverted triangles, 800 mg/m²; diamonds, 900 mg/m². Each point represents the mean with standard deviation.

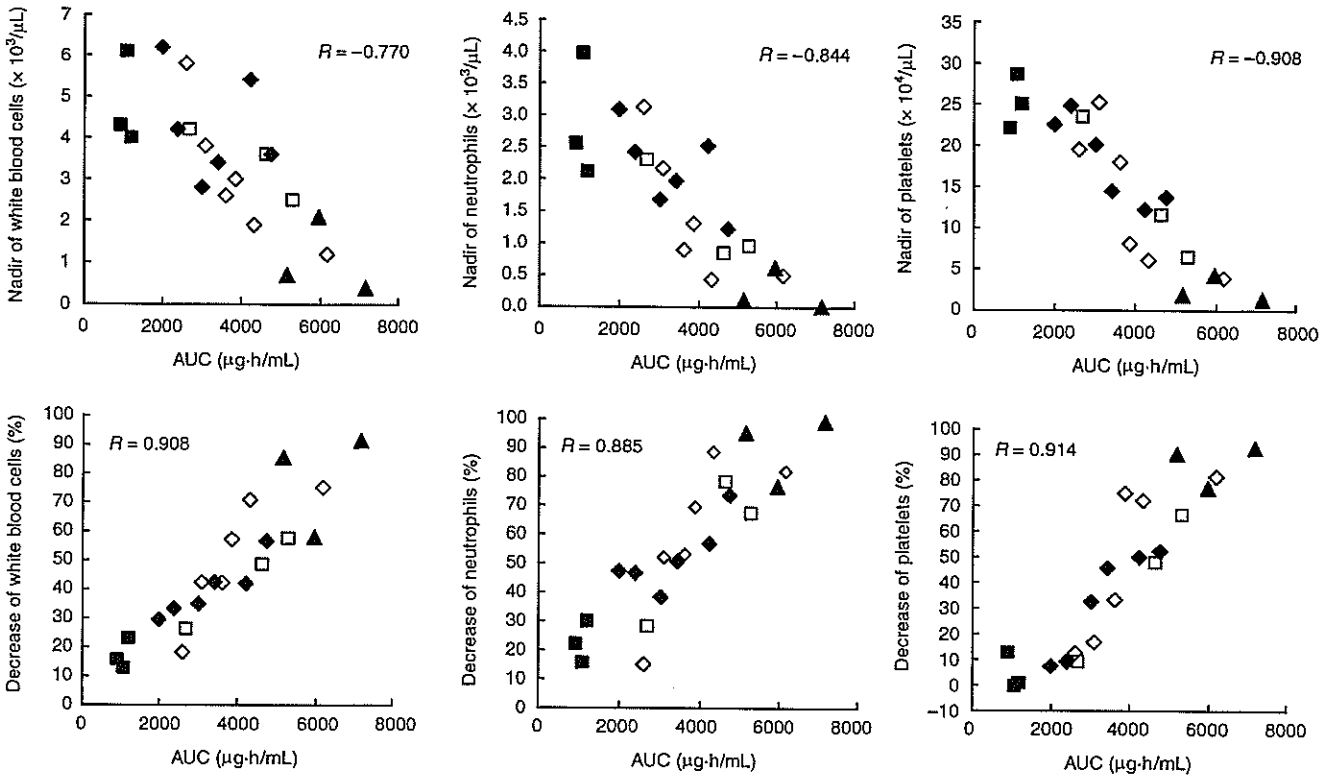

Fig. 2. Relationship between the area under the plasma concentration–time curve (AUC) of E7070 and white blood cells, neutrophil and platelet counts. Closed squares, 400 mg/m²; open squares, 600 mg/m²; closed diamonds, 700 mg/m²; open diamonds, 800 mg/m²; closed triangles, 900 mg/m². R, Pearson's correlation coefficient.

Genotyping of CYP2C9 and CYP2C19

The CYP2C9 and CYP2C19 genotypes were studied in 21 patients. Two (10%) were hetero EM for CYP2C9 (*1/*3), and 19 (90%) were homo EM for CYP2C9 (*1/*1). Five (24%) were PM for CYP2C19 (*2/*2 or *2/*3), eight (38%) were hetero EM for CYP2C19 (*1/*2 or *1/*3) and eight (38%) were homo EM for CYP2C19 (*1/*1). Figure 3 shows the relationship between dose and AUC of E7070 with respect to CYP2C9 and CYP2C19 genotypes. At a dose level of 600 mg/m² or more, the AUC of patients with mutant allele(s) (PM and hetero EM) of CYP2C9 or CYP2C19 were higher than those of the patients without mutant alleles (homo EM). DLT was observed in one CYP2C19 PM patient at 800 mg/m² and two CYP2C19 hetero EM or PM patients at 900 mg/m².

Antitumor activity

No objective clinical responses were observed, but liver metastasis was reduced by 22.5% at the 8th cycle of 700 mg/m² in one colorectal cancer patient, who had previously received 5-fluorouracil.

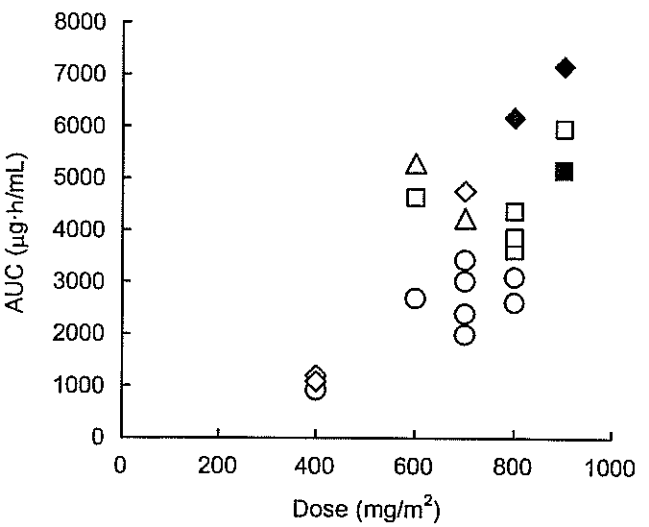

Fig. 3. Relationship between dose and area under the plasma concentration–time curve (AUC) of E7070 in relation to CYP2C9 and CYP2C19 genotypes. Circles, homo extensive metabolizers (EM) for both CYP2C9 and CYP2C19; squares, homo EM for CYP2C9 and hetero EM for CYP2C19; triangles, hetero EM for both CYP2C9 and CYP2C19; diamonds, homo EM for CYP2C9 and poor metabolizers (PM) for CYP2C19. Patients with dose-limiting toxicity are indicated with closed symbols.

Discussion

This phase I study was conducted to determine the MTD of E7070 administered by intravenous infusion over 1–2 h every 21 days, to determine the recommended single-agent dose for phase II studies, and to characterize the safety, pharmacokinetic and pharmacodynamic profiles of E7070. The

 doi: 10.1111/j.1349-7006.2005.00109.x

MTD in this study was estimated to be 900 mg/m^2, and the recommended dose for phase II studies is 800 mg/m^2. DLT observed at 900 mg/m^2 included leukopenia, neutropenia, thrombocytopenia, elevation of AST, and colitis.

The hematological abnormalities most commonly reported during the first cycle of treatment were neutropenia, leukopenia, and thrombocytopenia. Neutropenia, which tended to be dose-dependent, but not course-dependent, was the principal hematological toxicity in this study and was dose-limiting at 900 mg/m^2. At the recommended dose of 800 mg/m^2, the mean recovery time of neutrophils from day 1 to grade 1 neutropenia was 24.0 ± 6.1 days. Therefore, bone marrow recovery should be confirmed before the start of successive treatment cycles. Hematological toxicities were also dose-limiting in four previous phase I trials of E7070.[5–8]

The non-hematological toxicities most commonly reported during the first cycle of treatment were rash, fatigue, stomatitis, alopecia, injection site reaction, diarrhea and constipation. The type and incidence of the frequently noted events were generally consistent across dosages and cycles of treatment. Gastrointestinal toxicity, the most common non-hematological type of toxicity associated with E7070, was usually mild and well-controlled with medication. The frequency of diarrhea increased with dose, and grade 3 severe diarrhea and colitis were observed only in one patient at 900 mg/m^2; this patient had previously undergone intestinal surgery for colon cancer. Diarrhea was a dose-limiting toxicity in two previous phase I trials of E7070.[6,7] Because of the relatively high frequency and dose-dependency of diarrhea in this study, patients receiving E7070 should be carefully monitored for diarrhea. Grade 3 ileus followed by grade 4 constipation was reported in one patient treated with 800 mg/m^2 of E7070. Although this event appeared to be related to peritoneal dissemination, its onset after 7 days of E7070 infusion suggested that it might have been induced by E7070. None of the patients treated at less than 800 mg/m^2 had grade 2 or higher nausea, vomiting, or anorexia.

Grades 1–2 rash, commonly localized to the face, anterior chest, and upper back, with mild itching, was observed in 12 patients given 400–900 mg/m^2 of E7070. Its frequency and severity were not dose-dependent. Rashes recovered within a week after the administration of E7070, and skin toxicity did not interrupt the therapy in any patient. Injection site reaction (grades 1–2) was reported in nine (43%) patients. The frequency of this event did not seem to be dose-dependent, suggesting that it was related to infusion irritation by E7070, rather than hemolysis or thrombosis. E7070 shows similarities to chloroquinoxaline sulfonamide, which is known to cause hypoglycemia and cardiac tachycardia.[13] However, no hypoglycemia or cardiac arrhythmia was observed in this phase I trial of E7070.

The results of pharmacokinetic analysis suggested that the AUC of E7070 was non-linearly related to dose within the dose range of 400–900 mg/m^2. The clearance seemed to decrease, with a disproportionate increase in AUC. These results were in agreement with those obtained in other phase I trials with Caucasian patients.[5–8] This non-linearity was prominent at higher dose levels and is likely to be a complex consequence of saturation of metabolism, protein binding and distribution of E7070.[14] The absolute values of nadirs and the decrease ratios of WBC, neutrophil and platelet counts were apparently correlated with the AUC of E7070. *In vitro* experiments have shown that E7070 has the potential to inhibit CYP2C9 and CYP2C19, suggesting that these CYP may be involved in the metabolism of E7070.[10] In fact, other *in vitro* experiments have shown that CYP2C9 and CYP2C19 are responsible for the metabolism of E7070 (unpublished data). Since these CYP show genetic polymorphism, there is a possibility that subjects with one or more mutant alleles of these CYP have decreased clearance for any compound that is mainly metabolized by these polymorphic CYP. Therefore, we were prompted to investigate the relationship of the pharmacokinetics of E7070 with CYP2C9 and CYP2C19 genotype in this trial. At a dose level of 600 mg/m^2 or more, the AUC of patients with mutant allele(s) (PM and hetero EM) of CYP2C19 were higher than those of the patients without mutant alleles (homo EM). These results imply that the presence of mutant allele(s) of CYP2C19 may result in a decrease in the clearance of E7070 (Fig. 3), and support the involvement of CYP2C19 in the metabolism of E7070, as suggested from *in vitro* studies. The difference of AUC between CYP2C19 homo EM and PM was not clear at a dose of 400 mg/m^2. This was probably because metabolic capacity was less saturated at the low dose of 400 mg/m^2 compared with the higher doses, and so intergenotypic differences did not appear. The influence of the CYP2C9 genotype on the AUC of E7070 was not clarified because only two subjects had a mutant allele of this gene. The incidence of CYP2C9 PM is known to be less in Asian (< 1%) than in Caucasian (< 10%) people,[15,16] whereas CYP2C19 PM is more frequent in Asian (20%) than in Caucasian (< 1%) people.[17] Due to the low frequency of mutation of CYP2C9 in Asian populations, investigation of the effect of CYP2C9 on the pharmacokinetics of E7070 might be difficult in Japanese subjects. Research on subjects with various racial origins would be necessary for evaluation of the clinical impact of the CYP2C9 genotype. In any case, because of the small number of subjects in the present study, further studies should be taken into consideration to assess whether either the CYP2C9 or CYP2C19 genotype is of any clinical significance from the viewpoints of safety and efficacy of E7070. Urinary excretion of unchanged E7070, up to 72 h after the start of administration, was only 0.82–2.47% of the administered dose, indicating that renal clearance plays only a minor role in the elimination of E7070.

Although clinical efficacy (in terms of confirmed partial or complete responses) of E7070 was not demonstrated in this study, one patient with liver metastasis from colon cancer had a reduction in tumor size of ≤ 22.5% and demonstrated stable disease lasting 5 months. A phase II trial of E7070 as a single agent in 5-fluorouracil-resistant or refractory colorectal cancer showed limited activity with a 4% response rate,[18] and thus further clinical studies of combination therapy with irinotecan (CPT-11) are ongoing for the treatment of this tumor type.

In conclusion, the MTD of E7070 administered intravenously in a 1–2 h infusion every 3 weeks was estimated to be 900 mg/m^2 and the recommended dose for a phase II study is 800 mg/m^2. At 800 mg/m^2, hematological toxicities were manageable. Gastrointestinal toxicity was the most common non-hematological toxicity associated with E7070, but was generally well controlled with premedication. However, this recommended dose might be influenced by the CYP2C19

genotype and possibly by the CYP2C9 genotype as well. E7070 seems to be an interesting agent with novel cell-cycle-inhibitory effects. Additional phase I and II studies are currently ongoing in various tumor types to explore further the antitumor activity of this drug as a single agent and in combination with other chemotherapeutic agents.

References

1 Fukuoka K, Usuda J, Iwamoto Y *et al.* Mechanism of action of the novel sulfonamide anticancer agent E7070 on cell cycle progression in human non-small cell lung cancer cells. *Invest New Drugs* 2001; **19**: 219–27.
2 Sugi N, Ozawa Y, Watanabe T *et al.* A novel agent ER-35744, targeting G1 phase. II: Antitumor activities *in vitro* and *in vivo*. *Proc Am Assoc Cancer Res* 1996; **37**: A2668.
3 Yokoi A, Kuromitsu J, Kawai T *et al.* Profiling novel sulfonamide antitumor agents with cell-based phenotypic screens and array-based gene expression analysis. *Mol Cancer Ther* 2002; **1**: 275–86.
4 Ozawa Y, Sugi NH, Nagasu T *et al.* E7070, a novel sulphonamide agent with potent antitumour activity *in vitro* and *in vivo*. *Eur J Cancer* 2001; **37**: 2275–82.
5 Raymond E, ten Bokkel Huinink WW, Taieb J *et al.* Phase I and pharmacokinetic study of E7070, a novel chloroindolyl sulfonamide cell-cycle inhibitor, administered as a one-hour infusion every three weeks in patients with advanced cancer. *J Clin Oncol* 2002; **20**: 3508–21.
6 Punt CJ, Fumoleau P, van de Walle B, Faber MN, Ravic M, Campone M. Phase I and pharmacokinetic study of E7070, a novel sulfonamide, given at a daily times five schedule in patients with solid tumors. A study by the EORTC-early clinical studies group (ECSG). *Ann Oncol* 2001; **12**: 1289–93.
7 Dittrich C, Dumez H, Calvert H *et al.* Phase I and pharmacokinetic study of E7070, a chloroindolyl-sulfonamide anticancer agent, administered on a weekly schedule to patients with solid tumors. *Clin Cancer Res* 2003; **9**: 5195–204.
8 Terret C, Zanetta S, Roche H *et al.* Phase I clinical and pharmacokinetic study of E7070, a novel sulfonamide given as a 5-day continuous infusion repeated every 3 weeks in patients with solid tumours. A study by the EORTC Early Clinical Study Group (ECSG). *Eur J Cancer* 2003; **39**: 1097–104.
9 Thijssen HH, Flinois JP, Beaune PH. Cytochrome P4502C9 is the principal catalyst of racemic acenocoumarol hydroxylation reactions in human liver microsomes. *Drug Metab Dispos* 2000; **28**: 1284–90.
10 Van den Bongard HJGD, Sparidans RW, Critchley DJP, Beijnen JH, Schellens JHM. Pharmacokinetic drug-drug interaction of the novel anticancer agent E7070 and acenocoumarol. *Invest New Drugs* 2004; **22**: 151–8.
11 Therasse P, Arbuck SG, Eisenhauer EA *et al.* New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205–16.
12 Owa T, Yoshino H, Okauchi T *et al.* Discovery of novel antitumor sulfonamides targeting G1 phase of the cell cycle. *J Med Chem* 1999; **42**: 3789–99.
13 Rigas JR, Tong WP, Kris MG, Orazem JP, Young CW, Warrell RP Jr. Phase I clinical and pharmacological study of chloroquinoxaline sulfonamide. *Cancer Res* 1992; **52**: 6619–23.
14 Van den Bongard HJGD, Pluim D, van Waardenburg RC, Ravic M, Beijnen JH, Schellens JHM. *In vitro* pharmacokinetic study of the novel anticancer agent E7070: red blood cell and plasma protein binding in human blood. *Anticancer Drugs* 2003; **14**: 405–10.
15 Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 1999; **353**: 717–9.
16 Takahashi H, Echizen H. Pharmacogenetics of warfarin elimination and its clinical implications. *Clin Pharmacokinet* 2001; **40**: 587–603.
17 De Morais SM, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, Goldstein JA. Identification of a new genetic defect responsible for the polymorphism of (*S*)-mephenytoin metabolism in Japanese. *Mol Pharmacol* 1994; **46**: 594–8.
18 Mainwaring PN, van Cutsem E, van Laethem JL *et al.* A multicentre randomised phase II study of E7070 in patients with colorectal cancer who have failed 5-fluorouracil based chemotherapy. *Proc Am Soc Clin Oncol* 2002; **21**: 611.

Acknowledgments

We thank Mr Nozomu Koyanagi, Mr Makoto Shiba, Ms. Mayumi Suzuki, Mr Masaki Tanaka, Mr Masakiyo Kato, Mr Yuichi Inai and Dr Tatsuo Watanabe of Eisai Co. for data management and clinical research coordination.

 doi: 10.1111/j.1349-7006.2005.00109.x

Lung Cancer (2005) 49, 217–223

ELSEVIER

LUNG CANCER

www.elsevier.com/locate/lungcan

Clinical responses of large cell neuroendocrine carcinoma of the lung to cisplatin-based chemotherapy

Shigeo Yamazaki[a,d], Ikuo Sekine[a,*], Yoshihiro Matsuno[b], Hidefumi Takei[c], Noboru Yamamoto[a], Hideo Kunitoh[a], Yuichiro Ohe[a], Tomohide Tamura[a], Tetsuro Kodama[a], Hisao Asamura[c], Ryosuke Tsuchiya[c], Nagahiro Saijo[a]

[a] *Division of Thoracic Oncology and Internal Medicine, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan*
[b] *Division of Clinical Laboratory, National Cancer Center Hospital, Tokyo*
[c] *Division of Thoracic Surgery, National Cancer Center Hospital, Tokyo*
[d] *Department of Surgery, Keiyu-kai Sapporo Hospital, Sapporo, Japan*

Received 30 September 2004; received in revised form 3 January 2005; accepted 3 January 2005

KEYWORDS
Neuroendocrine carcinoma;
Lung cancer;
Chemotherapy;
Cisplatin

Summary
Background: The efficacy of chemotherapy in patients with large cell neuroendocrine carcinoma of the lung (LCNEC) remains unclear.
Methods: Patients with LCNEC who received cisplatin-based chemotherapy were identified by reviewing 567 autopsied and 2790 surgically resected lung cancer patients. The clinical characteristics and objective responses to chemotherapy in these patients were analyzed.
Results: Overall, 20 cases of LCNEC were identified, including stage IIIA ($n=3$), stage IIIB ($n=6$), stage IV ($n=6$) and postoperative recurrence ($n=5$) cases. Six patients had received prior chemotherapy, and 14 were chemo-naive patients. The patients had received a combination of cisplatin and etoposide ($n=9$), cisplatin, vindesine and mitomycin ($n=6$), cisplatin and vindesine ($n=4$), or cisplatin alone ($n=1$). One patient showed complete response and nine showed partial response, yielding an objective response rate of 50%. The response rate did not differ between patients with the initial diagnosis of SCLC and those with the initial diagnosis of NSCLC, however, the response rate in chemo-naive patients (64%) was significantly different from that in previously treated patients (17%).
Conclusions: Our results suggest that the response rate of LCNEC to cisplatin-based chemotherapy was comparable to that of SCLC.

* Corresponding author. Tel.: +81 3 3542 2511; fax: +81 3 3542 3815.
E-mail address: isekine@ncc.go.jp (I. Sekine).

0169-5002/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2005.01.008

1. Introduction

Pulmonary neuroendocrine tumors include a spectrum of four clinicopathological entities classified on the basis of the morphological and biological features: typical carcionoid and atypical carcinoid, which are tumors of low to intermediate grade malignancy, and large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCLC), which are high-grade malignant tumors. Travis et al. proposed the term LCNEC in 1991 [1], for classifying a type of poorly differentiated high-grade carcinoma characterized by a neuroendocrine appearance under light microscopy. LCNEC exhibits more prominent cellular pleomorphism and higher mitotic activity than the atypical carcinoid (AC), and is distinguished from SCLC by the tumor cell size and chromatin morphology. Although several different terminologies and classifications have been proposed previously, and even the present classification of pulmonary neuroendocrine tumors lacks uniform definition criteria, this class of tumors could become widely accepted and included in the updated histological classification of the World Health Organization [2].

The clinical features of LCNEC have not yet been completely clarified. The prognosis of patients with surgically resected LCNEC is reported to be intermediate between that of AC and SCLC [3–5], and the same as that of resected NSCLC, except that stage I LCNEC has a poorer prognosis than stage I non-small cell lung cancer (NSCLC) [6]. To the best of our knowledge, however, there are no studies that have examined the role of chemotherapy for LCNEC and the prognosis of patients with unresectable LCNEC, even though several reports have been published on the association between response to chemotherapy and the neuroendocrine differentiation of NSCLC [7–9]. The appropriate treatment of unresectable LCNEC, therefore, remains unclear. In the present study, we attempted to investigate the effectiveness of chemotherapy with cisplatin-based regimens for LCNEC in patients with unresectable and recurrent LCNEC.

2. Materials and methods

Eighty-seven of 2790 patients with primary lung cancer who underwent tumor resection from 1982 to 1999 at the National Cancer Center Hospital were found to have tumors with the histological characteristics of LCNEC [6]. Of these, five had received cisplatin-based chemotherapy at the time of recurrence, and were enrolled as subjects of this study. In addition, 303 of 567 patients who were autopsied from 1983 to 1997 at the National Cancer Center Hospital who had the following histological diagnoses were first selected: SCLC ($n=112$), poorly differentiated adenocarcinoma ($n=99$), large cell carcinoma ($n=58$), poorly differentiated squamous cell carcinoma ($n=29$), poorly differentiated adenosquamous carcinoma ($n=2$), LCNEC ($n=2$), and carcinoid ($n=1$). Of these, 161 had received cisplatin-based chemotherapy were selected for a pathological review. Finally, specimens from 17 of these161 cases were found to have histological characteristics consistent with the diagnosis of LCNEC, and were selected as subjects of this study. We focused on cisplatin, because since the 1980s, cisplatin has been the only anticancer agent with proven efficacy against both SCLC and NSCLC [10,11]; we, therefore, considered that the effectiveness of chemotherapy for LCNEC could be reasonably evaluated if cisplatin were included in the regimen. Cases which had received adjuvant chemotherapy without evaluable lesions were excluded from the analysis.

All the available paraffin-embedded tissue sections stained with hematoxylin–eosin were reviewed. We classified LCNEC according to the histopathological criteria in the WHO classification [2]. Immunohistochemical analysis was performed to confirm the neuroendocrine features of the tumors. For this purpose, formalin-fixed paraffin sections were stained for a panel of neuroendocrine markers, including chromogranin A (CGA), synaptophysin (SYN), and neural cell adhesion molecule (NCAM), using standard methods. The intensity of immunostaining for these markers was scored as follows: +, when the proportion of stained tumor cells was >50%; ±, when 10–50% of tumor cells were stained; and −, when <10% of tumor cells were stained, as previously described [6]. One case included in this study had the typical histological features of LCNEC, but no neuroendocrine features as determined by the immunohistochemical analysis. For specimens obtained after treatment, we routinely confirmed that the histopathological and morphological features showed no changes due to treatment as compared with the pretreatment biopsy or cytologic specimens. Such cases for which no pretreatment samples were available were excluded from the study; since it has been reported that histological changes may occur after treatment in SCLC [12], we were concerned that misdiagnosis might occur if the same were also true for LCNEC.

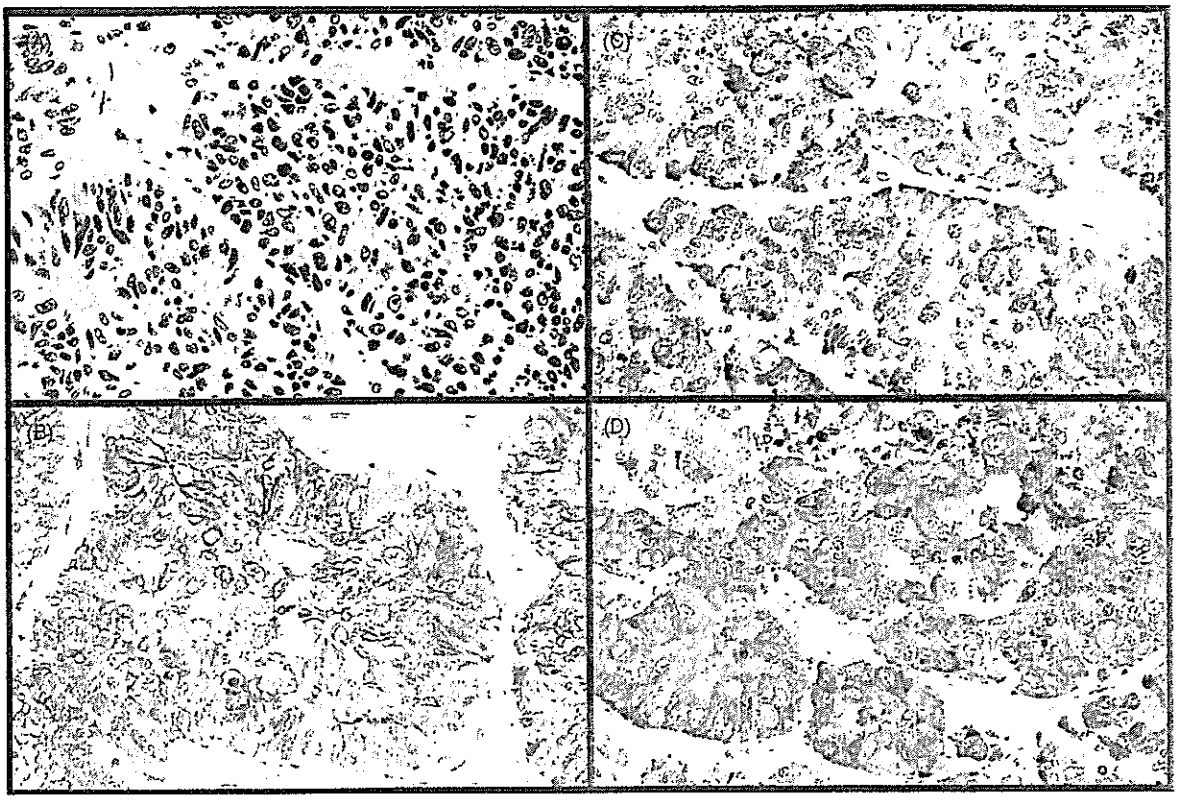

Fig. 1 Case no. 2, 57-year-old man. (A) The tumor cells which are large-sized, polygonal in shape and have a low nuclear-cytoplasmic ratio, are arranged in organoid nests and trabeculae (H&E stain, ×200). Positive staining for neural cell adhesion molecule (B), chromogranin A (C), and synaptophysin (D) (immunostain, ×400).

Clinical information about the cases was obtained from the medical records. The clinical disease staging was reassessed according to the latest International Union Against Cancer (UICC) staging criteria [13]. The response to chemotherapy and overall survival rate were assessed retrospectively. The objective tumor response was evaluated according to the WHO criteria published in 1979 (WHO, 1979) [14]. The survival time was measured from the date of start of chemotherapy with a cisplatin-containing regimen. Survival curves were drawn using the Kaplan–Meier method [15]. Drug toxicity could not be assessed as the study was a retrospective one and records were often incomplete.

3. Results

Overall, 22 cases were recognized as having tumors with histological characteristics consistent with LCNEC among the autopsied and surgically resected cases of primary lung cancer that had received cisplatin-based chemotherapy and had evaluable lesions; of these 17 were autopsied cases and five were surgically resected cases. Two of the autopsied cases were excluded, because no pretreatment pathological or cytological samples were available. The typical microscopic appearance of the tumor specimens is shown in Fig. 1A. The specimen sources for the prechemotherapy-diagnosis included surgically resected specimens ($n=5$), biopsy specimens ($n=9$), and cytology specimens ($n=6$). The histological and cytological findings in the specimens obtained before chemotherapy were consistent with those in the specimens obtained after chemotherapy. We therefore finally enrolled 20 cases in this study. The initial pathologic diagnoses in these patients were as follows: small cell carcinoma ($n=10$), poorly differentiated adenocarcinoma ($n=6$), large cell carcinoma ($n=2$), undifferentiated carcinoma ($n=1$), and poorly differentiated carcinoma ($n=1$) (Table 1). None of the cases had been labeled as LCNEC at the time of initial diagnosis, probably because the concept of LCNEC

Table 1 Patient characteristics

Characteristics	N	%
No. of patients	20	
Sex		
Male	18	90
Female	2	10
Age, median (range)	58 (37–74)	
Smoking history		
Yes	19	95
No	1	5
Performance status		
1–2	19	95
>2	1	5
Initial pathological diagnosis		
Small cell carcinoma	10	50
Adenocarcinoma	6	30
Large cell carcinoma	2	10
Others	2	10
Clinical stage at the start of chemotherapy		
IIIA	3	15
IIIB	6	30
IV	6	30
Postoperative recurrence	5	25
Prior treatment		
None	10	50
Surgery	4	20
Radiotherapy	2	10
Chemotherapy without cisplatin	6	30

Table 2 Staining for neuroendocrine markers in 20 LCNECs

Case	NCAM	CGA	SYN
1	+	+	+
2	+	+	+
3	+	+	+
4	±	+	+
5	+	+	+
6	+	+	+
7	−	+	−
8	+	−	−
9	−	−	−
10	−	+	±
11	+	−	+
12	+	+	+
13	+	+	+
14	+	−	±
15	+	+	+
16	+	−	NA
17	+	−	+
18	+	−	NA
19	+	−	+
20	−	+	+

NCAM, neural cell adhesion molecule; CGA, chromogranin A; SYN, synaptophysin; NA, not assessed.

was not completely accepted at our hospital at that time.

The results of the immunohistochemical staining are shown in Table 2, and a typical case showing positive staining is shown in Fig. 1B and D. Of the 20 LCNECs, 19 expressed at least one of the three general neuroendocrine markers, namely CGA, SYN, and NCAM. Sixteen of the 20 LCNECs exhibited positive staining for NCAM, while one showed equivocal staining. Twelve of the 20 LCNECs showed positive staining for CGA. Thirteen LCNECs showed positive staining for SYN and three showed equivocal staining. Only one case was negative for all the three general neuroendocrine markers, however, this case exhibited the typical histological features of LCNEC on light microscopy.

The clinical characteristics of the patients are summarized in Table 1. The extremely high predominance of men and smokers in this study was comparable to the demographic features of our LCNEC patients treated by surgical resection [6]. Previous chemotherapy was given in six patients: nedaplatin in one and cyclophosphamide-based regimen in five patients. The chemotherapy regimens used were as follows: cisplatin (80 mg/m^2, day 1) and etoposide (100 mg/m^2, days 1–3) (n = 9), cisplatin (80 mg/m^2, day 1), vindesine (3 mg/m^2, days 1 and 8) and mitomycin (8 mg/m^2, day 1) (n = 6), cisplatin (80 mg/m^2, day 1) and vindesine (3 mg/m^2, days 1 and 8) (n = 4), or cisplatin (100 mg/m^2, day 1) alone (n = 1). The median (range) number chemotherapy cycles were 2 (1–6). Of the 20 patients, one showed CR and nine showed PR, yielding an overall response rate of 50% (95% confidence interval, 27.2–72.8%). One CR and four PRs were observed among the cases treated with cisplatin and etoposide, two PRs were found among those treated with cisplatin, vindesine and mitomycin, and three PRs were found among those treated with cisplatin and vindesine. Seven patients showed NC, and three showed progressive disease. While the response rate did not differ between patients with an initial diagnosis of SCLC and those patients with an initial diagnosis of NSCLC, previous chemotherapy affected the response to cisplatin: the response rate in chemo-naive patients was 64%, whereas that in previously treated patients was 17%. The median progression-free survival in the 20 patients was 103 days, median survival was 239 days, 1-year survival rate was 35%, and 2-year survival rate was 15%.

4. Discussion

In this extensive review of over 3000 lung cancer patients, we found considerable difficulty in evaluating the response of LCNEC to systemic chemotherapy. The pathological diagnosis of LCNEC was established in 87 (3.1%) of 2790 patients treated by surgical resection. This low incidence of LCNEC in surgically treated lung cancer patients is comparable to that in other previously published reports: 2.4% (50/2070), 2.9% (22/766), and 3.6% (53/1530) [16–18]. Of the 87 patients, only five who had received cisplatin-based chemotherapy for recurrent tumor that was evaluable for the response. While LCNEC is difficult to diagnose prior to the start of treatment on the basis of the findings in biopsy or cytological specimens, the architectural neuroendocrine features may, more or less, be reflected in these small samples [19,20]. We, therefore, conducted a review of 567 autopsy cases of lung cancer, and identified 15 cases of LCNEC who had received cisplatin-based chemotherapy. We obtained a response rate to cisplatin-based chemotherapy of 50% in these 20 patients with LCNEC, however, the clinical characteristics of patients with medically treatable advanced LCNEC would still remain to be clarified, because autopsy is conducted only in highly selective cases.

Travis et al. suggested that immunohistochemical or electron-microscopic evidence of neuroendocrine features were important to diagnose LCNEC [1]. We assessed the neuroendocrine marker expression by immunohistochemical staining for CGA, SYN, and NCAM. Our cases included one that was negative for all the three neuroendocrine markers examined, but showed the typical histological features of LCNEC, which could be attributable to technical staining problems. Immunohistochemical staining for neuroendocrine tumors is generally recognized as only a supplementary diagnostic tool. In addition, the post-surgical survival rate did not differ between histologically diagnosed cases of LCNEC with neuroendocrine differentiation in marker expression as assessed by immunohistochemical staining and large cell carcinoma with neuroendocrine morphology where the neuroendocrine markers were negative (data not shown). Thus, we decided to include the case with negative staining as LCNEC on the basis of its typical neuroendocrine morphology.

To the best of our knowledge, only one study on the efficacy of chemotherapy in patients with LCNEC has been reported previously. In the study, 13 patients with LCNEC received chemotherapy when relapse was noted after surgical resection, and two (20%) of 10 evaluable patients showed an objective response. The evaluable lesion in these patients, however, was the brain in seven, liver in two, and bone in one patient [21]. Thus, the relatively low response rate in the report may be due to the site of the evaluable lesion. In addition, reports on the correlation between response to chemotherapy and neuroendocrine differentiation of NSCLC may be helpful. Graziano et al. reported that the proportion of NSCLC positive for neuroendocrine markers was higher in responders than in non-responders among 52 NSCLC patients treated by chemotherapy, and that the result suggested a correlation between positivity for neuroendocrine marker expression and the likelihood of response to chemotherapy [7]. On the other hand, others have reported the absence of any correlation between the presence of neuroendocrine differentiation and the response to chemotherapy [8,9]. The neuroendocrine differentiation in NSCLCs in the aforementioned studies was confirmed only by immunohistochemical staining and not on the basis of the morphological definition of LCNEC. Therefore, these groups might have potentially included heterogeneous subtypes of lung carcinoma, such as adenocarcinoma or squamous cell carcinoma, with components of neuroendocrine differentiation. The conflicting conclusions of these studies may, therefore, reflect differences in the biological characteristics of the tumors included in the analysis. Since the definition of LCNEC is based on morphological criteria as well as positivity for neuroendocrine marker expression, LCNEC is may be considered to be a clinically homogeneous group. Therefore, our study of LCNEC may endorse the former reports about the relationship between neuroendocrine differentiation and the sensitivity to chemotherapy.

Objective response to chemotherapy can be observed in only 15–30% of NSCLCs, even when they are treated with regimens containing cisplatin [10]. In SCLC, however, effective combination regimens yield objective response rates in the range of 80–90% [11]. Our study showed an overall response rate of LCNEC of 50% to cisplatin-based chemotherapy, and a response rate of 64% in chemo-naive patients, which seemed to be higher than the response rate of NSCLC to chemotherapy. Considered together, these results suggest that the chemosensitivity of LCNEC is intermediate between that of NSCLC and SCLC, although we were unable to obtain firm evidence from this retrospective study, which included only a small cohort of patients.

Since LCNEC is a relatively rare subtype of lung cancer, a prospective study is difficult to perform, and may only be possible as a multicenter study.

For this purpose, it is an urgent task to establish diagnostic criteria for LCNEC based on examination of biopsy or cytologic specimens. Although the histological definition of LCNEC in surgically resected specimens proposed by Travis et al. is commonly accepted, its diagnostic reproducibility is not satisfactory [22]. It is also difficult to apply the definition to biopsy specimens, in which artifacts can easily be produced and detailed examination may be difficult due to insufficient specimen size. Thus, definitive diagnostic criteria also applicable to biopsy and cytologic specimens are required.

Our study did not include any cases labeled as LCNEC at the time of initial diagnosis. One half of the cases was originally diagnosed as SCLC and the other half as NSCLC, including poorly differentiated adenocarcinoma and large cell carcinoma. This was attributed to the fact that the concept of LCNEC was not clearly defined prior to its being proposed by Travis et al. [1]. Thus, it is possible that patients with LCNEC were included in earlier clinical trials for NSCLC or SCLC. If LCNEC shares the poor prognosis of NSCLC, the reported results of chemotherapy for NSCLC may have been worse in studies in which cases of LCNEC were included. Similarly, the results of clinical studies of SCLC to study their objective response to chemotherapy may also have been worse because of the confounding effects of the inclusion of LCNECs among the cases.

In conclusion, our results suggest that the response rate of LCNEC to cisplatin-based chemotherapy was comparable to that of SCLC. However, because of the retrospective nature of this study and the small sample size, we could not arrive at any definitive conclusion; we, therefore, propose to conduct a prospective study in the future aimed at elucidating the efficacy of chemotherapy for LCNEC. To that end, firm diagnostic criteria for LCNEC need to be established, even when the diagnosis must be based only on examination of biopsy and cytology specimens.

Acknowledgment

We thank Ms. Yuko Yabe for kindly preparing this manuscript.

References

[1] Travis WD, Linnoila RI, Tsokos MG, Hitchcock CL, Cutler Jr GB, Nieman L, et al. Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma. An ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. Am J Surg Pathol 1991;15:529–53.

[2] Travis W, Corrin B, Shimosato Y, Brambilla E. Histological typing of lung and pleural tumours. Berlin: Springer-Verlag; 1999.

[3] Rusch VW, Klimstra DS, Venkatraman ES. Molecular markers help characterize neuroendocrine lung tumors. Ann Thorac Surg 1996;62:798–809 [discussions 09–10].

[4] Dresler CM, Ritter JH, Patterson GA, Ross E, Bailey MS, Wick MR. Clinical-pathologic analysis of 40 patients with large cell neuroendocrine carcinoma of the lung. Ann Thorac Surg 1997;63:180–5.

[5] Travis WD, Rush W, Flieder DB, Falk R, Fleming MV, Gal AA, et al. Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. Am J Surg Pathol 1998;22:934–44.

[6] Takei H, Asamura H, Maeshima A, Suzuki K, Kondo H, Niki T, et al. Large cell neuroendocrine carcinoma of the lung: a clinicopathologic study of eighty-seven cases. J Thorac Cardiovasc Surg 2002;124:285–92.

[7] Graziano SL, Mazid R, Newman N, Tatum A, Oler A, Mortimer JA, et al. The use of neuroendocrine immunoperoxidase markers to predict chemotherapy response in patients with non-small-cell lung cancer. J Clin Oncol 1989;7:1398–406.

[8] Schleusener JT, Tazelaar HD, Jung SH, Cha SS, Cera PJ, Myers JL, et al. Neuroendocrine differentiation is an independent prognostic factor in chemotherapy-treated nonsmall cell lung carcinoma. Cancer 1996;77:1284–91.

[9] Carles J, Rosell R, Ariza A, Pellicer I, Sanchez JJ, Fernandez-Vasalo G, et al. Neuroendocrine differentiation as a prognostic factor in non-small cell lung cancer. Lung Cancer 1993;10:209–19.

[10] Ginsberg RJ, Vokes EE, Rosenzweig K. Non-small cell lung cancer. In: DeVita VT, Hellman S, Rosenberg SA, editors. Cancer: principles & practice of oncology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 925–83.

[11] Murren J, Glatstein E, Pass HI. Small cell lung cancer. In: DeVita VT, Hellman S, Rosenberg SA, editors. Cancer: principles & practice of oncology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 983–1018.

[12] Fushimi H, Kikui M, Morino H, Yamamoto S, Tateishi R, Wada A, et al. Histologic changes in small cell lung carcinoma after treatment. Cancer 1996;77:278–83.

[13] Sobin LH, Wittenkind C. TNM classification of malignant tumors. New York: Wiley-Liss; 1997.

[14] Handbook for reporting results of cancer treatment. Geneva: World Health Organization (WHO); 1979.

[15] Kaplan ELMP. Non-parametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–81.

[16] Jiang SX, Kameya T, Shoji M, Dobashi Y, Shinada J, Yoshimura H. Large cell neuroendocrine carcinoma of the lung: a histologic and immunohistochemical study of 22 cases. Am J Surg Pathol 1998;22:526–37.

[17] Iyoda A, Hiroshima K, Toyozaki T, Haga Y, Fujisawa T, Ohwada H. Clinical characterization of pulmonary large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine morphology. Cancer 2001;91:1992–2000.

[18] Paci M, Cavazza A, Annessi V, Putrino I, Ferrari G, De Franco S, et al. Large cell neuroendocrine carcinoma of the lung: a 10-year clinicopathologic retrospective study. Ann Thorac Surg 2004;77:1163–7.

[19] Yang YJ, Steele CT, Ou XL, Snyder KP, Kohman LJ. Diagnosis of high-grade pulmonary neuroendocrine carcinoma by fine-needle aspiration biopsy: nonsmall-cell or small-cell type? Diagn Cytopathol 2001;25:292–300.