dermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. J Clin Oncol 2002;20:4292—302.

[2] Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, 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—46.

[3] Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, 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—84.

[4] Kris MG, Natale RB, Herbst RS, Lynch Jr TJ, Prager D, Belani CP, 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—58.

[5] Rusch V, Baselga J, Cordon-Cardo C, Orazem J, Zaman M, Hoda S, 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.

[6] Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. New Eng J Med 2004;350:2129—39.

[7] Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497—500.

[8] Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutation are common in lung cancer from "never smokers" and are associated with sensitivity of tumors to gefitinib and elrotinib. Proc Natl Acad Sci USA 2004;101:13306—11.

[9] Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 2004;305:1163—7.

[10] Tracy S, Mukohara T, Hansen M, Meyerson M, Johnson BE, Jänne PA. Gefitinib induces apoptosis in the EGFR L858R non-small cell lung cancer cell line H3255. Cancer Res 2004;64:7241—4.

[11] Hampe J, Wollstein A, Lu T, Frevel HJ, Will M, Manaster C, et al. An integrated system for high throughput TaqMan based SNP genotyping. Bioinformatics 2001;17:654—5.

[12] Mc Guigan FE, Ralston SH. Single nucleotide polymorphism detection: allelic discrimination using TaqMan. Psychiatr Genet 2002;12:133—6.

[13] Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, et al. High-throughput genotyping with single nucleotide polymorphisms. Genome Res 2001;11:1262—8.

[14] Shi MM, Bleavins MR, de la Iglesia FA. Technologies for detecting genetic polymorphisms in pharmacogenomics. Mol Diagn 1999;4:343—51.

[15] Shi MM. Enabling large-scale pharmacogenetic studies by high-throughput mutation detection and genotyping technologies. Clin Chem 2001;47:164—72.

[16] Tanaka C, Kamide K, Takiuchi S, Miwa Y, Yoshii M, Kawano Y, et al. An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. Hypertens Res 2003;26:301—6.

[17] Robledo R, Beggs W, Bender P. A simple and cost-effective method for rapid genotyping of insertion/deletion polymorphisms. Genomics 2003;82:580—2.

[18] Sasaki H, Endo K, Konishi A, Takada M, Kawahara M, Iuchi K, et al. EGFR mutation status in Japanese lung cancer patients: genotyping analysis using LightCycler. Clin Cancer Res 2005;11:2924—9.

[19] Jänne PA, Gurubhagavatula S, Yeap BY, Lucca J, Ostler P, Skarin AT, et al. Outcomes of patients with advanced non-small cell lung cancer treated with gefitinib (ZD1839, "Iressa") on an expanded access study. Lung Cancer 2004;44:221—30.

[20] Hirsch FR, Varella-Garcia M, Bunn Jr PA, Di Maria MV, Veve R, Bremmes RM, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 2003;21:3798—807.

[21] Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 2005;97:643—55.

[22] Chen YC, Chen JH, Richard K, Chen PY, Christiani DC. Lung adenocarcinoma and human papillomavirus infection. Cancer 2004;15:1428—36.

[23] Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919—23.

[24] Huang S-F, Liu HP, Li LH, Ku YC, Fu YN, Tsai HY, et al. High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. Clin Cancer Res 2004;10:8195—203.

[25] Arao T, Fukumoto H, Takeda M, Tamura T, Saijo N, Nishio K. Small in-frame deletion in the epidermal growth factor receptor as a target for ZD6474. Cancer Res 2004;64:9101—4.

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Smoking history before surgery and prognosis in patients with stage IA non-small-cell lung cancer—a multicenter study

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Summary   The prognosis of lung cancer patients with surgically resected non-small-cell lung cancer (NSCLC) can be predicted generally from age, sex, histologic type, stage at diagnosis, and additional treatment. Nine studies have reported that a history of smoking before diagnosis influences the prognosis of the disease in lung cancer patients. In this study, a total of 3082 patients who underwent surgery and were diagnosed with primary pathological stage IA NSCLC at 36 national hospitals from 1982 to 1997 were analyzed for the effect of smoking on survival. Smoking history and other factors influencing either the overall survival or the disease-specific survival rates of patients were estimated with the Cox proportional hazards model. Multivariate analysis demonstrated significant associations between overall

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survival and age ($P < 0.0001$), sex ($P = 0.0002$), and performance status (PS) ($P < 0.0001$). Disease-specific survival was associated with age ($P = 0.0063$), sex (0.00161), and PS ($P = 0.0029$). In males, disease-specific survival was associated with age ($P = 0.0120$), PS ($P = 0.0022$), and pack-years (number of cigarette packs per day, and years of smoking) ($P = 0.0463$). These results indicate that smoking history (pack-years) is important clinical prognostic factor in estimating disease-specific survival, in male patients with stage IA primary NSCLC that has been surgically resected.

1. Introduction

The worldwide incidence and mortality from lung cancer have increased rapidly in recent decades [1]. Non-small-cell lung cancer (NSCLC) constitutes approximately 85% of all lung cancers [2]. Even after 30 years of improvements in therapeutic approaches, the 5-year mortality rate of all lung cancer remains an alarmingly high 85% [3]. The 5-year survival rate, even in the optimum surgical stage IA (T1N0M0), is 67% [4]. These poor survival rates are due primarily to recurrences [5] and second lung cancers [6].

The prognosis of lung cancer patients with surgically resected NSCLC can be predicted generally from age, sex, histologic type, stage at diagnosis, and additional treatment [4,7].

The impact of smoking history on survival is controversial. Nine studies have reported that smoking history is a negative prognostic factor in lung cancer [8–16]; whereas, others studies did not find an association [7,17–20].

Recently, Fujisawa et al. have reported that preoperative smoking history is an important clinical postoperative prognostic factor in estimating overall long-term survival in patients with primary resected stage I NSCLC [14].

The aim of this study was to evaluate the effect of smoking history on survival in patients with primary resected stage IA NSCLC.

2. Patients and methods

2.1. Patients

A Central registry for all lung cancer patients has been established in which 33,161 cases have been registered at 36 national hospitals that belong to the Japan National Chest Hospital Study Group for Lung Cancer from 1982 to 1997. We used the central registry data of surgical patients with NSCLC who had been newly diagnosed and undergone surgery. The study group comprised 3217 patients who underwent complete resection and were pathologi-

cally confirmed stage IA NSCLC. Ninety-one patients who were lack of smoking history or follow-up interval were excluded from survival analysis. In order to focus on long-term survival, 44 patients (11 with squamous cell carcinoma, 32 with adenocarcinoma, and 1 with large cell carcinoma; a total of 25 men and 19 women) who died within 1 month after surgery were excluded from the survival analyses [21]. Finally, 3082 patients were analyzed for survival analysis. The cancer histologic types included 840 squamous cell carcinomas, 2161 adenocarcinomas, and 81 large cell carcinomas. The patient group consisted of 1221 women and 1861 men who ranged in age from 22 to 89 years (mean age, 64.4 years). Histologic type and TNM classification were classified according to the criteria of World Health Organization. Performance status (PS) was classified according to the criteria of Eastern Cooperative Oncology group (ECOG). The data on smoking history (pack-years, number of packs per day, and years of smoking) were obtained from hospital records. Cause of death was reported by the doctor who followed the patient. At the last follow-up, for overall survival curves, an observation was censored if the patient was alive; for disease-specific curves, data were censored if the patient was alive or had died from a cause other than NSCLC.

2.2. Survival rate and statistical analysis

Overall survival was defined as the time between surgery and death or last follow-up evaluation. Disease-specific survival was defined as the time between surgery and cancer death or last follow-up evaluation.

Bivariate analysis was performed with Fisher's exact test. The difference in age between the two groups was analyzed with the Student's t-test. Overall survival and disease-specific survival were calculated with the Kaplan–Meier method, and the difference between survival curves was analyzed with the log-rank test. Variables in this study consist of age, sex, histologic type, tumor classification, and cigarette smoking before surgery. Multivariate analysis was performed with the Cox proportional

hazards model. All statistical analysis in this study was performed with StatView statistical software (StatView version 5.0 for Macintosh; SAS institute Inc., Cary, NC, USA). Statistical significant P-values were considered to be less than 0.05.

3. Results

3.1. Association between clinical features and smoking pack-years

Clinical features, including age, sex, PS, and histology, were evaluated according to smoking pack-years (Table 1). The heavy smokers group also had significantly higher population of older age, male patients, poor PS, and squamous cell carcinomas than smokers with less than 40 pack-years or non-smokers.

3.2. Cause of death

Forty-four patients died within 1 month after surgery (1.4% of 3126 patients). After a median follow-up of 3.9 years, of 3082 patients used for survival analysis, 491 patients died from recurrent or second lung cancer, and 159 patients died from non-recurrent diseases. Non-recurrent causes consisted of 27 second primary malignancies.

3.3. Overall survival and disease-specific survival

The overall and disease-specific 5- and 10-year survival curves are shown in Fig. 1. Fig. 2 demonstrates the overall survival and disease-specific survival curves according to cigarette smoking,
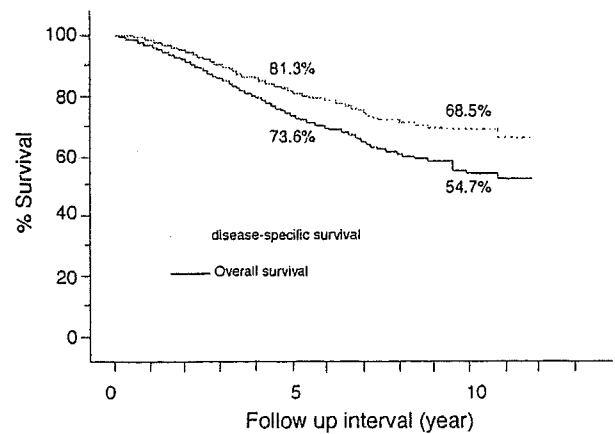
Fig. 1 Overall survival and disease-specific survival curves in patients with primary, surgically resected stage IA NSCLC.
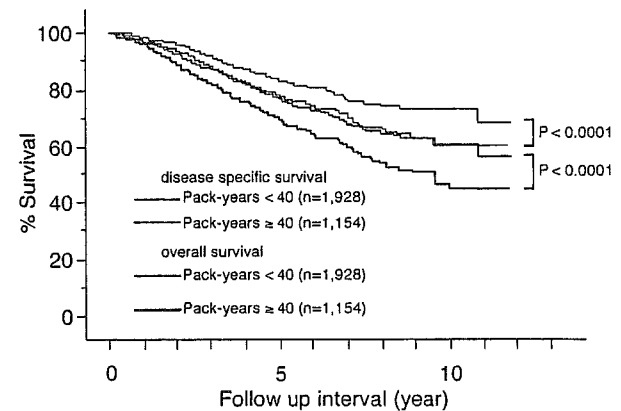
Fig. 2 Overall survival and disease-specific survival curves in patients with primary, surgically resected stage IA NSCLC, evaluated by pack-years.

Table 1   Distribution of clinical features, according to smoking pack-years

Clinical feature	Pack-years		P^a
	<40	≥40	
Age (mean ± S.D.)	62.8 ± 10.3	66.9 ± 8.2	<0.0001
Sex			
Male	770	1091	<0.0001
Female	1158	63	
PS			
0	1612	855	<0.0001
≥1	306	289	
Histology[b]			
Nonsquamous cell carcinoma	1660	582	<0.0001
Squamous cell carcinoma	268	572	

[a] P-value for age are by Student's t-test and for the remainder are for Fisher exact test.
[b] Nonsquamous cell carcinoma is comprised of adenocarcinoma and large cell carcinoma.

Table 2   Overall survival and disease-specific survival, according to clinical prognostic factors

Clinical feature	No. of patients	Overall survival (%)			Disease-specific survival (%)		
		5 years	10 years	P[a]	5 years	10 years	P[a]
Age (years)							
<70	2115	77.3	60.6	<0.0001	83.1	70.9	0.0003
≥70	961	64.4	36.3		76.1	60.7	
Sex							
Male	1861	70.7	47.3	<0.0001	79.2	63.0	<0.0001
Female	1221	78.1	66.7		84.5	76.7	
Histology[b]							
Squamous cell carcinoma	840	69.8	49.8	0.0041	79.8	61.5	0.0831
Nonsquamous cell carcinoma	2242	75.1	56.6		81.8	71.2	
Performance status							
0	2467	76.8	58.1	<0.0001	82.3	69.9	<0.0001
≥1	595	61.0	41.8		76.5	62.0	
Pack-years							
<40	1928	76.5	60.5	<0.0001	83.4	73.6	<0.0001
≥40	1154	69.0	45.7		77.8	60.3	

[a] P-value for the log-rank test.
[b] Nonsquamous cell carcinoma is comprised of adenocarcinoma and large cell carcinoma.

and the 5- and 10-year survival rates between heavy smokers (pack-years ≥ 40) and light smokers (pack-years < 40) are both significantly different ($P < 0.0001$).

Table 2 shows the overall and disease-specific 5- and 10-year survival rates according to several variables. Significant differences in overall survival were demonstrated with age ($P < 0.0001$), sex ($P < 0.0001$), histologic type ($P = 0.0041$), PS ($P < 0.0001$), and pack-years ($P < 0.0001$). But no significant difference in disease-specific survival was found with histologic type ($P = 0.0831$). With regard to cigarette smoking, the difference between heavy smokers and light smokers was statistically significant ($P < 0.0001$) in the both overall survival and disease-specific survival.

3.4. Multivariate analysis

Multivariate analysis was conducted with the Cox proportional hazards model with the five variables. Multivariate analysis demonstrated a significant association between overall survival and age ($P < 0.0001$), sex ($P = 0.0002$), and PS ($P < 0.0001$), but no association was observed with histologic type ($P = 0.3807$) or pack-years ($P = 0.1742$) (Table 3).

Next, multivariate analysis for disease-specific survival was performed with the five variables. Multivariate analysis demonstrated a significant association of disease-specific survival with age ($P = 0.0063$), sex ($P = 0.0161$), and PS ($P = 0.0029$),

and no significant association with histologic type ($P = 0.3935$) or pack-years ($P = 0.0741$) (Table 4).

We conducted a subgroup analysis for overall survival and disease-specific survival according to sex. In a subgroup analysis (Tables 3 and 4), disease-specific survival demonstrated a significant association with age ($P = 0.0120$), PS ($P = 0.0022$), and pack-years ($P = 0.0463$), and no significant correlation with histologic type ($P = 0.1971$). Similar trends were observed for overall survival among males, but pack-years was not a significant prognostic factor ($P = 0.1410$). On the other hand, the analyses for females, a considerably small proportion of heavy smokers (5.1%) gave an unstable odds ratio estimation (Tables 1, 3 and 4).

4. Discussion

In this study, more than 3000 patients with stage IA primary NSCLC that has been surgically resected were analyzed, and we found that older age, poor PS, male, and smoking history, in male, were significant unfavorable prognostic factors. We demonstrated the significant inverse correlation between cigarette smoking and long-term disease-specific survival in stage IA NSCLC patients using multivariate analysis. Even if a curative surgery has been underwent in a very early stage NSCLC, previous smoking history still was disadvantage.

The impact of smoking history on survival is still confusing. Earlier studies found no associ-

Table 3 Multivariate cox proportional hazards model analyses of various factors affecting overall survival in primary, resected stage IA NSCLC

Variable	Total			Male			Female		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Sex, male vs. female	1.422	1.179–1.715	0.0002	—	—	—	—	—	—
Age, ≥70 years vs. <70 years	1.548	1.325–1.808	<0.0001	1.605	1.333–1.605	<0.0001	1.470	1.106–1.470	0.0080
Performance status, ≥1 vs. 0	1.727	1.461–2.037	<0.0001	1.869	1.545–1.869	<0.0001	1.383	0.994–1.926	0.0543
Pack-years, ≥40 vs. <40	1.129	0.948–1.344	0.1742	1.148	0.955–1.379	0.1410	0.841	0.463–1.526	0.5697
Histologic type[a], squamous vs. nonsquamous	1.080	0.909–1.284	0.3807	1.168	0.973–1.401	0.0950	1.697	1.049–2.739	0.0309

[a] Nonsquamous cell carcinoma is comprised of adenocarcinoma and large cell carcinoma.

Table 4 Multivariate cox proportional hazards model analyses of various factors affecting disease-specific survival in primary, resected stage IA NSCLC

Variable	Total			Male			Female		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Sex, male vs. female	1.325	1.054–1.664	0.0161	—	—	—	—	—	—
Age, ≥70 years vs. <70 years	1.314	1.080–1.597	0.0063	1.350	1.068–1.706	0.0120	1.265	0.886–1.808	0.1949
Performance status, ≥1 vs. 0	1.387	1.119–1.718	0.0029	1.475	1.151–1.890	0.0022	1.088	0.705–1.680	0.7018
Pack-years, ≥40 vs. <40	1.218	0.981–1.511	0.0741	1.263	1.004–1.587	0.0463	0.768	0.351–1.675	0.5069
Histologic type[a], squamous vs. nonsquamous	1.098	0.886–1.360	0.3935	1.160	0.926–1.455	0.1971	1.517	0.810–2.849	0.1926

[a] Nonsquamous cell carcinoma is comprised of adenocarcinoma and large cell carcinoma.

ation between smoking history and lung cancer survival [17,18]; however, these studies did not utilize multivariate analysis. Harpole et al. reported a multivariate model that quantified the risk of recurrence and cancer death for patients with stage I NSCLC, but they demonstrated no significant impact on univariate analysis using smoking history [7]. Some other studies showed smoking history is negative prognostic factor [19,20]. However, their studies included relatively small cases and therefore may be insufficient power to detect smoking effects.

Hinds et al. reported model-predicted survival curves in women for smokers and never-smokers after adjustments for age, disease stage at diagnosis, and tumor histology [8]. The curves were significantly different. But, they did not use the Cox proportional hazards model and did not have information on pretreatment PS. Sobue et al. reported that current smokers who smoked 50 pack-years or more showed a 2.38 times higher risk of death than non-smokers for patients who undergo operations for adenocarcinoma of the lung [10]. Isobe et al. reported similar results [11]. In contrast, Sioris et al. showed that smoking history is one of the prognostic factors in squamous cell carcinoma for overall survival but not in adenocarcinoma [13]. Fujisawa et al. provided answer to this conflict. They employed multivariate analysis and showed smoking history is prognostic factor in evaluating overall long-term survival in patients with stage I primary resected NSCLC [14]. Nevertheless, they did not show important information on pretreatment PS. Furthermore, smoking history is not prognostic factor in evaluating disease-specific survival. It has been said that comorbidity may be one of the most important prognostic factor [22], as smoking is strongly associated with numerous serious disease such as chronic obstructive pulmonary disease, coronary heart disease, and stroke. To offset the influence of comorbidities on survival, disease-specific survival is superior to overall survival. On the other hand, the large number of patients at the multicenter gave us considerable confidence in the reliability of our data on smoking history and prognosis.

In our study, smoking status was investigated only at the time of admission to the hospital, and no information on smoking status was obtained after the operation. Richardson et al. reported that patients treated for SCLC who continue to smoke cigarettes increase their rate of developing second lung cancers [23]. In Japan, Kawahara et al. reported the same results [24]. In NSCLC, Fujisawa et al. reported no significant differences between postoperative smoking status and out-

come in the population of patients, alive or dead, due to recurrent disease, second malignancy, or non-malignant disease [14]. Further study is necessary to determine the prognosis and incidence of recurrence among patients who continue to smoke.

Although the mechanisms by which smoking affects the prognosis of lung cancer patients independently of other factors are not yet clear, some recent reports on oncogene suggest the clinical influence of cigarette smoking. Molecular changes that have been demonstrated in lung cancer include the activation of oncogenes such as ras, myc, bcl-2, and c-erbB-2, and the loss of tumor suppressor genes such as p53, RB and p16$^{INK4\alpha}$ [25–27]. Recently, Vahakangas et al. reported that p53 mutations occur more commonly in smokers and ex-smokers than in never-smokers [28]. Furthermore, Tammemagi et al. reported that p53 alteration and smoking history are negative prognostic factor [15]. Heavy smokers may have these molecular changes. These reports support our data showing poor prognosis in heavy smokers.

Among patients with resectable tumors, advanced age is generally described as an unfavorable prognostic factor, possibly because of higher postoperative mortality rates [29]. Our findings confirmed that age is a prognostic factor in a curative resection setting.

Gender and smoking history were closely correlated in Japan. In this series, we observed striking differences in smoking history between men and women; that is, 82.5% of women were non-smokers and 88.2% of men were smokers. Furthermore, smokers with more than 40 pack-years made up only 5.1% of all females and 58.6% of all males. That is, mean cigarette consumption was significantly lower in smoking women than in men. Such differences in smoking history likely resulted in different clinical presentation, histology, and treatment. It has been reported that the differences in smoking history may explain the better prognosis in females [8,30]. That is to say, gender is potential confounding factor in this study. In order to avoid systemic bias, we analyzed subgroup analyses.

In conclusion, the results of the current study found a preoperative smoking history to be a significant predictor of prognosis by univariate and multivariate analyses, in males. We showed a significant correlation between cigarette smoking and long-term disease-specific survival in stage IA NSCLC patients in numerous cases. The poor prognosis makes patients with smoking history an important population for creating stratification levels in clin-

ical trials and for the study of chemoprevention or smoking cessation study.

References

[1] Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer 1999;83:18—29.

[2] Fry WA, Menck HR, Winchester DP. The National Cancer Data Base report on lung cancer. Cancer 1999;77:1947—55.

[3] Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics. CA Cancer J Clin 1999;49:8—31.

[4] Mountain CF. Revisions in the international system for staging lung cancer. Chest 1997;111:1710—7.

[5] Harpole Jr DH, Herndon Jr JE, Young Jr WG, Wolfe WG, Sabiston Jr DC. Stage I nonsmall cell lung cancer. A multivariate analysis of treatment methods and patterns of recurrence. Cancer 1995;76:787—96.

[6] Johnson BE. Second lung cancers in patients after treatment for an initial lung cancer. J Natl Cancer Inst 1998;90:1335—45.

[7] Harpole Jr DH, Herndon Jr JE, Wolfe WG, Iglehart JD, Marks JR. A prognostic model of recurrence and death in stage I non-small cell lung cancer utilizing presentation, histopathology, and oncoprotein expression. Cancer Res 1995;55:51—6.

[8] Hinds MW, Yang HY, Stemmermann G, Lee J, Kolonel LN. Smoking history and lung cancer survival in women. J Natl Cancer Inst 1982;68:395—9.

[9] Kato I, Tominaga S, Ikari A. Lung cancer prognostic factors from the Aichi Cancer Registry. Jpn J Clin Oncol 1990;20:238—45.

[10] Sobue T, Suzuki T, Fujimoto I, Doi O, Tateishi R, Sato T. Prognostic factors for surgically treated lung adenocarcinoma patients, with special reference to smoking habit. Jpn J Cancer Res 1991;82:33—9.

[11] Isobe T, Hiyama K, Yoshida Y, Fujiwara Y, Yamakido M. Prognostic significance of p53 and ras gene abnormalities in lung adenocarcinoma patients with stage I disease after curative resection. Jpn J Cancer Res 1994;85:1240—6.

[12] Suzuki K, Nagai K, Yoshida J, Nishimura M, Takahashi K, Yokose T, et al. Conventional clinicopathologic prognostic factors in surgically resected non-small cell lung carcinoma A comparison of prognostic factors for each pathologic TNM stage based

on multivariate analyses. Cancer 1999;86:1976—84.

[13] Sioris T, Husgafvel-Pursiainen K, Karjalainen A, Anttila S, Kannio A, Salo JA, et al. Survival in operable non-small-cell lung cancer: role of p53 mutations, tobacco smoking and asbestos exposure. Int J Cancer 2000;86:590—4.

[14] Fujisawa T, Iizasa T, Saitoh Y, Sekine Y, Motohashi S, Yasukawa T, et al. Smoking before surgery predicts poor long-term survival in patients with stage I non-small-cell lung carcinomas. J Clin Oncol 1999;17:2086—91.

[15] Tammemagi MC, McLaughlin JR, Mullen JB, Bull SB, Johnston MR, Tsao MS, et al. A study of smoking, p53 tumor suppressor gene alterations and non-small cell lung cancer. Ann Epidemiol 2000;10:176—85.

[16] Lippman SM, Lee JJ, Karp DD, Vokes EE, Benner SE, Goodman GE, et al. Randomized phase III intergroup trial of isotretinoin to prevent second primary tumors in stage I non-small-cell lung cancer. J Natl Cancer Inst 2001;93:605—18.

[17] Linden G, Dunn Jr JE, Hom PH, Mann M. Effect of smoking on the survival of patients with lung cancer. Cancer 1972;30:325—8.

[18] Shimizu H, Tominaga S, Nishimura M, Urata A. Comparison of clinico-epidemiological features of lung cancer patients with and without a history of smoking. Jpn J Clin Oncol 1984;14:595—600.

[19] Ebina M, Steinberg SM, Mulshine JL, Linnoila RI. Relationship of p53 overexpression and up-regulation of proliferating cell nuclear antigen with the clinical course of non-small cell lung cancer. Cancer Res 1994;54:2496—503.

[20] Holli K, Visakorpi T, Hakama M. Smoking and survival from lung cancer. Acta Oncol 1999;38:989—92.

[21] Moores LK. Smoking and postoperative pulmonary complications. An evidence-based review of the recent literature. Clin Chest Med 2000;21:139—46.

[22] Ogle KS, Swanson GM, Woods N, Azzouz F. Cancer and comorbidity: redefining chronic diseases. Cancer 2000;88:653—63.

[23] Richardson GE, Tucker MA, Venzon DJ, Linnoila RI, Phelps R, Phares JC, et al. Smoking cessation after successful treatment of small-cell lung cancer is associated with fewer smoking-related second primary cancers. Ann Intern Med 1993;119:383—90.

[24] Kawahara M, Ushijima S, Kamimori T, Kodama N, Ogawara M, Matsui K, et al. Second primary tumours in more than 2-year disease-free survivors of small-cell lung cancer in Japan: the role of smoking cessation. Br J Cancer 1998;78:409—12.

[25] Kwiatkowski DJ, Harpole Jr DH, Godleski J, Herndon Jr JE, Shieh DB, Richards W, et al. Molecular pathologic substaging in 244 stage I non-small-cell lung cancer patients: clinical implications. J Clin Oncol 1998;16:2468—77.

[26] Taga S, Osaki T, Ohgami A, Imoto H, Yoshimatsu T, Yoshino I, et al. Prognostic value of the immunohistochemical detection of p16INK4 expression in nonsmall cell lung carcinoma. Cancer 1997;80:389—95.

[27] Niklinski J, Niklinska W, Laudanski J, Chyczewska E, Chyczewski L. Prognostic molecular markers in non-small cell lung cancer. Lung Cancer 2001;34(Suppl. 2):S53—8.

[28] Vahakangas KH, Bennett WP, Castren K, Welsh JA, Khan MA, Blomeke B, et al. p53 and K-ras mutations in lung cancers from former and never-smoking women. Cancer Res 2001;61:4350—6.

[29] Foucher P, Coudert B, Arveux P, Boutron MC, Kisterman JP, Bernard A, et al. Age and prognosis of non-small cell lung cancer. Usefulness of a relative survival model. Eur J Cancer 1993;29A:1809–13.

[30] Johnston-Early A, Cohen MH, Minna JD, Paxton LM, Fossieck Jr BE, Ihde DC, et al. Smoking abstinence and small cell lung cancer survival. An association. JAMA 1980;244:2175–9.

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EGFR Mutation Status in Japanese Lung Cancer Patients: Genotyping Analysis Using LightCycler

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Abstract   **Purpose:** Recently, somatic mutations of the epidermal growth factor receptor (*EGFR*) gene were found in ~25% of Japanese lung cancer patients. These *EGFR* mutations are reported to be correlated with clinical response to gefitinib therapy. However, DNA sequencing using the PCR methods described to date is time-consuming and requires significant quantities of DNA; thus, this existing approach is not suitable for a routine pretherapeutic screening program.

Experimental Design: We have genotyped *EGFR* mutation status in Japanese lung cancer patients, including 102 surgically treated lung cancer cases from Nagoya City University Hospital and 16 gefitinib-treated lung cancer cases from Kinki-chuo Chest Medical Center. The presence or absence of three common *EGFR* mutations were analyzed by real-time quantitative PCR with mutation-specific sensor and anchor probes.

Results: In exon 21, *EGFR* mutations (CTG → CGG; L858R) were found from 8 of 102 patients from Nagoya and 1 of 16 from Kinki. We also detected the deletion mutations in exon 19 from 7 of 102 patients from Nagoya (all were deletion type 1a) and 4 of 16 patients from Kinki (one was type 1a and three were type 1b). In exon 18, one example of G719S mutation was found from both Nagoya and Kinki. The L858R mutation was significantly correlated with gender (women versus men, $P < 0.0001$), Brinkman index ($600 \leqq$ versus 600), $P = 0.001$), pathologic subtypes (adenocarcinoma versus nonadenocarcinoma, $P = 0.007$), and differentiation status of the lung cancers (well versus moderately or poorly, $P = 0.0439$), whereas the deletion mutants were not. *EGFR* gene status, including the type of *EGFR* somatic mutation, was correlated with sensitivity to gefitinib therapy. For example, some of our gefitinib-responsive patients had L858R or deletion type 1a mutations. On the other hand, one of our gefitinib-resistant patients had a G719S mutation.

Conclusions: Using the LightCycler PCR assay, the *EGFR* L858R mutation status might correlate with gender, pathologic subtypes, and gefitinib sensitivity of lung cancers. However, further genotyping studies are needed to confirm the mechanisms of *EGFR* mutations for the sensitivity or resistance of gefitinib therapy for the lung cancer.

Lung cancer is a major cause of death from malignant diseases because of its high incidence, malignant behavior, and lack of major advancements in treatment strategy (1). Lung cancer was the leading indication for respiratory surgery (42.2%) in 1998 in Japan (2). More than 15,000 patients underwent surgical operation at Japanese institutions in 1998 (2). The clinical behavior of the lung cancer is largely associated with its stage.

The cure of the disease by surgery is only achieved in cases representing an early stage of lung cancer (3).

The epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitor, gefitinib, has been approved in Japan for the treatment of non–small cell lung cancer from 2002. Although *EGFR* is more abundantly expressed in lung carcinoma (4, 5), *EGFR* expression, as detected by immunohistochemistry, did not reveal any obvious relationship with response to gefitinib (6). Clinical trial have revealed significant variability in the response to gefitinib, with higher response in Japanese patients than in predominantly European-derived population (27.5% versus 10.4%; ref. 7). The partial clinical responses to gefitinib have been observed most frequently in women, in nonsmokers, and in patients with adenocarcinoma (8–10). More recently, we have collaborated with Dana-Farber Cancer Institute and found that novel *EGFR* mutations status at ATP binding pockets in Japanese non–small cell lung cancer patients were correlated with the clinicopathologic features related to good response to gefitinib (11). Actually, *EGFR* mutations in lung cancer have been correlated with clinical response to gefitinib therapy *in vivo* and *in vitro* (11–13).

The standard for experimental detection of mutations is direct sequencing of DNA samples from the tissues. For known mutations, real-time polymerase chain detection followed by melting curve analysis, using hybridization probes, is highly sensitive, rapid, and an efficient alternative approach to mutation detection (14-16).

To determine the *EGFR* mutation status in Japanese lung carcinoma for screening and diagnostic purposes, we wanted to develop a faster and easy method to detect *EGFR* mutations. In this report, we investigated *EGFR* mutation status by the real-time reverse transcription-PCR assay using LightCycler (17) mutation-specific sensor and anchor probes. With this method, 32 samples were genotyped within 1 hour without the need of any post-PCR sample manipulation. The findings were compared with the clinicopathologic features of lung cancer.

Materials and Methods

Patients. The study group included 102 lung cancer patients who had undergone surgery (but did not receive gefitinib) at the Department of Surgery II, Nagoya City University Medical School, between 1997 and 2000. The study group also included 16 lung cancer patients who had undergone surgery at the Department of Surgery, National Hospital Organization, Kinki-chuo Chest Medical Center, and were subsequently treated with gefitinib. These 16 samples were sequenced by ABI prism 3100 analyzer (Applied Biosystems Japan, Ltd., Tokyo, Japan; data not shown) and analyzed by ABI prism Seq Scape version 2.1.1. The lung tumors were classified according to the general rule for clinical and pathologic record of lung cancer in Japan (18). All tumor samples were immediately frozen and stored at −80°C until assayed.

The clinical and pathologic characteristics of the 102 lung cancer patients are as follows: 52 cases at stage I, 16 at stage II, and 34 at stage III to IV. The mean age was 65.5 years (range, 42-85). Among the 102 lung cancer patients, 49 (48%) were diagnosed as having adenocarcinoma, 32 (31.4%) squamous cell carcinoma, 9 (8.8%) adenosquamous cell carcinoma, and 7 (6.9%) small cell carcinoma.

PCR assays for EGFR. The genomic DNA was extracted from lung cancer tissues and matched normal lymphocytes from the peripheral blood using the Wizard SV Genomic DNA purification system (Promega Corporation, Madison, WI). Initially, 58 DNA samples were also extracted from lung cancer tissues from Nagoya City University and sequenced as reported in our previous paper (11). These sets of DNA were used as a positive and negative control for genotyping. DNA concentration was determined by spectrophotometry and adjusted to a concentration of 50 ng/mL. We then used 1 μL of each DNA for LightCycler analyses. To ensure the fidelity of DNA extraction, all samples were subjected to PCR amplification with oligonucleotide primers specific for exon 18 of the *EGFR* gene and then digested by *Sac*I enzyme. The primer sequences for *EGFR* gene in exon 18 were as follows: the forward primer, 5-TCCAAATGAGCTGGCAAGTG-3, and the reverse primer, 5-TCCCAAACACTCAGTGAAACAAA-3 (397 bp). The cycling conditions were as follows: initial denaturation at 95°C for 15 minutes followed by 35 cycles at 95°C for 20 seconds, 57°C for 20 seconds, 72°C for 30 seconds, and one cycle of 72°C for 3 minutes. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA) and then digested with restriction enzyme at 37°C for 2 hours. The genotyping PCR reactions were done using LightCycler DNA Master Hybridization probes kit (Roche Molecular Biochemicals, Mannheim, Germany) in a 20 μL reaction volume. The primer sequences for *EGFR* gene in exon 18 were as follows: the forward primer, 5-TCCAATGAGCTGGCAAGTG-3, and the reverse primer, 5-TCCCAAACACTCAGTGAAACAAA-3 (397 bp). For the exon 18 genotyping, sensor (LC Red 640-GCACCGGAGCCCAGCA) and anchor (GCCAGGGACCTTATACACGTGCCGAA-Fluorescein) probes were used. The cycling conditions were as follows: initial denaturation at

95°C for 10 minutes, followed by 45 cycles at 95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 16 seconds. The primer sequences for *EGFR* gene in exon 19 were as follows: the forward primer, 5-CGTCTTCCTTCTCTCTCTGTC-3, and the reverse primer, 5-GACATGA-GAAAAGGTGGGC-3 (175 bp). For the exon 19 genotyping, sensor (GCTATCAAAACATCTCC-Fluorescein) and anchor (LC Red 640-AAAGCCAACAAGGAAATCCTCGATGTGAGTTCTGCTTTGCTGT-GTGGGG) probes were used. The cycling conditions were as follows: initial denaturation at 95°C for 10 minutes followed by 45 cycles at 95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 7 seconds. The primer sequences for *EGFR* gene in exon 21 were as follows: the forward primer, 5-GCTCAGAGCCTGGCATGAA-3, and the reverse primer, 5-CATCC-TCCCCTGCATGTGT-3 (349 bp). The cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 45 cycles at 95°C for 10 seconds, 57°C for 10 seconds, and 72°C for 14 seconds. For the exon 21 genotyping, sensor (Fluorescein-AGTTTGGCCCGCCCA) and anchor (LC Red 640-CCTCCTTCTGCATGGTATTCTTTCTCTTCCG-CACCCAG) probes were used.

Statistical methods. Statistical analyses were done using the Mann-Whitney U test for unpaired samples and Wilcoxon's singed rank test for paired samples. Linear relationships between variables were determined by simple linear regression. Correlation coefficients were determined by rank correlation using Spearman's test and χ^2 test. The overall survival of lung cancer patients was examined by the Kaplan-Meier methods and differences were examined by the log-rank test, Breslow-Gehan-Wilcoxon test, and Cox proportional hazard regression model. All analyses were done using the StatView software package (Abacus Concepts, Inc., Berkeley, CA) and results were considered significant when $P < 0.05$.

Results

Fidelity of allele-specific PCR confirmed by conventional PCR assay in lung cancer tissues. Using the exon 18 primer sets, a PCR product of 397 bp was obtained. We have analyzed the product using PCR-RFLP method. The wild-type DNA does not have a *Sac*I site within the 397 bp. The PCR products digested with *Sac*I were loaded with 2% agarose gel and wild-type DNA should be visualized as one band. However, if the substitution mutation G719S were present, the PCR products digested by *Sac*I will be visualized as three bands. Using this method, PCR products were visualized from all lung cancer patients studied. In exon 18, a G719S mutation was found from one Nagoya specimen (stage Ia, well-differentiated adenocarcinoma with bronchioloalveolar carcinoma pattern at the edge of tumor, female, nonsmoker patient) and one Kinki specimen (Fig. 1A). These mutants were also analyzed by LightCycler. The anchor probe was matched for wild type. As shown in Fig. 1B for the G719S mutation in exon 18, the homozygous wild-type PCR product showed a single peak at 69°C, whereas the heterozygous products (mutant) showed an additional peak at 59°C. The LightCycler method using the mutation-specific probes confirmed the results with the restriction fragment analysis.

Genotyping of EGFR at exon 19 and exon 21 in lung cancer tissues. For exon 21 genotyping, the anchor probe was matched for L858R mutation. As shown in Fig. 2, for the L858R mutation in exon 21, the homozygous wild-type PCR product showed a single peak at 53°C, whereas the heterozygous products (mutant) showed an additional peak at 65°C. From the 102 lung cancer patients, 8 patients had the L858R mutation. One was male and seven were female. Seven were nonsmokers and one was a smoker (Brinkman index was 600). All eight patients had adenocarcinoma, one was moderately differentiated, and
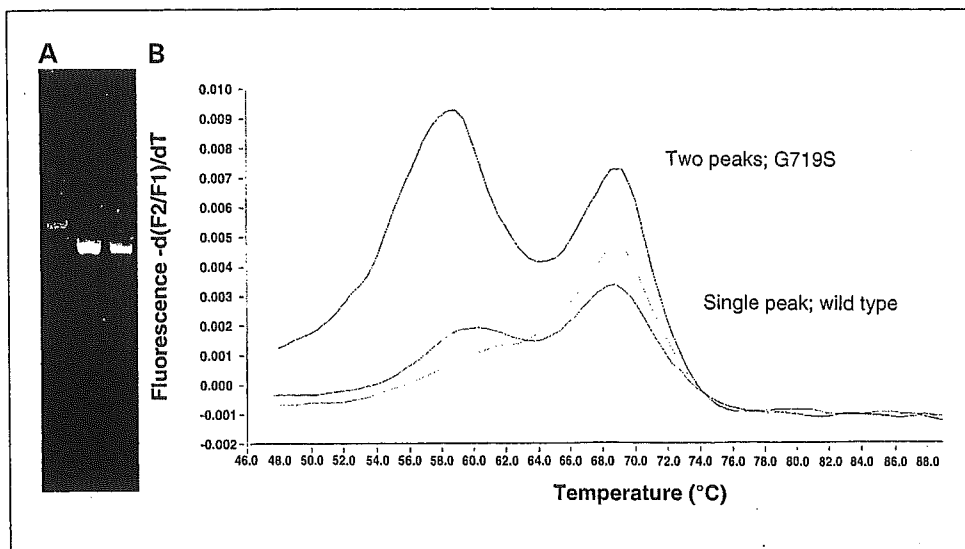
Fig. 1. *A*, analyzed data using PCR-RFLP. Left lane, the wild-type DNA within the 397 bp does not have *Sac* I site. The PCR products restricted with *Sac* I were loaded with 2% agarose gel and was visualized as one band. Right lane, the substitution mutation G719S caused *Sac* I site, and the PCR products restricted by *Sac* I was visualized as three bands. *B*, detection of a G719S mutation in the *EGFR* gene in genomic DNA extracted from lung cancer tissues. The negative derivative of the fluorescence ($-dF/dT$) versus temperature graph shows peaks with different T_m. The wild-type sample showed a single T_m at 69°C. The heterozygous mutant sample showed an additional peak at 59°C.

seven were well differentiated. Five of eight adenocarcinomas showed bronchioloalveolar carcinoma pattern at the edge of tumor. Thus, L858R mutation status was significantly correlated with gender, Brinkman index, pathologic subtypes, and differentiation of lung cancer (Table 1). Eight of eight PCR products from matched peripheral lymphocyte DNA showed a single peak, suggesting that the mutations were somatic. L858R mutation was also found in one nonsmoking female adenocarcinoma patient from Kinki-chuo Chest Medical Center.

For exon 19 genotyping, the anchor probe was matched for deletion type 1a (2,235-2,249 nucleotides deletion; deletion GGAATTAAGAGAAGC) mutation. As shown in Fig. 3, for the deletion 1a mutation in exon 19, the PCR product showed a single peak at 56°C, whereas the deletion 1b products (2,236-2,250 nucleotides deletion; deletion GAATTAAGAGAAGCA) showed a peak at 47°C. From the 102 lung cancer patients, seven patients had the deletion 1a mutation. Four were males and three were females. Three were nonsmokers and four were smokers. Four patients had adenocarcinoma, two had squamous cell carcinoma, and one had adenosquamous cell carcinoma. One of the tumors was moderately differentiated,

two were poorly differentiated, and three were well differentiated. One of four adenocarcinomas showed bronchioloalveolar carcinoma pattern at the edge of tumor. Thus, deletion 1a mutation status was not significantly correlated with gender, Brinkman index, pathologic subtypes, and differentiation of lung cancer (Table 2). Five of seven PCR products from matched peripheral lymphocyte DNA were available and showed a single peak, suggesting that these mutations were somatic.

The mutations detected in lung cancer specimens from Kinki-chuo Chest Medical Center are summarized in Table 3. L858R mutation and deletion type 1a were found from partial response patients. On the other hand, G719S mutation was found from a patient with no response to gefitinib (progressive disease). A total of six mutations were found from 16 gefitinib-treated patients (37.5%). Taken together, 22 mutations were found from 117 examined samples in our analysis (18.8%).

The overall survival of 102 lung cancer patients from Nagoya City University, with follow-up through December 30, 2003, was studied in reference to the *EGFR* mutation status. There was no significant difference in the prognosis between the patients with wild-type *EGFR* ($n = 86$, 22 were dead) and the patients with
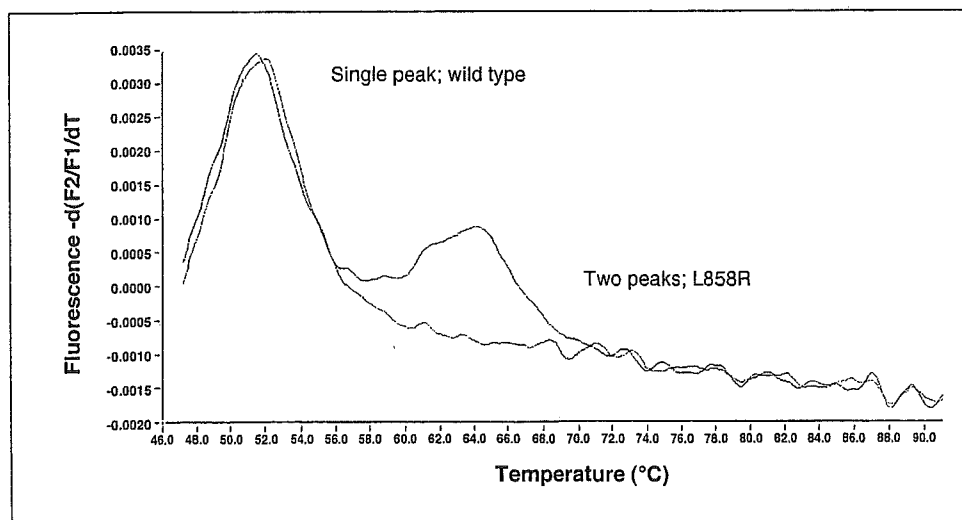

Fig. 2. The L858R mutation in exon 21 of the homozygous wild-type PCR product showed a single peak at 53°C, whereas the heterozygous products (mutant) showed an additional peak at 65°C.

Table 1. Clinicopathologic data of 102 lung cancer patients

Factors	L858R		P
	Mutation patients (%)	Wild-type patients (%)	
Mean age (y), 65.5 ± 9.3	8	94	
Stage			
I	7 (87.5)	45 (47.9)	0.0744
II-IV	1 (12.5)	49 (52.1)	
Lymph node metastasis			
N0	7 (87.5)	60 (63.8)	0.3341
N+	1 (12.5)	34 (36.2)	
BI			
≦600	8 (100)	32 (34.0)	0.001
>600	0 (0)	62 (66.0)	
Differentiation			
Well	7 (87.5)	31 (43.1)	0.0439
Moderately or poorly	1 (12.5)	41 (56.9)	
Pathologic subtypes			
Adenocarcinoma	8 (100)	41 (43.6)	0.007
Nonadenocarcinoma	0 (0)	53 (56.4)	
Age			
≦60	2 (25.0)	26 (27.7)	0.9999
>60	6 (75.0)	68 (72.3)	
Gender			
Male	1 (12.5)	80 (85.1)	<0.0001
Female	7 (87.5)	14 (14.9)	

Abbreviations: N+, lymph node metastasis positive; BI, Brinkman index.

common in lung cancers from never smokers (13) and females with adenocarcinoma (11). Our analysis also suggested that the type of *EGFR* mutation might be correlated with the sensitivity of gefitinib therapy for lung cancers.

When the PCR is used for the detection of mutations in very small amounts of DNA, although we would like to start from biopsy samples in the future, it is usually necessary to use "nested PCR." In this case, a DNA fragment is amplified with a first set of primers and part of the product is reamplified with a second set of primers complementary to sequences in the product. Recent developments in fast PCR and real-time detection of products make a more sensitive approach to detection of mutations possible (14–16, 19). We have optimized mutation detection, without nested PCR, using the LightCycler. This instrument measures fluorescence during PCR and can detect the SYBR Green dye when it is intercalated in double-stranded DNA, allowing the detection of double-stranded PCR product formation. The use of labeled probes homologous to the PCR product permits specific identification of PCR products (17). In the LightCycler, two adjacent probes were used, labeled with different fluorescent molecules. When the probes were bound to the single-stranded target, one to five bases apart, the 3'-end label of the 5' probes came close to the 5'-end label of the 3' probe, resulting in resonance and strong fluorescence at a specific wavelength. An advantage of this strategy is that hybridization of the probe is not restricted to the temperature range required for Taq polymerase to remove a base (19, 20). Further melting curves can be produced after PCR to assess the dissociation temperature of the probe. Mutations covered by the probe can be detected by a shift in melting temperature. The one-cycle analysis took ~1 hour and could examine 32 samples.

Because so many *EGFR* mutation phenotypes were discovered, it would be of interest to determine whether resistance to *EGFR* inhibition emerges through secondary mutation as is the case in imatinib-treated chronic myelogenous leukemia (21). Our data showed that L858R mutation and deletion type 1a were found in gefitinib-sensitive patients; on the other hand, a G719S mutation was found in a gefitinib-resistant patient. Interestingly, recent data reported that L858R mutant (transfected cell) was inhibited at 10-fold lower concentrations of tyrosine kinase inhibitor; however, the deletion mutant seemed to have similar sensitivities as wild-type *EGFR*

mutation in the *EGFR* gene (*n* = 16, two were dead; log-rank test, *P* = 0.3608; Breslow-Gehan-Wilcoxon test, *P* = 0.4761), although the observation period was short.

Discussion

We obtained findings that L858R *EGFR* mutation status was significantly correlated with gender, smoking history, and pathologic subtypes of lung cancers. This was in agreement with the recent reports that *EGFR* gene mutations are

Fig. 3. Detection of the deletion mutations in the *EGFR* gene in genomic DNA extracted from lung cancer. The deletion 1a – type sample showed a single T_m at 56°C. The deletion type 1b sample showed a single peak at 47°C.
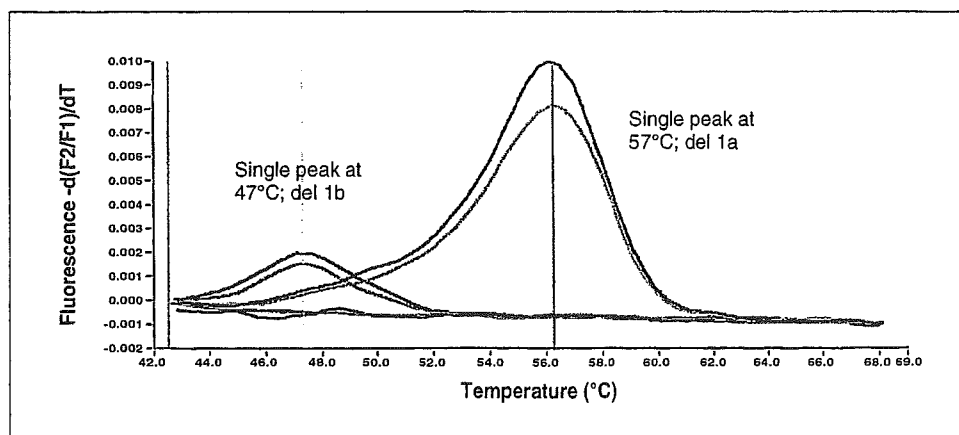
Table 2. Clinicopathologic data of 102 lung cancer patients

Factors	Exon 19 deletion		
	Mutation patients (%)	Wild-type patients (%)	P
Mean age (y), 65.5 ± 9.3	7	95	
Stage			
I	3 (42.9)	49 (51.6)	0.9571
II-IV	4 (57.1)	46 (48.4)	
Lymph node metastasis			
N0	3 (42.9)	64 (67.4)	0.3650
N+	4 (57.1)	31 (32.6)	
BI			
≦600	5 (71.4)	35 (36.8)	0.1592
>600	2 (28.6)	60 (63.2)	
Differentiation			
Well	3 (50.0)	35 (47.3)	0.9999
Moderately or poorly	3 (50.0)	39 (52.7)	
Pathologic subtypes			
Adenocarcinoma	4 (57.1)	45 (47.4)	0.9143
Nonadenocarcinoma	3 (42.9)	50 (52.6)	
Age			
≦60	2 (28.6)	26 (27.4)	0.9999
>60	5 (71.4)	69 (72.6)	
Gender			
Male	4 (57.1)	77 (81.1)	0.3051
Female	3 (42.9)	18 (18.9)	

receptor tyrosine kinase substantially increases the ability of the unphosphorylated kinase to bind ATP (23). From our data, this mutation pattern (L858R) might be more correlated with the populations, such as women, smoking, and adenocarcinoma.

DNA sequencing using the PCR methods described to date is time-consuming and, therefore, may not be suitable for a regular pretherapeutic screening program. Genechip technology is promising but still in its infancy, and adapting this technology to new polymorphisms is time-consuming and expensive. Real-time PCR, on the other hand, allows for easy adoption of new polymorphisms and possibly provides the best means for pretherapeutic genotyping in a clinical setting at present. We, therefore, developed three different PCRs to detect *EGFR* gene mutations and deletions. The advantages of real-time PCR are extensive. The faster PCR method and elimination of additional steps to analyze PCR products save time and minimize the risks of DNA contamination. Handling is facilitated and potentially toxic reagents, such as ethidium bromide stain, are avoided. We have only found 16 of 101 surgically removed samples from Nagoya City University and 6 of 16 gefitinib-treated samples from Kinki-chuo Chest Medical Center. Other mutations might have existed for these patients, although we have only checked the three most frequent mutations. The difference in the ratio of *EGFR* mutation between Nagoya and Kinki patients might have been caused by selection bias because gefitinib was known to be sensitive for female, nonsmoker, and adenocarcinoma patients. Actually, we have checked seven small cell carcinoma and three large cell carcinoma patients from Nagoya and no mutations were found from these patients.

to drug (13). Thus, mutation phenotypes might be correlated with sensitivity for gefitinib therapy. Substitution mutation L858R is located adjacent to the highly conserved DFG motif in the activation motif. The activation loop was known to be important for autoregulation in many kinases (22). For example, the mutation in the activation loop of insulin

Using the LightCycler reverse transcription-PCR assay described here, the determination of *EGFR* mutation status may be of clinical importance in predicting the sensitivity or resistance to gefitinib therapy for lung cancer. With this method, 32 samples were genotyped within 1 hour without the need of any post-PCR sample manipulation. Mutation detection using real-time PCR with hybridization probes and

Table 3. Genotyping analyses data for the non – small cell lung cancer patients from Kinki-chuo Chest Medical Center

Age	Gender	Mutation	Exon	Mutation type	Pathology	Smoking history
59	F	+	19	del 1a	Adenocarcinoma	N
69	F	+	18	G719S	Adenocarcinoma	N
76	M	+	19	del 1b	Adenocarcinoma	N
56	M	+	19	del 1b	Adenocarcinoma	F/C
33	M	+	19	del 1b	Adenocarcinoma	F/C
59	F	+	21	L858R	Adenocarcinoma	N
47	M	−			Adenocarcinoma	F/C
65	F	−			Adenocarcinoma	N
51	F	−			Adenocarcinoma	N
66	M	−			Adenocarcinoma	F/C
82	M	−			Adenocarcinoma	F/C
71	F	−			BAC	N
66	F	−			BAC	N
71	F	−			Adenocarcinoma	N

Abbreviations: F, female; M, male; del, deletion; BAC, bronchioloalveolar carcinoma; N, never smoker; F/C, former or current smoker.

melting curve analysis can be used for the sensitive detection of DNA mutations. The fast detection of single base substitutions in small amounts of DNA has great potential in pretreated diagnosis and in oncology.

Acknowledgments

References

1. Ginsberg RJ, Kris K, Armstrong G. Cancer of the lung. 4th ed. In: Principles and Practice of Oncology. Philadelphia: Lippincott 1993;1993. p. 673–82.

2. Yasuda K, Ayabe H, Ide H, Uchida Y; on behalf of the Japanese Association for Thoracic Surgery: Thoracic and cardiovascular surgery in Japan during 1998. Annual report by the Japanese association for thoracic surgery. Jpn J Cardiothorac Surg 1998;48:401–15.

3. Postus PE; on behalf of the Lung Cancer Cooperative Group of the EORTC. The experience of the lung cancer cooperative group of the European Organization for Research and Treatment of Cancer. Chest Suppl 1997;113:28–31S.

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; 53:2379–85.

5. Sasaki H, Yukiue H, Mizuno K, et al. Elevated serum epidermal growth factor receptor level is correlated with lymph node metastasis in lung cancer. Int J Clin Oncol 2003;8:79–82.

6. Parra HS, Cavina R, Latteri F, et al. Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib ("Iressa," ZD1839) in non-small cell lung cancer. Br J Cancer 2004;91:208–12.

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;21:2237–49.

8. Janne PA, Gurubhagavatula S, Yeap BY, et al. Outcomes of patient wit advanced non-small cell lung cancer treated with gefitinib (ZD1839,"Iressa") on an expanded access study. Lung Cancer 2004;44:221–30.

9. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth facto receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA 2003;290: 2149–58.

10. Miller VA, Kris MG, Shah N, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. J Clin Oncol 2004;22:1103–9.

11. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;81:61–9.

12. 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;53:1192–202.

13. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and elrotinib. Proc Natl Acad Sci U S A 2004;101:13306–11.

14. Wittwer CT, Herrmann MG, Moss AA, Rasmussen RP. Continuous fluorescence monitoring of rapid cycle DNA amplification. BioTechniques 1997;22: 130–8.

15. Wittwer CT, Reed GB, Ririe KM. Rapid cycle amplification. In: Mullis KB, Ferre F, Gibbs RA, editors. The polymerase chain reaction. Boston: Birkhauser; 1994. p. 174–81.

16. Pals G, Pindolia K, Worsham MJ. A rapid and sensitive approach to mutation detection using real-time polymerase chain reaction and melting curve analyses, using BRCA1 as an example. Mol Diagn 1999;4:241–6.

17. Wittwer T, Ririe M, Andrew V, David A, Gungry A, Bali J. The LightCycler: a microvolume multisample fluorimeter with rapid temperature control. BioTechniques 1997;22:176–81.

18. Japan Lung Cancer Society. General rule for clinical and pathological record of lung cancer. 5th ed. Tokyo: Japan Lung Cancer Society 1999; p. 1–177.

19. Pals G, Young C, Mao HS, Worsham MJ. Detection of a single base substitution in a single cell using the LightCycler. J Biochem Biophys Methods 2001;47: 121–9.

20. Heid CA, Stevens J, William PM. Real time quantitative PCR. Genome Res 1996;6:986–94.

21. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001; 293:876–80.

22. Huse M, Kuriyan J. The conformational plasticity of protein kinases. Cell 2002;109:275–82.

23. Till JH, Ablooglu AJ, Frankel M, Bishop SM, Kohanski RA, Hubbard SR. Crystallographic and solution studies of an activation loop mutant of the insulin receptor tyrosine kinase: insights into kinase mechanism. J Biol Chem 2001;276:10049–55.

ORIGINAL ARTICLE

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A phase I study of 3-day topotecan and cisplatin in elderly patients with small-cell lung cancer

Abstract *Purpose*: The aim of this phase I study was to determine the maximum-tolerated dose (MTD) in elderly patients with small-cell lung cancer (SCLC). *Patients and methods*: Patients aged over 75 years with previously untreated SCLC were enrolled in this study. Both topotecan and cisplatin were administered on days 1–3 and repeated every 3 weeks. The starting dose of topotecan was 0.5 mg/m^2/day, while cisplatin was fixed at the dose of 20 mg/m^2/day. Patients with limited disease (LD) SCLC received thoracic irradiation after the completion of chemotherapy. *Results*: Twenty-one elderly patients were enrolled in this study and received a total of 59 cycles. The major hematological toxicity was neutropenia and non-hematological toxicities including diarrhea were generally mild and reversible. The MTD of topotecan was determined as 1.2 mg/m^2/day. The recommended phase II study dose of topotecan was determined as 1.0 mg/m^2/day with cisplatin 20 mg/m^2/day daily for 3 days. An objective response was observed in 6 of 10 patients (60%) with LD-SCLC and 6 of 11 (55%) with extensive disease (ED) SCLC. The median survival time in patients with LD-SCLC and those with ED-SCLC were 16.0 and 11.0 months, respectively. *Conclusion*: The combination chemotherapy of 3-day topotecan and cisplatin appears to be tolerable and effective in elderly patients with SCLC.

Keywords Small-cell lung cancer · Elderly patients · Topotecan · Cisplatin · Phase I study · 3-day schedule

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Introduction

The standard chemotherapy for extensive disease small-cell lung cancer (ED-SCLC) has been considered to be a combination of etoposide and cisplatin [1, 9, 11]. Recently, a randomized phase III study comparing a combination of irinotecan, one of the topoisomerase I inhibitors, and cisplatin with a standard combination of etoposide and cisplatin in patients with previously untreated ED-SCLC, demonstrated a significant survival benefit in a combination with irinotecan and cisplatin [18]. Thus, the combination of a topomerase-I inhibitor and cisplatin is an attractive strategy for the treatment of SCLC.

However, elderly patients were excluded from these previous trials [11, 18]. In general, elderly patients are

considered to have an increased risk of chemotherapy-related morbidity and mortality due to comorbid diseases, deterioration of organ functions, or poor performance status (PS) [6, 21]. In addition, frequent dose reductions due to excessive toxicities may be required in elderly patients because of poor functional reserves, resulting in an insufficient dose-intensity of the chemotherapy [27]. Regarding the toxicity profile of the irinotecan and cisplatin combination, one of the major toxicities seems to be high incidence of diarrhea (grade 2 or more: 44% [18]), which may lead to low treatment compliance in the elderly patients. Therefore, it is desirable to establish the optimal treatment for elderly patients with SCLC.

Topotecan is a semi-synthetic derivative of camptothecin, which is a potent inhibitor of the topoisomerase I enzyme and involved in DNA unwinding needed for DNA replication and transcription [8]. In the previous phase II monotherapy trial in the 5-day administration schedule, the overall response rate for previously untreated SCLCs was 39% [25]. Non-hematological toxicities were relatively mild. In particular, diarrhea has been reported to be rare, which is the dose-limiting toxicity (DLT) of irinotecan [14, 17]. Additionally, the safety and efficacy of a 3-day topotecan regimen have recently been reported in patients with ovarian cancer [4, 13], and this modified regimen seemed to be less toxic than a 5-day topotecan regimen [7] with a comparable antitumor activity in patients with ovarian cancer [4, 13]. These findings suggest that a 3-day topotecan might be safely administered to elderly patients with SCLC.

Based on these background data, we designed a phase I study of topotecan administered for three consecutive days in combination with cisplatin, a key drug for SCLCs in elderly patients with SCLC. The primary objective was to determine the maximum-tolerated dose (MTD) for each drug, with a secondary objective of assessing antitumor activity.

Patients and methods

Eligibility

The eligibility criteria for entry into this study were as follows: (1) pathologically proven SCLC, (2) age of 76 years or more, (3) no prior anticancer therapy,(4) PS of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale [20], (5) presence of evaluable lesions, (6) adequate reserves of hematological function (white blood cell [WBC] count $\geq$ 4,000/μl, neutrophil count $\geq$ 2,000/μl, hemoglobin level $\geq$ 9.5 g/dl, platelet count $\geq$ 10×10^4/μl), renal function (serum creatinine $\leq$ 1.5 mg/dl), hepatic function (total bilirubin $\leq$ 1.5 mg/dl, serum transaminases < 2.5×upper limit of normal range) and pulmonary function (PaO$_2$ $\geq$ 60 Torr at rest), and (7) acquisition of a written informed consent. Patients with symptomatic brain metastasis were excluded from the study. The baseline pretreatment evaluations included a complete history, physical examination, laboratory tests,

a chest radiograph, computed tomography (CT) scans of the chest and abdomen, fibreoptic bronchoscopy, magnetic resonance imaging (MRI) of the brain, and a radionuclide bone scan, if medically indicated. The protocol was approved by the institutional review board of each participating institute.

Treatment scheme

Topotecan, diluted in 100 ml of physiological saline, was intravenously administered for 30 min on days 1–3. After the completion of the topotecan infusion, a fixed dose of cisplatin (20 mg/m^2/day), diluted in 300 ml of physiological saline, was intravenously administered over 1 h on the same days. The treatment was repeated every 3 weeks and six dose levels were planned (Table 1).

Four cycles of chemotherapy were planned. Patients were treated with at least two cycles of chemotherapy unless there was a disease progression, unacceptable toxicity in the first cycle, or withdrawal of their consent. Initiation of the next cycle of chemotherapy was delayed until recovery of the WBC count to 3,000/μl, the neutrophil count to $\geq$ 1,500/μl, the platelet count to $\geq$ 10×10^4/μl, hemoglobin $\geq$ 8.0 g/dl, and resolution of non-hematologic toxicities to $\leq$ grade 1. If grade 4 leukopenia, grade 4 neutropenia, or febrile neutropenia was noted, the use of granulocyte colony-stimulating factor (G-CSF) was permitted. Patients with limited-disease (LD)-SCLC received thoracic irradiation at a total of 45 Gy in 25 fractions after the completion of chemotherapy. In addition, patients achieving complete response received prophylactic cranial irradiation.

Assessment of toxicity and dose escalation

Toxicities were graded according to the National Cancer Institute-Common Toxicity Criteria Version 2.0. All treatment cycles were analyzed to determine DLT, although the decision to elevate the dose level was based on the toxicities in the first cycle. The DLT was defined as development of at least one of the following toxicities: any non-hematological toxicities $\geq$ grade 3 other than nausea, vomiting, and alopecia; grade 4 neutropenia or leukopenia lasting for 4 days or more; platelet count $\leq$ 1–10^4/μl. At least three patients were scheduled to enter the study at each dose level and if all three patients developed the DLT, the dose level was determined to be

Table 1 Planned dose level

Dose levels	Cisplatin (mg/m^2/day)	Topotecan (mg/m^2/day)
1	20	0.5
2	20	0.65
3	20	0.8
4	20	1.0
5	20	1.2
6	20	1.4

the MTD. If one or two of the three patients experienced the DLT, three additional patients were subjected to the same dose level. The MTD was defined as a dose level that produced any of the DLTs developed in three or more patients among a maximum of six patients, and further dose escalation was not permitted. Dose escalation in the individual patient was not allowed. The recommended dose was defined as the dose level below the MTD for safe administration of the both drugs.

Assessment of efficacy

The response was evaluated according to the Standard Response Evaluation Criteria in Solid Tumors [28]. The time to progression and the overall survival time were calculated from the date of registration to this trial until the first document of disease progression and death, respectively, using the Kaplan–Meier method. Statistical analyses were performed using the STATVIEW 5.0 program (Brainpower, Calabasas, CA).

Results

Patient characteristics

Between November 2001 and September 2004, a total of 21 elderly SCLC patients were enrolled in this study (Table 2). In ED-SCLC patients, most frequent metastatic sites were the liver and adrenal gland. A total of 59 cycles were administered, with median number of three cycles per patients (range 1–4). Seven of the 10 LD-SCLC patients received thoracic irradiation after completion of chemotherapy with a median-delivered dose of 45 Gy. One patient received only one cycle of chemotherapy because of withdrawal of consent. All patients and cycles were assessable for toxicity and response.

Hematological toxicity

The hematological toxicities in 21 patients are listed in Table 3. The main toxicity was neutropenia, which was

Table 2 Patient characteristics

No. of patients	21
Age	
Median (range)	78 (76–82)
Gender	
Male	19
Female	2
Performance status	
0	5
1	13
2	3
Stage	
Limited disease	10
Extensive disease	11

observed in 54 (91.5%) of 59 cycles. G-CSF was required in 34 (58%) cycles for grade 4 neutropenia (31 cycles) or febrile neutropenia (three cycles). Grade 4 anemia was observed in seven (12%) cycles, and blood transfusion was required in four cycles at dose levels 3 and 5. Grade 2 or 3 thrombocytopenia was frequently observed and platelet transfusion was required in one cycle at dose level 5, however, no severe hemorrhage complications were experienced.

Non-hematological toxicity

Table 4 shows non-hematological toxicities of grade 2 or greater in all treatment cycles. Diarrhea was extremely mild and grade 1 diarrhea occurred in 7 (12%) of 59 cycles and no grade 2 or more diarrhea was observed in this study. Febrile neutropenia was experienced in one and two cycles at dose levels 3 and 5, respectively, however, it was reversible with appropriate supportive care including G-CSF and antibiotics. Grade 3 hepatic dysfunction and grade 4 hyponatremia occurred in one cycle each, and these toxicities were considered to be the DLT. However, these conditions spontaneously recovered. There were no treatment-related deaths.

Maximum-tolerated dose

Dose limiting toxicity was observed in one of six patients at dose level 3 (hepatic toxicity), and in three of six patients at dose level 5 (febrile neutropenia, persistent neutropenia, and hyponatremia). Thus, we determined the MTD of 3-day topotecan and cisplatin to be 1.2 and 20 mg/m^2/day, respectively (dose level 5). The recommended doses were considered to be 1.0 mg/m^2/day for topotecan and 20 mg/m^2/day for cisplatin (dose level 4).

Antitumor activity

An objective response was observed in 6 (60%) of 10 patients with LD-SCLC and 6 (55%) of 11 patients with ED-SCLC. The median follow-up time of the surviving patients was 11.0 months, and the median survival time was 12.8 months. When stratified by disease extent, the median survival times in patients with LD-SCLC and those with ED-SCLC were 16.0 and 11.0 months, respectively.

Discussion

The present phase I study demonstrated that the combination chemotherapy of 3-day topotecan and cisplatin was well tolerated in elderly SCLC patients. The major toxicity in our study was myelosuppression, whereas diarrhea was rarely observed. All the toxicities were reversible and no life-threatening toxicities occurred.

Table 3 Hematological toxicity of grade 2 or greater (all cycles)

		Dose levels				
		1	2	3	4	5
No. of treated patients		3	3	6	3	6
No. of cycles evaluated		9	5	19	7	19
	Grades	No. of cycles (%)				
Leukopenia	2	4 (44)	2 (40)	7 (37)	4 (57)	10 (53)
	3	1 (11)	1 (20)	10 (53)	0	6 (32)
	4	0	0	0	0	2 (11)
Neutropenia	2	0	1 (20)	2 (11)	1 (14)	0
	3	4 (44)	3 (60)	6 (32)	1 (14)	9 (47)
	4	3 (33)	1 (20)	10 (53)	3 (43)	10 (53)
Anemia	2	3 (33)	0	6 (32)	2 (29)	3 (16)
	3	1 (11)	2 (40)	3 (16)	2 (29)	3 (16)
	4	0	0	2 (11)	0	5 (26)
Thrombocytopenia	2	3 (33)	2 (40)	4 (21)	0	2 (11)
	3	2 (22)	2 (40)	6 (32)	0	11 (58)
	4	0	0	0	0	0

Table 4 Non-hematological toxicity of grade 2 or greater (all cycles)

		Dose levels				
		1	2	3	4	5
No. of treated patients		3	3	6	3	6
No. of cycles evaluated		9	5	19	7	19
	Grades	No. of cycles (%)				
Nausea/vomiting	2	2 (22)	1 (20)	2 (11)	2 (29)	2 (11)
	3	2 (22)	0	6 (32)	0	2 (11)
Fatigue	2	1 (11)	1 (20)	0	0	0
	3	0	0	0	0	8 (42)
Hepatotoxicity	2	0	0	0	1 (14)	0
	3	0	0	1 (5)	0	0
Infection	3	1 (11)	0	2 (11)	1 (14)	2 (11)
Febrile Neutropenia	3	0	0	1 (5)	0	2 (11)
Hyponatremia	4	0	0	0	0	1 (5)

The MTDs for topotecan and cisplatin were determined to be 1.2 and 20 mg/m^2/day, respectively (dose level 5), and this regimen yielded a favorable antitumor activity.

It is of note that diarrhea was extremely mild in our regimen without any grade 2 or over. Diarrhea was a major toxicity in the irinotecan and cisplatin arm of the

Table 5 Response

	Dose level					Total
	1	2	3	4	5	
LD-SCLC						
No. of patients evaluated	1	2	3	1	3	10
CR	1	0	2	1	0	4 (40%)
PR	0	0	0	0	2	2 (20%)
NC	0	2	1	0	0	3 (30%)
PD	0	0	0	0	1	1 (10%)
ED-SCLC						
No. of patients evaluated	2	1	3	2	3	11
CR	0	0	0	0	0	0 (0%)
PR	1	0	2	1	2	6 (55%)
NC	1	1	0	1	1	4 (36%)
PD	0	0	1	0	0	1 (9%)

LD-SCLC limited disease small-cell lung cancer, *ED-SCLC* extensive disease small-cell lung cancer, *CR* complete response, *PR* partial response, *NC* no change, *PD* progressive disease

recent randomized phase III study. Indeed, grade 2 or more diarrhea occurred in 44% of the evaluable patients [18]. Topotecan has the advantage of a lower incidence of diarrhea compared to irinotecan when combined with cisplatin. However, Lilenbaum et al.[12] also demonstrated in a phase I study of topotecan combined with cisplatin that grade 2 diarrhea occurred in 3 (9.7%) of 31 patients despite the fact that no grade 3 or 4 diarrhea was experienced. In addition, Ardizzoni et al.[3] reported grade 3 or 4 diarrhea to be 4% in a phase II trial of topotecan with cisplatin. Accordingly, the 3-day administration schedule in the present study may be superior to prevent diarrhea.

In the previous phase I studies of topotecan and cisplatin, the major toxicity was myelosuppression [12, 16, 22–24]. In a phase I study of 5-day topotecan with cisplatin conducted by Miller et al.[16], dose-limiting grade 4 neutropenia lasting for more than 7 days occurred in three (30%) of nine patients, whereas our 3-day-schedule regimen did not show such a durable toxicity. Additionally, in a phase II study comparing a 3-day regimen of topotecan and cisplatin with a 5-day regimen, the incidence of grade 3 or more leukopenia was somewhat lower in the former regimen (22 and 33%, respectively) [26]. These observations suggest that a 3-day topotecan regimen may be less toxic than a 5-day one, although other clinical factors possibly affected the difference of the toxicity profiles. Furthermore, the frequency of neutropenia in our trial was almost comparable with that in the irinotecan and cisplatin arm of the randomized trial [18], and that in the combination chemotherapy of carboplatin and etoposide in elderly patients with SCLC [19]. Thus, our regimen is considered to be safely administrable in terms of both hematological and non-hematological toxicity when compared with the previous results.

With regard to the efficacy, our regimen seems to have potential antitumor activity in elderly patients with SCLC, with response rates of 60% in LD-SCLC and 55% in ED-SCLC. In addition, the median survival times for LD- and ED-SCLC were 16.0 and 11.0 months, respectively. In the previous clinical trials, median survival times in the treatment of elderly LD- and ED-SCLCs were reported to be 12–15 and 9–11 months, respectively, with combination chemotherapy consisting of carboplatin and etoposide [5, 10, 15, 19]. Ardizzoni et al.[2] recently conducted a phase II study of cisplatin and etoposide in elderly patients with LD- and ED-SCLC. They demonstrated that the overall response rate and survival time were 60.0% and 9.5 months, respectively. The clinical outcome in our study seems to be comparable with these studies, suggesting that this regimen has considerable antitumor activity in elderly patients with SCLC. Because of the small sample size in this study, it is necessary to verify the efficacy of this regimen in a subsequent phase II study.

In conclusion, combination chemotherapy consisting of topotecan and cisplatin on days 1–3 is well tolerated for elderly patients with SCLC, which seems to show reasonable efficacy. The phase II study of this regimen is now under investigation.

References

1. Ardizzoni A, Grossi F (2000) Update on the treatment of small cell lung cancer (SCLC). Ann Oncol 11(Suppl 3):101–108
2. Ardizzoni A, Favaretto A, Boni L, Baldini E, Castiglioni F, Antonelli P, Pari F, Tibaldi C, Altieri AM, Barbera S, Cacciani G, Raimondi M, Tixi L, Stefani M, Monfardini S, Antilli A, Rosso R, Paccagnella A (2005) Platinum-etoposide chemotherapy in elderly patients with small-cell lung cancer: results of a randomized multicenter phase II study assessing attenuated-dose or full-dose with lenograstim prophylaxis—a Forza Operativa Nazionale Italiana Carcinoma Polmonare and Gruppo Studio Tumori Polmonari Veneto (FONICAP-GSTPV) study. J Clin Oncol 23(3):569–575
3. Ardizzoni A, Manegold C, Gaafar R, Buchholdz E, Debruyne C, Damen S, Curran D, King K, Giaconne G (1999) Combination chemotherapy with cisplatin and topotecan as second-line treatment of sensitive and refractory small cell lung cancer (SCLC): An EORTC LCCG phase II study. Proc Am Soc Clin Oncol 18:471a (Abstr 1817)
4. Brown JV 3rd, Peters WA 3rd, Rettenmaier MA, Karlan BY, Dillman RA, Smith MR, Drescher CW, Micha JP (2000) A phase I trial of a 3-day topotecan Q 21 days for recurrent epithelial cancers of the ovary, fallopian tube, and peritoneum. Gynecol Oncol 79(3):495–498
5. Evans WK, Radwi A, Tomiak E, Logan DM, Martins H, Stewart DJ, Goss G, Maroun JA, Dahrouge S (1995) Oral etoposide and carboplatin. Effective therapy for elderly patients with small cell lung cancer. Am J Clin Oncol 18(2):149–155
6. Findlay MP, Griffin AM, Raghavan D, McDonald KE, Coates AS, Duval PJ, Gianoutsos P (1991) Retrospective review of chemotherapy for small cell lung cancer in the elderly: does the end justify the means? Eur J Cancer 27(12):1597–1601
7. Greco FA (2003) Topotecan as first-line therapy for small cell lung cancer. Lung Cancer 41(Suppl 4):S9–S16
8. Hsiang YH, Liu LF (1988) Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. Cancer Res 48(7):1722–1726
9. Johnson BE (2002) Management of small cell lung cancer. Clin Chest Med 23(1):225–239
10. Jeremic B, Shibamoto Y, Acimovic L, Milisavljevic S (1998) Carboplatin, etoposide, and accelerated hyperfractionated radiotherapy for elderly patients with limited small cell lung carcinoma: a phase II study. Cancer 82(5):836–841
11. Kosmidis PA, Samantas E, Fountzilas G, Pavlidis N, Apostolopoulou F, Skarlos D (1994) Cisplatin/etoposide versus carboplatin/etoposide chemotherapy and irradiation in small cell lung cancer: a randomized phase III study. Hellenic Cooperative Oncology Group for Lung Cancer Trials. Semin Oncol 21(3 Suppl 6):23–30
12. Lilenbaum RC, Miller AA, Batist G, Bernard S, Hollis DR, Rosner GL, Egorin MJ, Schilsky RL, Ratain MJ (1998) Phase I and pharmacologic study of continuous infusion topotecan in combination with cisplatin in patients with advanced cancer: a Cancer and LeukemiaGroup B study. J Clin Oncol 16(10):3302–3309
13. Markman M, Kennedy A, Webster K, Kulp B, Peterson G, Belinson J (2000) Phase 2 evaluation of topotecan administered on a 3-day schedule in the treatment of platinum- and paclitaxel-refractory ovarian cancer. Gynecol Oncol 79(1):116–119
14. Masuda N, Fukuoka M, Kusunoki Y, Matsui K, Takifuji N, Kudoh S, Negoro S, Nishioka M, Nakagawa K, Takada M (1992) CPT-11: a new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 10(8):1225–1229