TABLE 1. Patient Characteristics and Sample Procurement According to EGFR Mutation Status

	EGFR Mutation Status			
	All	Mutation	Wild type	*p*
All cases	66	27 (21)	39	
Sex				0.175
Male	36	10 (8)	26	
Female	30	17 (13)	13	
Age (yr)				0.5084
≤64	31	14 (11)	17	
>64	35	13 (10)	22	
Histology				0.0199
Adenocarcinoma[a]	59	27 (21)	32	*p* ([a] vs. [b])
Squamous cell[b]	2	0	2	
Large cell[b]	2	0	2	
Pleomorphic[b]	1	0	1	
NSCLC NOS[b]	2	0	2	
Smoking status				0.0002
Never smoker[c]	24	17 (13)	7	*p* ([c] vs. [d])
Former smoker[d]	17	9 (7)	8	
Current smoker[d]	25	1 (1)	24	
Stage at initial diagnosis				0.6348
IA[e]	2	1	1	*p* ([e] vs. [f])
IIB[e]	4	2 (2)	2	
IIIA[f]	3	0	3	
IIIB[f]	16	3 (2)	13	
IV[f]	41	21 (17)	20	
Performance status				0.6059
0/1	51	20 (14)	31	*p* (0/1 vs. ≥2)
2	7	3 (3)	4	
3	3	1 (1)	2	
4	5	3 (3)	2	
Prior first treatment				ND
No	8	5 (5)	3	
Surgery	3	3 (1)	0	
Thoracic irradiation	4	2 (2)	2	
Chemoradiotherapy	10	2 (1)	8	
Bone irradiation	6	3 (3)	3	
Brain irradiation	6	3 (2)	3	
Sclerotherapy for effusion	1	1 (1)	0	
Chemotherapy	28	8 (6)	20	
Prior chemotherapy				0.4337
0	28	13 (12)	15	*p* (0 vs. ≥1)
One regimen	28	10 (6)	18	
Two regimens	8	4 (3)	4	
Three regimens	2	0	2	
Method for sample procurement				ND
Bronchoscopic biopsy	23	11	12	
CT/US-guided needle biopsy	22	6	16	
Pleural effusion aspiration	7	4	3	
LN/skin aspiration	6	2	4	
Tonsil/skin biopsy	2	0	2	
Thoracotomy	3	2	1	
VATS	2	1	1	
Mediastinoscopy	1	1	0	

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; NOS, not otherwise specified; ND, not done; CT/US, computed tomography/ultrasound; LN, lymph node; VATS, video-assisted thoracoscopy. Superscript letters indicate groups compared in the statistical analysis. Numbers in parentheses represent the numbers of patients receiving gefitinib treatment.

17 were the point mutation at codon 858. As previously reported,[12–14,17] the EGFR mutations were significantly associated with adenocarcinoma histology and never-smoking status (Table 1). However, the EGFR mutation status was not significantly correlated with sex, age, PS, stage at initial diagnosis, or prior chemotherapy. Twelve patients received gefitinib treatment as the first-line chemotherapy; five patients desired first-line gefitinib therapy, and the other seven were unfit for conventional chemotherapy because of age (one patient, age 84 yr), cardiac disease (one patient), widespread bone metastases (two patients), and poor PS (3–4 in three patients).

Clinical Response and Survival

Of 27 patients harboring EGFR mutation, 21 were treated with gefitinib and were assessable for objective responses (Table 2) and adverse events (Table 3). The median interval of gefitinib treatment was 5.9 months (range, 0.67 to 11.4 mo). Of the assessable 21 patients, 19 patients achieved objective responses (three complete response and 16 partial response), for an overall response rate of 90.5% (95% CI, 69.6–98.8%). One patient had stable disease, giving an overall disease control rate of 95.2% (95% CI, 76.2–99.9%). According to EGFR mutation classes and PS, the objective responses were seven of eight for the exon 19 deletion, 12 of 13 for the L858R point mutation, 13 of 14 in PS 0 to PS 1 patients, and 6 of seven in PS 2 to PS 4 patients. The response to gefitinib did not differ significantly according to the mutation class or PS.

The median PFS was 7.7 months (95% CI, 6.0 mo to not reached) (Figure 1A). The median OS has not been reached at present (Figure 1B). Subset analyses showed that PFS was greater in patients with the exon 19 deletion than in those with the L858R point mutation (log rank test, $p = 0.04$; Fig 2A). The median PFS for the exon 19 deletion group was 7.8 months (95% CI, 7.6 mo to not reached); for the L858R mutation group, median PFS was 6.0 months (95% CI, 2.6 to 7.7 mo). OS did not differ significantly between the two types of mutations (Figure 2B). No difference was observed in PFS

TABLE 2. Response of EGFR Mutation-Positive Patients to Gefitinib Treatment

	EGFR Mutation Status		
	Exon 19 Deletion (n = 8)	L858R Mutation (n = 13)	Total (n = 21)
CR	1 (12.5%)	2 (15.4%)	3 (14.3%)
PR	6 (75%)	10 (76.9%)	16 (76.2%)
Overall response rate (CR + PR)	7 (87.5%)	12 (92.3%)	19 (90.5%)
SD	1 (12.5%)	0	1 (4.8%)
Disease control (CR + PR + SD)	8 (100%)	12 (92.3%)	20 (95.2%)
Progressive disease	0	1 (7.7%)	1 (4.8%)

EGFR, epidermal growth factor receptor; CR, complete response; PR, partial response; SD, stable disease.

TABLE 3. Number (%) of Patients with Treatment-Related Adverse Events (n = 21)

	Grade				
	0	1	2	3	4
Skin toxicity	15 (71)	4 (19)	2 (10)	0	0
Diarrhea	13 (62)	3 (14)	3 (14)	2 (10)	0
Elevated aspartate aminotransferase/ alanine aminotransferase	15 (71)	1 (5)	2 (10)	3 (14)	0
Nail changes	17 (81)	3 (14)	1 (5)	0	0
Mucositis	20 (95)	1 (5)	0	0	0
Joint pain	20 (95)	1 (5)	0	0	0

and OS between never-smokers and current/former smokers (data not shown).

Adverse Events

All 21 patients were evaluated for drug-related adverse events. The most common adverse events were skin toxicity, diarrhea, and elevated asparatate aminotransferase/alanine aminotransferase (AST/ALT) (Table 3). The grade 3 adverse events of diarrhea and elevated AST/ALT occurred in two (10%) and three (14%) patients, respectively. These events occurred slightly more frequently than in previous studies.[8,9] No grade 4 adverse events or pulmonary toxicity were observed. Seven patients required an interruption of treatment, lasting 2 to 4 weeks, because of grade 2/3 diarrhea or grade 3 elevated transaminases. Two patients withdrew: one after 3 weeks of gefitinib treatment because of grade 3 diarrhea, and the other after 9 weeks of gefitinib treatment because of grade 2 nail changes.

DISCUSSION

In the present study, we have observed that the objective response rate in our patients was similar to that in previous reports. We also found that PFS and OS seem promising in identifying gefitinib-sensitive patients regardless of whether the study includes patients unsuited for conventional cytotoxic chemotherapy because of age, cardiac disease, widespread bone metastases, or poor PS (3 to 4). Our favorable data might have resulted because we selected patients harboring one of two hotspot mutations (exon 19 deletion and exon 21 L858R mutation). Greulich et al.[31] examined NIH-3T3 cells transformed with various EGFR mutants and showed that a distinct EGFR mutation confers differential sensitivity to TKIs. They demonstrated greater sensitivity to TKIs in cell lines with the two hotspot mutations than with the G719S mutation, and insensitivity to TKIs in cell lines with exon 20 insertion (D770-N771 ins) mutation. These in vitro data may explain, at least partially, our promising results for detecting these two sensitive mutations.

We previously reported that patients with the EGFR exon 19 deletion respond significantly better to gefitinib than those with the L858R mutation ($p = 0.0108$).[17] Our current data show no difference in gefitinib sensitivity and OS after
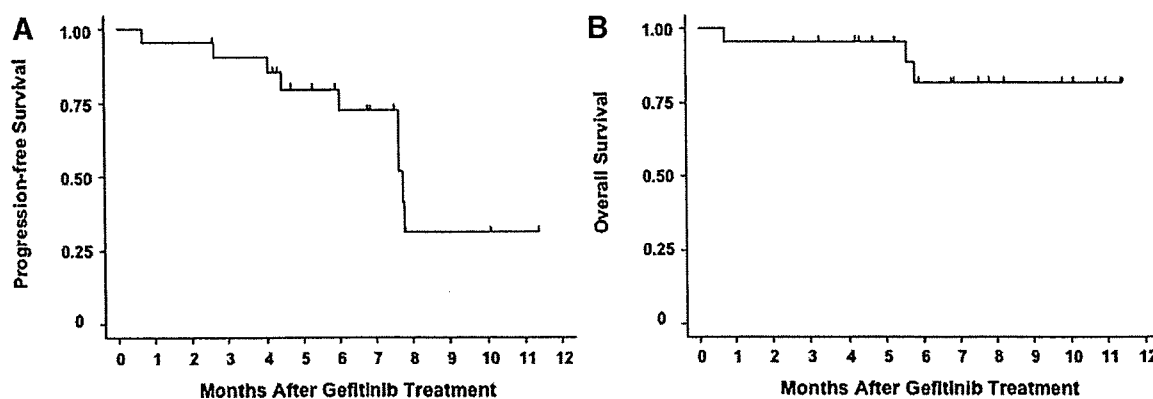
FIGURE 1. Kaplan–Meier estimates of (*A*) progression-free survival and (*B*) overall survival for patients with EGFR mutations (*n* = 21). The median progression-free survival was 7.7 months (95% CI, 6.0 mo to not reached). The median survival was not reached.
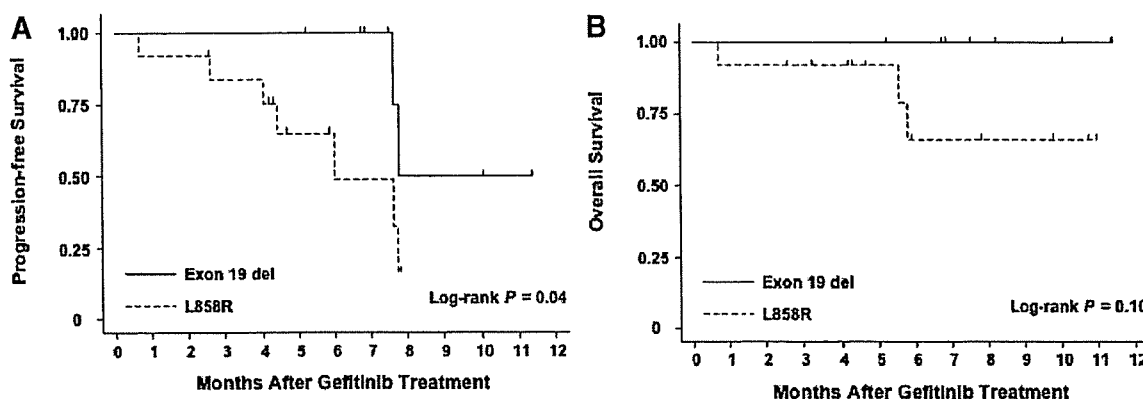

FIGURE 2. Kaplan–Meier estimates of (*A*) progression-free survival and (*B*) overall survival for patients with EGFR mutations according to the exon 19 deletion (*n* = 8) and L858R mutation (*n* = 13). The median PFS for the exon 19 deletion group was 7.8 months (95% CI, 7.6 mo to not reached); for the L858R mutation group, median PFS was 6.0 months (95% CI, 2.6 to 7.7 mo).

gefitinib treatment between these two groups of patients, although we observed a greater PFS in the EGFR exon 19 deletion group than in the L858R group. It is possible that the number of patients (eight with exon 19 deletion and 13 with L858R) was too small to detect a statistically significant difference in OS. Riely et al.[32] reported recently that patients with exon 19 deletion have a significantly longer survival after TKI treatment than those with the L858R mutation (*p* = 0.01). These findings suggest that the EGFR exon 19 deletion might be a better predictor of the efficacy of TKIs than the L858R mutation.

EGFR mutations are significantly associated with patients with adenocarcinomas, patients of Asian origin, females, and patients who had never smoked—clinical factors also associated with patients who respond to gefitinib.[13,14,24,33] A phase II trial using gefitinib monotherapy as the first-line therapy for patients with adenocarcinoma histology and never-smoking status was recently completed in South Korea and reported promising data (e.g., an objective response rate of 69% and estimated 1-year survival rate of 73%).[34] However, this trial did not select patients using

biomarkers, and we believe the benefit of gefitinib therapy could be enhanced by selecting individual patients according to appropriate biomarkers. Very recently, two prospective phase II studies that had selected patients based on molecular biomarkers demonstrated that EGFR mutations[35] and gene copy number assessed by fluorescence in situ hybridization (FISH)[36] can predict clinical outcomes in TKI-treated NSCLC patients.

The grade 3 adverse events of diarrhea and elevated AST/ALT were observed in five patients (24%); this is a higher rate than that reported in two previous phase II studies that reported rates of adverse events of 1.5%[8] and 7%[9] at a gefitinib dose of 250 mg per day. The reasons for our higher rate of adverse events are unknown. Although adverse events related to gefitinib treatment are generally thought to be mild and tolerable, they should not be discounted.

Most studies have detected EGFR mutations using direct sequencing or single-strand conformation polymorphism analysis for exons 18 to 21.[37] These techniques are less sensitive when applied to a small amount of tumor cells from the biopsy or aspiration samples.[38] We were able to detect

two hotspot mutations with our sensitive rapid screening assay in most biopsy or aspiration samples in the routine clinical setting. Although this assay needs precise assessment of tumor samples by a pathologist to enrich the tumor cells, it is very sensitive and accurate for detection, and it can be completed within 4 hours without need for microdissection or nested PCR process.[29]

The key genetic event for TKI sensitivity has not been perfectly identified and is the subject of a growing debate about the role of EGFR mutations versus EGFR gene amplification/copy number in NSCLC. EGFR mutant NSCLC cell lines are strongly associated with increased EGFR gene copy number.[39,40] Cappuzzo et al.[27] and Takano et al.[22] found that EGFR mutations in NSCLC patients correlate significantly with gene copy number assessed by FISH and quantitative real-time PCR, respectively. However, Cappuzzo et al.[27] demonstrated that in patients treated with gefitinib, a high EGFR gene copy number is a better predictor of survival than EGFR mutations.[27] In contrast, Takano et al.[22] reported that the status of the EGFR mutations, rather than gene copy number, is the major determinant of gefitinib efficacy. Recent reports of the molecular analyses from the largest phase III TKI monotherapy trials failed to show that the EGFR mutation is superior to gene copy number in predicting the efficacy of TKIs.[23,26] These conflicting results on EGFR mutations and gene amplification/copy number could be explained by (i) differences in the detection methodologies and assessment of mutation and gene amplification/copy number (e.g., direct sequence versus PCR-based DNA testing for detecting EGFR mutations, or FISH versus PCR-based amplification for detecting EGFR gene amplification/copy number), (ii) failure to reconfirm these results in other institutions, and (iii) other unknown factors underlying drug sensitivity, especially those related to ethnicity. Further prospective studies are needed to investigate the crucial molecular markers involved in the EGFR network, using adequate tissue samples and assays to more precisely detect molecular events.

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Original contribution

Gemcitabine/Carboplatin in a Modified 21-Day Administration Schedule for Advanced-Stage Non–Small-Cell Lung Cancer

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Abstract

PURPOSE: Gemcitabine/carboplatin is active for advanced-stage non–small-cell lung cancer. Although it has a better toxicity profile than gemcitabine/cisplatin, severe thrombocytopenia can be a problem. We conducted a phase II study of gemcitabine/carboplatin on a 21-day schedule with administration of carboplatin delayed until day 8, intending to decrease the severity of thrombocytopenia and evaluate the feasibility and efficacy of this schedule. PATIENTS AND METHODS: Thirty-one patients with stage IIIB or stage IV non–small-cell lung cancer received gemcitabine 1000 mg/m^2 on days 1 and 8 and carboplatin at an area under the curve of 5 mg × minute/mL on day 8, every 21 days. RESULTS: The response rate was 22.6%, including 1 complete response. The median time to progression was 161 days, and the median survival was 454 days. Grade 3/4 thrombocytopenia, according to the National Cancer Institute Common Toxicity Criteria, version 3.0, was observed in 2 patients (6.5%) in the first 2 cycles. Nonhematologic toxicity included rash, depression, fever, nausea/vomiting and increased hepatic transaminase. The median courses of delivery were 3, and 13 patients (42%) received the first 3 courses without treatment delay. Dose intensity for each drug was 638 mg/m^2 per week for gemcitabine and 1.56 mg × minute/mL per week for carboplatin area under the curve, respectively. CONCLUSION: This study suggests that gemcitabine/carboplatin with a day-8 administration of carboplatin in a 21-day schedule reduces the severity of thrombocytopenia without having a detrimental effect on efficacy.

Introduction

Non–small-cell lung cancer (NSCLC) constitutes 75%-80% of lung cancer cases and currently represents a leading cause of cancer-related death throughout the world.[1] Significant proportions of the patients present with locally advanced or metastatic disease at the time of diagnosis.[2] Although a recent overview suggested that platinum agent–based chemotherapy improves survival and quality of life,[3] the long-term prognosis of these patients is still generally poor. In the past 2 decades, several new chemotherapeutic agents have been developed and have proven to be active in advanced-stage NSCLC. Gemcitabine, a pyrimidine antimetabolite, is one of the most promising among these agents,

showing definite efficacy and mild toxicity profiles. Initial phase I studies using a schedule of weekly administrations of 3 weeks for every 4 weeks established 790 mg/m^2 weekly as the maximum tolerated dose. Dose-limiting toxicity was myelosuppression, with thrombocytopenia more significant than granulocytopenia.[4] Later phase I/II studies have established 1250 mg/m^2 weekly as an optimal tolerated dose.[5-7] Several phase II studies of single-agent gemcitabine in advanced-stage NSCLC have demonstrated response rates of 20%-26% and a median survival of 7-9.4 months.[8-13] In these studies, 800-1250 mg/m^2 gemcitabine was administered weekly for 3 weeks every 4 weeks. Toxicities reported in these studies were myelosuppression, such as granulocytopenia and thrombocytopenia, transient increase of hepatic transaminases, rash, flu-like symptoms, and lethargy.

The combination of gemcitabine and a platinum compound has demonstrated a synergistic effect in preclinical settings, and a number of phase II/III studies of gemcitabine/cisplatin have been performed.[14-22] This combination chemotherapy has proved to be very promising, showing

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an objective response rate (ORR) of 28%-54% and a median survival of 8.4-15.4 months. Gemcitabine/cisplatin is now one of the standard chemotherapy combinations for advanced-stage NSCLC. However, the toxicity profile of cisplatin, such as nausea/vomiting, nephrotoxicity, and neurotoxicity, can be troublesome for patients with advanced-stage NSCLC, who generally have poor prognosis. Moreover, cisplatin is often intolerable for certain patients, especially the elderly and/or those with concomitant severe diseases. Carboplatin is a cisplatin analogue, and its nonhematologic toxicity is milder compared with cisplatin. Carboplatin is also expected to exert a synergistic effect with gemcitabine. Several phase II studies of gemcitabine/carboplatin have been reported. The early studies adopted a schedule of weekly administration of gemcitabine for 3 weeks (day 1, 8, and 15 administrations) and day-1 administration of carboplatin every 4 weeks.[23-29] However, those studies reported high incidences of thrombocytopenia, prompting the investigation of other schedules that are less myelosuppressive. Iaffaiolli et al recommended a 28-day schedule that decreased myelotoxicity around day 15 by administrating carboplatin on day 8 and eliminating the administration of gemcitabine on day 15.[30] Edelman et al recommended a 21-day schedule that decreased myelotoxicity around day 15 by simply eliminating the administration of gemcitabine on day 15.[31] Several large phase II studies have been performed using these schedules. Among them, Mott et al reported a phase II study with a 28-day schedule described by Iaffaiolli et al, with an ORR of 10% and a median survival of 8.3 months.[32] On the other hand, Yamamoto et al reported the results of a comparative phase II study in which a 21-day schedule described by Edelman et al was compared with gemcitabine/vinorelbine as a control arm.[33] The ORR of gemcitabine/carboplatin was 20%, and the median survival of 432 days was favorable. However, a high incidence of dose reduction as a result of myelosuppression and early withdrawal from the study were reported. These studies suggest that the schedule for gemcitabine/carboplatin still needs improvement. In the present article, we report another 21-day schedule, with the intent to be more dose intense than Mott et al and less myelosuppresive than Yamamoto et al.

Patients and Methods

Eligibility Criteria

Eligibility criteria of patients were as follows: age 20-80 years, a histologic or cytologic diagnosis of clinical stage IIIB NSCLC with malignant pleural effusion or clinical stage IV NSCLC, and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2. Patients were required to have adequate bone marrow reserve (leukocyte count > 4000/μL, platelet count > 100,000/μL, and hemoglobin > 10 g/dL), normal hepatic function (serum bilirubin < 1.5 mg/dL, transaminases < 2 times the upper limit of normal), normal renal function (serum creatinine < 1.2 mg/dL), and a life expectancy of > 3 months. Patients who did not have measurable disease based on Response Evaluation Crite-

ria in Solid Tumors[34] were excluded from the study. Neither previous chemotherapy nor thoracic irradiation was allowed. Patients were excluded from the study when they met one of the following conditions: active uncontrolled infection, unstable concomitant disease (ischemic heart disease, hypertension, or diabetes mellitus), active concomitant malignant disease, pregnancy, or breastfeeding. Written informed consent was obtained from all patients.

Study Design

This was a single-arm phase II study. Because the response rate of gemcitabine/carboplatin has been reported by a variety of authors, we determined the primary endpoint of our study as the rate of treatment completion without treatment delay. It has been reported that the median courses of delivery of platinum-doublet chemotherapy was approximately three[35] and that there was no statistical significance in survival of patients between 3 and 6 courses of platinum agent–containing chemotherapy.[36] Therefore, we analyzed drug delivery in the first 3 courses to evaluate the feasibility of the schedule and defined the treatment completion rate to be the percentage of patients who received the first 3 courses with no delay from the intended schedule. The expected and threshold value of the treatment completion rates were 90% and 70%, respectively. The number of patients required was determined with an α risk of 0.05 and a β risk of 0.2. Simon's optimal design was applied to recruit the patients[37]: if completion of treatment was observed in < 5 patients among the first 6 patients, the study was to be terminated; if it was observed in ≥ 5 patients, recruitment of as many as 27 patients was allowed. This schedule was judged to be feasible when, in an analysis of 27 patients, treatment completion was observed in > 22 patients. The secondary endpoints included the evaluation of response rate, toxicities, median time to progression (TTP), and overall survival. This study was approved by the Institutional Review Board of Osaka Medical Center for Cancer and Cardiovascular Diseases.

Treatment Plan

Patients received carboplatin at an area under the curve (AUC) of 5 mg × minute/mL, calculated using the Calvert formula[38] with creatinine clearance evaluation by the Cockcroft formula.[39] Carboplatin was administrated in a 60-minute infusion on day 8 of a 21-day cycle. Gemcitabine was administrated at 1000 mg/m^2 in a 30-minute infusion on days 1 and 8. The planned dose intensity for each drug was 667 mg/m^2 per week for gemcitabine and 1.67 mg × minute/mL every week for carboplatin AUC. Four cycles of treatment were intended. On day 1 and day 8 of each cycle, complete blood count was evaluated. Drug administration was delayed until recovery in cases with leukocyte count < 3000/μL or platelet count < 100,000/μL on day 8.

The hematologic criteria to start the next cycles were loosened to increase dose intensity (leukocyte count > 2500/μl,

Table 1 Patient Characteristics (N = 31)

Characteristic	Number of Patients
Median Age, Years (Range)	63 (42-76)
Sex	
Male	12
Female	19
Stage	
IIIB	8
IV	23
Histology	
Adenocarcinoma	25
Squamous cell carcinoma	6
ECOG PS	
0	22
1	9

Table 2 Hematologic Toxicities

Adverse Event	Grade 3	Grade 4	N (%)
Leukopenia	10	0	10 (32.2)
Neutropenia	16	5	21 (67.7)
Anemia	3	0	3 (9.7)

and platelet count > 75,000/μL). The start of the new cycles was postponed until blood count met these criteria. Doses of gemcitabine were adjusted according to leukocyte, neutrophil, and platelet counts. If grade 4 leukopenia or neutropenia continued > 3 days despite the use of granulocyte colony-stimulating factor or if platelet count decreased to < 25,000/μL, the gemcitabine dose was reduced by 200 mg/m² intervals until 600 mg/m². Patients were withdrawn from the study in cases of disease progression, development of grade > 3 nonhematologic toxicities, unacceptable treatment delay as a result of hematologic toxicities, or necessity of gemcitabine dose reduction to < 600 mg/m². After withdrawal from the study, subsequent treatment was to be decided by the investigator.

Evaluation

Response was evaluated by chest and abdominal computed tomography (CT) scans after the second and fourth cycles of chemotherapy according to Response Evaluation Criteria in Solid Tumors. Brain magnetic resonance imaging, chest CT scan, and abdominal CT scan were performed at any time if assessment for the disease progression was necessary. Confirmation was necessary to determine partial and complete response. During the study, all enrolled patients were evaluated weekly by physical examination, complete blood count, and blood chemistries. Toxic effects were graded according to National Cancer Institute Common Toxicity Criteria, version 3.0.

Statistical Analysis

Time to progression was calculated from the date of enrollment to the date of progression using the Kaplan-Meier method.[40] Overall survival was calculated from the date of enrollment until the date of death or last known contact using the Kaplan-Meier method. Statistical analysis in the study was carried out using the SPSS program.

Results

From June 2003 to April 2005, 31 eligible patients were enrolled in the study. There were 12 men and 19 women; 6 patients with squamous cell carcinoma and 25 with adenocarcinoma; 8 patients with clinical stage IIIB and 23 with clinical stage IV; 22 patients with an ECOG PS of 0 and 9 with a PS of 1. Sixteen patients had a smoking history. Patient characteristics are summarized in Table 1. Tumor response was assessable in all 31 patients. One complete response and 6 partial responses were observed, resulting in a response rate of 22.6%. Median TTP was 161 days (95% confidence interval, 109-213 days). At the time of analysis, when the median follow-up time was 356 days (range, 40-946 days), 12 patients were alive, 16 patients were dead, and 3 patients were lost to follow-up. Median survival time was 454 days (95% confidence interval, 230-678 days).

Toxicity profiles are summarized in Tables 2, 3, and 4. Table 2 shows hematologic toxicities except thrombocytopenia in the first 2 cycles. Neutropenia was frequently observed, with grade 3/4 neutropenia occurring in 51.6% (16 of 31 patients) and 16.1% (5 of 31 patients) of the patients, respectively. However, febrile neutropenia was not observed. Grade 3 anemia was observed in 9.7% of patients (3 of 31 patients), and grade 4 anemia was not observed. The incidence of red blood cell and platelet transfusions was 3.2% (1 of 31 patients) and 3.2% (1 of 31 patients), respectively. Because the grading of thrombocytopenia is substantially different among versions of the National Cancer Institute Common Toxicity Criteria, we show detailed results of platelet numbers in Table 3. Thrombocytopenia was relatively mild; grade 3/4 thrombocytopenia occurred in 3.2% (1 of 31 patients) and 3.2% (1 of 31 patients) of patients in the first 2 cycles, without serious hemorrhagic events. The lowest platelet count was 15,000/μL and was observed in the first cycle in a 74-year-old man. Grade 2/3 nausea/vomiting occurred in 3.2% (1 of 31 patients) and 3.2% (1 of 31 patients) of patients, respectively, grade 2 and 3 rash in 6.5% (2 of 31 patients) and 12.9% (4 of 31 patients), grade 3 depression in 3.2% (1 of 31 patients), grade 1 fever (in the absence of neutropenia) in 3.2% (1 of 31 patients), and grade 1 hepatic transaminase increase in 9.7% (3 of 31 patients). A total of 94 cycles with a median of 3 cycles for each patient were administered. Treatment was delayed in 42.6% of cycles and required dose reduction in 6.4% of cycles. The median number of days per cycle was 24 days (22, 29, and 26 days for the first, second, and third cycles, respectively). The dose intensity was 638 mg/m² per week for gemcitabine and 1.56 mg × minute/mL per week for carboplatin AUC.

Table 3	Thrombocytopenia Incidence			
Thrombocytopenia	N	Overall	1/2 Cycles	≥3 Cycles
Grade 3/4	31	2/3 (16.2%)	1/1 (6.5%)	1/2 (9.7%)

Nadir platelet counts in 5 cases with grade > 3 thrombocytopenia ($\times 10^4$) were 1.5, 2, 2.5, 3.9, and 4.9.

Table 4	Nonhematologic Toxicities			
Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	2	1	1	0
Rash	0	2	4	0
Depression	0	0	1	0
Fever (Absence of Neutropenia)	1	0	0	0
Transaminase	3	0	0	0

Among the first 6 patients, 5 had ≥ 3 treatment cycles without treatment delay (4, 3, 2, 8, 4, and 4 cycles for the first, second, third, fourth, fifth, and sixth patients, respectively). Final analysis revealed that 21 of 31 patients received ≥ 3 treatment cycles, but 8 of these patients experienced treatment delay in the first 3 cycles. The treatment completion rate was not sufficiently high at 42%. Ten patients were withdrawn from the study early; the reason for withdrawal was progressive disease for 2 patients, hematologic toxicity for 3 (all were neutropenic but did not have thrombocytopenia), and nonhematologic toxicity for 5 (grade 3 depression in 1 patient and grade 3 rash in 4 patients; 1 was caused by carboplatin, and the others were caused by gemcitabine).

Discussion

Third-generation chemotherapy, consisting of a platinum agent and a third-generation chemotherapeutic agent, including gemcitabine, is considered a standard treatment for advanced-stage NSCLC worldwide. Many studies were carried out to compare the toxicity and efficacy of each regimen of third-generation chemotherapy. According to the ECOG 1594 study, a significant difference in efficacy is difficult to demonstrate among the regimens.[41] In contrast, the profiles of toxicities were demonstrably different among the regimens.

Although platinum compounds, such as cisplatin and carboplatin, are still key drugs in chemotherapy for NSCLC, a recent metaanalysis suggested that treatment with regimens containing gemcitabine showed small but statistically significant improvement in patient survival.[42] With its mild toxicity and easiness in administration, gemcitabine is becoming another key drug in chemotherapy for NSCLC. In a Japanese phase III trial in which gemcitabine/vinorelbine/paclitaxel in combination with a platinum agent were compared with irinotecan/cisplatin, a Japanese standard for NSCLC, gemcitabine/cisplatin exerted the best result; however, the difference was not statistically significant.[35] Recent trials showed that the gemcitabine/carboplatin improved patient survival compared with gemcitabine alone and mitomycin/ifosfamide/cisplatin.[43,44] Taking these results together, gemcitabine/carboplatin is a reasonable combination and becoming widely used for NSCLC.

Early studies of gemcitabine/carboplatin used a 28-day schedule in which gemcitabine was administered on days 1, 8, and 15 and carboplatin was administered on day 1.[23-29] However, because of a high incidence of severe thrombocytopenia, 2 alternate schedules were proposed: one is a 21-day schedule treatment in which gemcitabine is administered on days 1 and 8 with carboplatin administered on day 1,[31] and the other is a 28-day schedule in which gemcitabine is administered on day

1 and 8 with carboplatin on day 8.[30] Obasaju et al conducted a randomized phase II study comparing these 2 schedules.[45] Although the study was not powered to show a statistically significant difference between these 2 regimens, the 21-day schedule seemed to be superior to the 28-day schedule in terms of efficacy. However, grade 3/4 thrombocytopenia was observed in 14% of cycles in the 21-day schedule, higher than that in the 28-day schedule. The 21-day schedule has been used in several other studies, in which thrombocytopenia was still the main problem, accompanied by bleeding episodes, although not frequently.[27,46,47] In the Japanese phase II study described previously, thrombocytopenia was again a major issue, resulting in a high incidence of dose reduction and early withdrawal from the study.[33] Nevertheless, good median survival time of the patients treated with gemcitabine/carboplatin (432 days) and low incidences of nonhematologic toxicities were impressive. Meanwhile, the 28-day schedule in which carboplatin was administered on day 8 appeared to be less myelotoxic than the 21-day schedule but has the problem of low dose intensity.

Our study was designed to evaluate the feasibility and efficacy of gemcitabine/carboplatin in a modified administration schedule. Gemcitabine/carboplatin were administered at 1000 mg/m^2 on days 1 and 8 and at AUC 5 on day 8 of each 21-day cycle, respectively. The main aim of this study was to decrease the severity of thrombocytopenia with minimal effect on dose intensity. The low incidence of grade 3/4 thrombocytopenia was notable, observed in only 2 of 31 patients in the first 2 cycles. This result suggested that the nadir of thrombocytopenia of gemcitabine and carboplatin occur around day 15, and that incidence of severe thrombocytopenia could be decreased even in a 21-day schedule by delaying administration of carboplatin until day 8. We were concerned whether this 3-weekly chemotherapy would become possible by adopting looser criteria (leukocyte count > 2500/μl and platelet count > 75,000/μL) to start new cycles. Other hematologic and nonhematologic toxicities were also mild, and altogether, the treatment was well tolerated. The incidence of stressful toxicities represented by nausea/vomiting, neurologic toxicities, and alopecia was relatively low in the gemcitabine/carboplatin combination.

The planned dose intensities and actual dose intensities were 667 mg/m^2 per week and 638 mg/m^2 per week (95.7% of planned dose intensity) for gemcitabine and 1.67 mg -

minute/mL per week and 1.56 mg × minute/mL per week (93.4% of planned dose intensity) for carboplatin AUC, respectively. Dose intensity for each drug in the 28-day schedule described previously[30,32] was estimated to be 550 mg/m² per week for gemcitabine and 1.25 mg × minute/mL per week for carboplatin AUC, respectively. The median cycles of delivery were 3, which was comparable with those of platinum-doublet chemotherapy.[35] Therefore, our main purpose to decrease the incidence of thrombocytopenia and increase dose intensity was achieved, although there are still problems to be solved.

Drug administrations were frequently delayed, treatment time tended to be protracted, and the treatment completion rate we defined was 42%. Unfortunately, early withdrawal from the study was seen in 10 patients (32%). Among these patients, 3 experienced grade > 2 leukopenia (leukocyte count < 3000/µL) on day 8 of the first course, and the other 3 patients developed grade 3 rash after administration of day 1 gemcitabine. For these 6 patients, gemcitabine/carboplatin chemotherapy was considered inappropriate regardless of the schedule. This schedule, which delays carboplatin administration until day 8, would enable early exclusion of the patients who are inappropriate for this combination chemotherapy, avoiding severe hematologic and nonhematologic toxicities. Response rate, median TTP, and median survival time were favorable. However, this might be biased by the small number of patients and the high percentage of patients with good prognostic factors such as female sex and PS of 0 in this study.

Recently, prolonged administration of gemcitabine combined with carboplatin has been tested.[48,49] Because gemcitabine/carboplatin combination chemotherapy has become a widely used regimen, further improvement of this regimen is necessary.

Conclusion

The present study suggests that carboplatin administered on day 8 in a 21-day schedule of gemcitabine/carboplatin reduces severity of thrombocytopenia without having a detrimental effect on efficacy. However, further evaluation is still needed to estimate the efficacy and feasibility of this regimen. The ongoing randomized phase II study compares day-1 and day-8 administration of carboplatin in a 21-day schedule of gemcitabine/carboplatin. In clinical practice, this regimen will be one of the treatment options suitable for outpatients.

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Improved Diagnostic Efficacy by Rapid Cytology Test in Fluoroscopy-Guided Bronchoscopy

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Background: Fluoroscopy-guided bronchoscopy is a safe and routine method used to obtain a histologic or cytologic specimen of peripheral lung nodules, but it has low sensitivity in diagnosing malignant tumors. Although feedback from rapid cytology tests are expected to improve diagnostic rates, the value of the routine use of rapid cytology tests has not been established.

Materials and Methods: We prospectively studied 657 patients with suspected peripheral malignant lung lesions on chest computed tomography who underwent fluoroscopy-guided bronchoscopy between January 2002 and December 2004. Rapid on-site cytopathologic examinations (ROSE) were performed during bronchoscopic examinations. The additional approach to the lesions was performed immediately after conventional bronchoscopic examinations when ROSE was not considered diagnostic.

Results: There were 528 patients diagnosed as having malignant lesions. In 477 of these patients (90.3%), final malignant diagnosis was established by the initial bronchoscopy. Among these, 84 patients (15.9%) were diagnosed only with the additional feedback from ROSE. Of 240 peripheral lesions ≤2 cm, 174 were found to be malignant. Without ROSE, 110 (63.2%) of peripheral malignant lesions were diagnosed by bronchoscopy. The integration of ROSE enabled us to diagnose an additional 40 patients (23.0%) by bronchoscopy. ROSE improved diagnostic yield independent of the site and histology of the lesions and experience of the operators.

Conclusion: ROSE increased the diagnostic yield of bronchoscopy from 74.4% to 90.3% and therefore is an effective reinforcement in bronchoscopic diagnosis of peripheral pulmonary malignancies. The use of ROSE in routine bronchoscopy should be encouraged.

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Examinations used to diagnose pulmonary malignant lesions should be safe, accurate, and optimal for obtaining adequate information. A flexible fiberoptic bronchoscope has

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become prevalent in obtaining specimen from lung lesions. Although central visible tumors can be diagnosed at high sensitivity, it is reported that the diagnostic rate for peripheral lung lesions is low, from 62% to 86%, even in combination with various techniques.[1–4] Brush, curette, forceps, and aspiration needles have been investigated as tools to obtain diagnostic specimens. Other reports recommend rapid on-site cytopathologic examinations (ROSE) in transbronchial needle aspiration of lymph nodes.[5–7] However, ROSE has not been introduced for diagnosing peripheral lung lesions. Recently, the combination of ultra-fast Papanicolaou staining and multiplanar reconstruction images has been recommended to improve diagnostic accuracy and safety in fluoroscopy-guided transbronchial biopsy.[8] In this prospective study, we integrated ROSE into routine bronchoscopy and evaluated the benefit of bronchoscopy combined with ROSE.

BRONCHOSCOPY

In our hospital, we foremost recommend bronchoscopy with a flexible bronchoscope in the diagnosis of pulmonary nodules because of its safety. If the lesions are not bronchoscopically invisible, procedures to obtain diagnostic materials are performed under fluoroscopic guidance. Transcutaneous fine-needle biopsy (TCNB) is recommended for patients with a negative result of preceding bronchoscopy or with negligible risk of pneumothorax by percutaneous puncture, such as those with lesions invading the thoracic wall. Video-assisted thoracic surgery (VATS) is usually recommended for patients with negative results of bronchoscopy and/or TCNB or lesions unrecognizable under fluoroscopy. For pure GGO, we recommend computed tomographic (CT) follow-up, otherwise VATS.

In bronchoscopy, the specimen for cytology was obtained by curetting or brushing. The material was smeared on two glass slides: one was subjected to ROSE (ROSE sample) and the other to conventional Papanicolaou staining. During ROSE, forceps biopsy was performed to obtain the specimen for histology and cytology. When ROSE was not diagnostic, additional bronchoscopic examinations, such as transbronchial needle aspiration (TBNA), bronchial washing, or ultra-thin bronchoscopy, were performed to obtain additional samples just after conventional bronchoscopy. For the analysis, we defined both the material subjected to Papanicolaou staining and the material obtained by biopsy as conventional

samples. The material obtained by additional bronchoscopic examinations after ROSE was defined as additional samples.

CYTOLOGY AND HISTOLOGY EXAMINATION

We used rapid Shorr stain as a rapid cytology test, which we have recently developed by modifying the Shorr stain.[9] Rapid Shorr stain completes staining very fast (approximately 1 minute) and presents similar coloring to Papanicolaou staining; therefore, it is familiar to the cytoscreeners in our institute. The cytopathologist was able to provide a preliminary diagnosis within a few minutes. Papanicolaou staining was performed after bronchoscopic examination. Tissue specimens obtained by forceps biopsy were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin. Additional specific staining was performed when necessary.

PATIENTS

We performed 1900 flexible bronchoscopic examinations between January 2001 and December 2004. Based on the results of chest radiograph and CT, 795 patients were thought to have central lesions and underwent bronchoscopy without fluoroscopy; 1105 patients underwent fluoroscopy-guided bronchoscopy. ROSE was not performed in the examinations to obtain samples for bacterial testing, for visible lesions, or to evaluate lesions diagnosed before, etc. ROSE was not used for the patients entered into another study performed during the same period in which ROSE was not integrated. Other patients' samples were not subjected to ROSE because only a single trial to obtain bronchoscopic material was possible because of patients' stress during bronchoscopy. Excluding these from the 1105 patients who underwent fluoroscopy-guided bronchoscopy, 657 patients received fluoroscopy-guided bronchoscopy with ROSE. ROSE was repeated when we thought it possible and necessary. Despite negative ROSE results, the lesions of very likely malignant or difficulty except for bronchoscopy, we tend to repeat ROSE. If a diagnosis could not be made via bronchoscopy, further work-up for the lesions included surgical procedures, TCNB, follow-up by bronchoscopy, chest radiograph and CT, and sputum investigations.

RESULTS

Bronchoscopic examinations with ROSE were performed under fluoroscopic guidance for 657 peripheral lung lesions. Patient characteristics are listed in Table 1. The final diagnosis of malignant and benign disease was determined in 528 and 117 lesions, respectively. The remaining 12 lesions were not diagnosed and subjected to careful follow-up. Malignant lesions consisted of adenocarcinoma (n = 328), squamous cell carcinoma (n = 87), small cell carcinoma (n = 32), carcinoid (n = 20), large call carcinoma (n = 7), lymphoma (n = 3), metastatic carcinoma (n = 22), and other malignancies (n = 29).

As shown in Table 2, 393 lesions were diagnosed as malignant by using conventional samples alone. ROSE definitively detected malignant cells in 357 malignant lesions but failed to detect atypical cells in 36 malignant lesions. The

TABLE 1. Patient characteristics

Sex	All patients	Patients with malignancy
Male	411	344
Female	246	184
Age (year)		
Range	25–89	27-87
Average	65.7	66.5
Chance of discovery		
Annual screening	250	183
Tests for other diseases	223	176
Subjective symptoms	163	151
Others	21	18
Smoking status		
Smoker	223	190
Ex-smoker	161	136
Non-smoker	210	156
Unknown	63	46

false-negative rate of ROSE was 9.2% compared with diagnosis based on conventional samples. In ROSE, a limited time period is permitted for screening and diagnosis. However, cancer cells were detected in only one sample with a negative ROSE result by subsequent re-diagnosis with sufficient time. There was no false-positive result in ROSE. However, final diagnosis was obtained with the additional samples in 84 of 135 malignant lesions that were not diagnosed with conventional samples alone. Therefore, the integration of ROSE into bronchoscopic examination improved the diagnostic sensitivity from 74.4% to 90.3% (Figure 1A). The improvement of sensitivity was statistically significant ($p < 0.05$) and enabled effective diagnosis for peripheral lung lesions.

Additional samples for diagnosis were collected by brushing, curetting, forceps biopsy, TBNA, ultra-thin-bronchoscopy, and washing from the same or other bronchi. Sometimes, several methods were combined for obtaining a specimen. The methods to obtain additional specimens were determined based on the bronchoscopic access to the lesions and the condition of patients. We analyzed additional approaches contribute to the improvement of diagnostic accuracy (Table 3). Whereas brushing showed low diagnostic yield, curetting or forceps biopsy from the other branch, TBNA, and forceps biopsy with ultra-thin bronchoscope yielded more than a 65% positive rate in additional approaches. Washing was also useful for diagnosis in additional approaches, but malignant cells were usually detected by the other methods conducted at the same time.

Surprisingly, ROSE provided more benefit for the diagnosis of small-sized lesions (≤ 2 cm) (Figure 1B). With conventional samples, 110 of 174 small-sized malignant lesions (63.2%) were diagnosed by bronchoscopy. With the help of ROSE, 40 lesions (23.0%) were diagnosed only with an additional sample. Improvement of diagnostic rate for small lesions was significantly greater than that for larger lesions (23.0% versus 12.4%; $p < 0.05$). No significant improvement was observed among the other factors in exam-
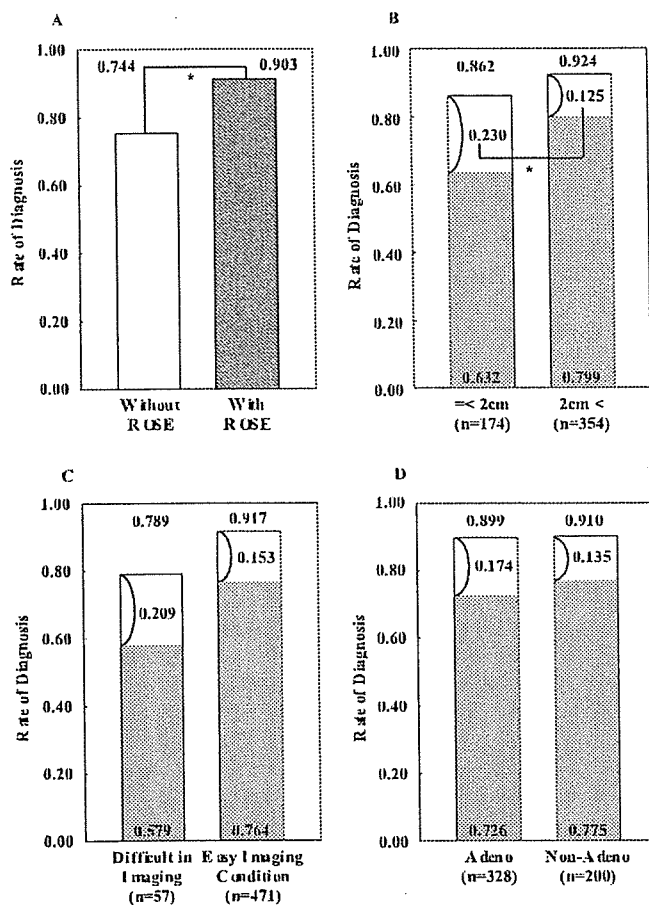
FIGURE 1. ROSE improved diagnostic yield and lesion features. *A*, ROSE improved diagnostic sensitivity. The *gray bar* shows the diagnostic sensitivity of fluoroscopy-guided bronchoscopy with ROSE; the *white bar* shows the diagnostic sensitivity of bronchoscopy without ROSE. The sensitivity is significantly different ($p < 0.05$). *B*, Tumor size and improvement of diagnostic sensitivity by ROSE. The *shaded area* indicates diagnostic sensitivity without ROSE. The improvement in small lesions was better than that in large lesions ($p < 0.05$). *C*, Imaging conditions of the lesions under fluoroscopy revealed diagnostic yield but little difference in improvement by ROSE. The *shaded area* indicates diagnostic sensitivity without ROSE. *D*, Histology type made little difference in diagnostic sensitivity and improvement by ROSE. The *shaded area* indicates diagnostic sensitivity without ROSE.

inations (Figure 1, *C* and *D*). Examination of poorly visible lesions in fluoroscopy had low sensitivity (n = 57, 78.9%) compared with that of clearly visible lesions (n = 471, 91.7%). The improvement by ROSE was slightly higher in examinations for poorly visible lesions (21.1% versus 15.3%), although the difference was not statistically significant. Little improvement by ROSE was shown between histology types of the lesions: adenocarcinoma 52.3%, squamous cell carcinoma 56.3%, small cell carcinoma 50%, and metastatic carcinoma 40.0% of ROSE-negative lesions. Our results also showed the difficulty in diagnosing lesions in the

upper lobe and S6, especially in right lung with conventional samples. However, a comparable improvement of diagnostic yield was achieved with ROSE in most areas (from 40% to 60% of ROSE-negative lesions). We calculated the diagnostic yields with conventional samples and additional samples for each examiner to determine the effect of skill level of examiners on usefulness of ROSE. Although the skill level of the examiner tends to correlate to diagnostic yield with conventional samples, improved diagnosis by ROSE was observed similarly in almost all of the examiners (approximately 40% to 52% of ROSE-negative cases).

ROSE was repeated to make a decision for further examinations when access to the lesion was not satisfactory and an additional approach was considered to be possible. We calculated the effect of repeated ROSE on the diagnostic yield of peripheral lung cancer by fluoroscopy-guided bronchoscopy and found that a diagnostic improvement of 89.4% was attained by the first ROSE and 3.2% by the second ROSE (Table 4). Repeated ROSE improved diagnosis in only five of 107 examinations.

DISCUSSION

Bronchoscopic examination with fluoroscopic guidance is often used to obtain a diagnostic specimen of lung nodules. However, most reports have shown relatively low accuracy of diagnosing peripheral lesions by bronchoscopy.[10–12] Bandoh et al.[8] reported refined accuracy up to 91% by combining multiplanar reconstruction images and ultra-fast Papanicolaou staining. They used a historical control for comparison and multiplanar images for another tool. Our study was designed to improve the bronchoscopic diagnosis of peripheral malignant lesions by introducing only ROSE and was performed prospectively in routine bronchoscopic examinations. Therefore, more precise analysis could be performed to estimate ROSE's effectiveness. Our result shows that diagnostic sensitivity of peripheral malignant lesions was improved from 74.4% to 90.3% with ROSE only.

To obtain rapid diagnosis during bronchoscopy, the staining method should be convenient and fast and should present suitable coloring for diagnosis. Several staining methods are applied in ROSE.[8,14,15] We selected rapid Shorr staining for ROSE that we established recently[9] because it is simple, rapid, and similar in coloring to Papanicolaou staining, which is familiar to cytoscreeners and cytopathologists. Additionally, rapid Shorr staining requires only a small area for staining. Rapid Shorr staining is reliable, with low false-positive and false-negative rates.

To improve sensitivity, a method for obtaining additional samples should be carefully determined. When another visible bronchus could be a suitable path to the lesion, we selected this path. When the visible route to the lesion could not be improved, we changed the method for approaching to lesions to TBNA, ultra-thin bronchoscopy, or washing. Comparison among the methods indicates that TBNA and ultra-thin bronchoscopy were most effective in the approach through the same bronchus. In the approach through different bronchi, curetting and biopsy were effective for diagnosis, whereas TBNA was a good alternative (Table 3). Therefore,

TABLE 2. Results of bronchoscopic examinations with ROSE

ROSE	Final diagnosis	Diagnosis by conventional samples	Diagnosis by additional samples	Diagnosis by different examinations
Negative	279			
Malignant	154	26	80	48
Benign	113	13	2	98
Unknown	12	0	0	12
Positive suspected	21			
Malignant	17	10	4	3
Benign	4	1	0	3
Unknown	0	' 0	0	0
Positive	357			
Malignant	357	357	0	0
Benign	0	0	0	0
Unknown	0	0	0	0

ROSE, rapid on-site cytopathologic examinations.

TABLE 3. Methods of additional sampling for diagnosing malignant lesions

	Tested lesions	Sole positive	Positive
Brushing	16	0 (0.0%)	4 (26.7%)
(from other branch)	4	0 (0.0%)	1 (25.0%)
Curetting and forceps	101	33 (32.7%)	51 (50.5%)
(from other branch)	14	12 (85.7%)	13 (92.9%)
TBNA	35	16 (45.7%)	25 (71.4%)
(from other branch)	7	4 (57.1%)	6 (85.7%)
Washing	29	3 (10.3%)	12 (41.4%)
(from other branch)	4	1 (25.0%)	2 (50.0%)
Forceps with ultra-thin bronchoscope	20	14 (70.0%)	20 (100%)
Washing with ultra-thin bronchoscope	16	0 (0.0%)	11 (68.8%)

TABLE 4. Diagnostic yield of malignant lesions by repeated ROSE

ROSE	Bronchoscopic examinations	Additional examination	Diagnostic yield	Accumulated sensitivity
0	657		393	74.4%
1	657	214	79	89.4%
2	126	94	3	90.0%
3	20	12	2	90.3%
4	1	1	0	90.3%

ROSE, rapid on-site cytopathologic examinations.

alternative routes or methods such as TNBA or ultra-thin bronchoscopy should be considered when ROSE is not diagnostic. We do not recommend brushing and washing.

It has been reported that the size of the lesion has negative correlation to the sensitivity of bronchoscopy. Our results also showed low sensitivity for small lesions (≤2 cm). Surprisingly, however, improvement of diagnostic yield by ROSE was more prominent in diagnosing small lesions (Figure 1B). We analyzed the relationship between the size of lesions and the methods by which diagnosis could be made with additional samples. There was no distinct difference in

frequency of usage of each method and its ability to yield additional diagnoses between the small and large lesions. Therefore, the reason why diagnostic yield improved more in smaller lesions is not known. One possible explanation is poor fluoroscopic targeting for smaller lesions in bronchoscopy. We used biplane fluoroscopy, but not CT, to determine whether the tip of sampling tools reached the lesions. It is reasonable that the error in targeting by this method is greater for small lesions than for large lesions. ROSE may have improved diagnostic yield partly by correcting the error in targeting.

There are several factors other than the size of tumors related to diagnostic yields. The experience of the examiners relates to the diagnostic sensitivity of bronchoscopic examinations.[16] The location of the lesion, histology type, and visibility under fluoroscopy can influence the yield. We analyzed the relationship between these factors and diagnostic yield. Experience of examiners, location of the lesion, and fluoroscopic visibility of lesions showed some relation to the diagnostic yield. However, improvement of diagnosis by ROSE was similarly observed for all examiners. Diagnostic yield of the lesions in the upper lobe and S6 was relatively low. However, we did not observe a clear difference of improvement by ROSE by location. Examinations for poorly

visible lesions under fluoroscopy showed low sensitivity compared with clearly visible lesions. The improvement by ROSE was slightly higher in the examinations for poorly visible lesions, although not statistically significant. Comparison among histology types of the lesions showed little difference in sensitivity and improvement by ROSE. We encourage the use of ROSE for diagnosing peripheral lesions, especially those of small size, regardless of their location, fluoroscopic visibility, or experience of the examiners.

We usually performed curetting and forceps biopsy only once before ROSE. Although repeated curetting and biopsy were thought to improve sensitivity, we repeated the collection of specimens only in negative ROSE cases, including false negatives. We performed additional examinations for only 214 cases with ROSE and showed an increased sensitivity by 14.9% instead of performing repeated curetting and biopsy in most of the 657 cases without ROSE. ROSE enabled us to avoid unnecessary examinations, even including false-negative cases. Considering the low effectiveness of repeated ROSE, single ROSE is recommended. Recently, CT screening and positron emission tomography have been experimentally introduced for the early detection of lung cancer.[16–18] We expect to diagnose peripheral lung nodules more safely and accurately in the future. The combination of ROSE with fluoroscopy-guided bronchoscopy is encouraged as a conventional method to enhance its safety and sensitivity.

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Case Reports

Relapse of Stage I Small Cell Lung Cancer Ten or More Years after the Start of Treatment

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Most patients with small cell lung cancer (SCLC) usually show relapse within 1 or 2 years. Relapses after a 5-year disease-free survival are extremely rare. This report describes two patients with stage I SCLC in whom the disease recurred 10 or more years after the start of initial therapy. Because the recurrence of SCLC was noted in the mediastinal lymph nodes of the same side, we concluded that the patients had a late relapse of SCLC rather than a metachronous lung cancer.

Key words: 10-year disease-free survival – late relapse – second malignancy – small cell lung cancer

INTRODUCTION

Small cell lung cancer (SCLC) is characterized by early and widespread metastases, but good responsiveness to both chemotherapy and radiotherapy. The percentage of long-term disease-free survival was reported in 1983 (1) to be in the range of 15–20% in cases of limited disease (LD) and only a few percent in those with extensive disease, and a recent report suggested an expected 5-year survival rate of ~25% in cases with LD SCLC (2). Previous analyses of long-term disease-free survivors of SCLC (3,4) revealed that relapses usually occurred by 1.5 years after the beginning of combination chemotherapy. However, recent data indicate that as many as one-fourth of the patients who are disease-free at 30 months after the initial therapy develop late relapses (5). Furthermore, in his series, Vogelsang et al. (6) reported that 18 of the 25 long-term survivors (>2 years) eventually showed relapse, sometimes as late as 8 years after the initial diagnosis. In 1993, we reported the course of a patient with SCLC who showed relapse 9.4 years after the initial treatment (7). In this paper, we report two cases of SCLC in whom relapse occurred after 10 or more years' disease-free survival, along with a review of the total of seven cases of SCLC reported until

now, who developed a second SCLC or relapse after 10 years' disease-free survival.

CASE REPORTS

CASE 1

A 61-year-old man participated in a mass screening for lung cancer by chest roentgenography (CXR) in June 1994. The Brinkman index was 1200, however, he stopped smoking after the first diagnosis. Fiberoptic bronchoscopy with transbronchial tumor biopsy confirmed the diagnosis of SCLC (Fig. 1a and b). The primary tumor was located in the B^{1+2} segment of the left upper lobe (Fig. 2a). Surgical resection of the left upper lobe was conducted, followed by combination chemotherapy with four cycles of cisplatin and etoposide. Pathologically, the tumor was determined to be stage IA SCLC and had no components of non-SCLC or large cell carcinoma with neuroendocrine properties.

The patient underwent transurethral resection for early-stage bladder cancer (second malignancy) in January 2002 and received radiotherapy (75 Gy) for A2 (early) prostate carcinoma (third malignancy) in March 2004.

In June 2004, when he was 71 years old, a follow-up chest computed tomography (CT) and MRI (Fig. 2b) revealed para-aortic mediastinal lymphadenopathy (40 × 50 mm in size). The serum levels of pro-gastrin-releasing peptide, neuron-specific enolase (NSE) and carcinoembryonic antigen

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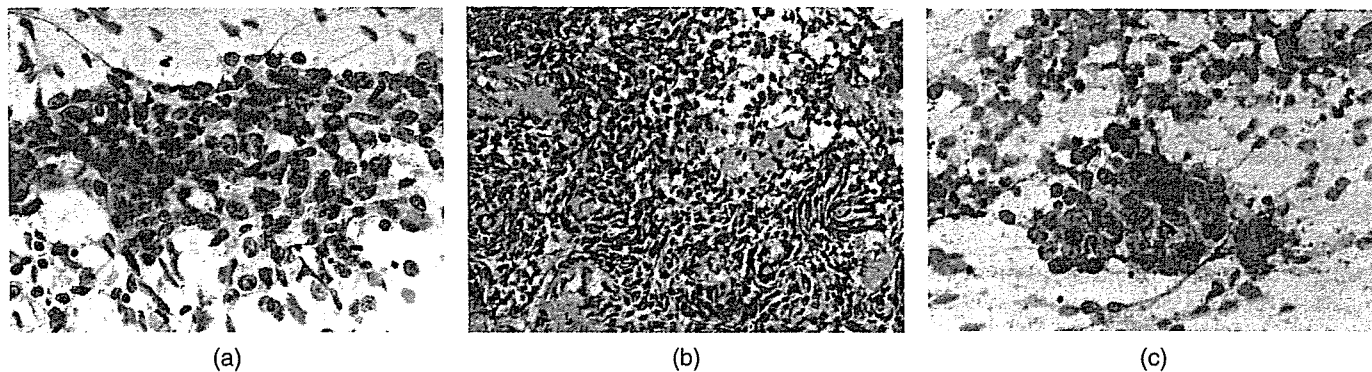
Figure 1. Cytological (a) and histological (b) appearance of the first tumor in July 1994 and aspiration biopsy (c) of cervical lymph node in September 2005 at relapse in Case 1.
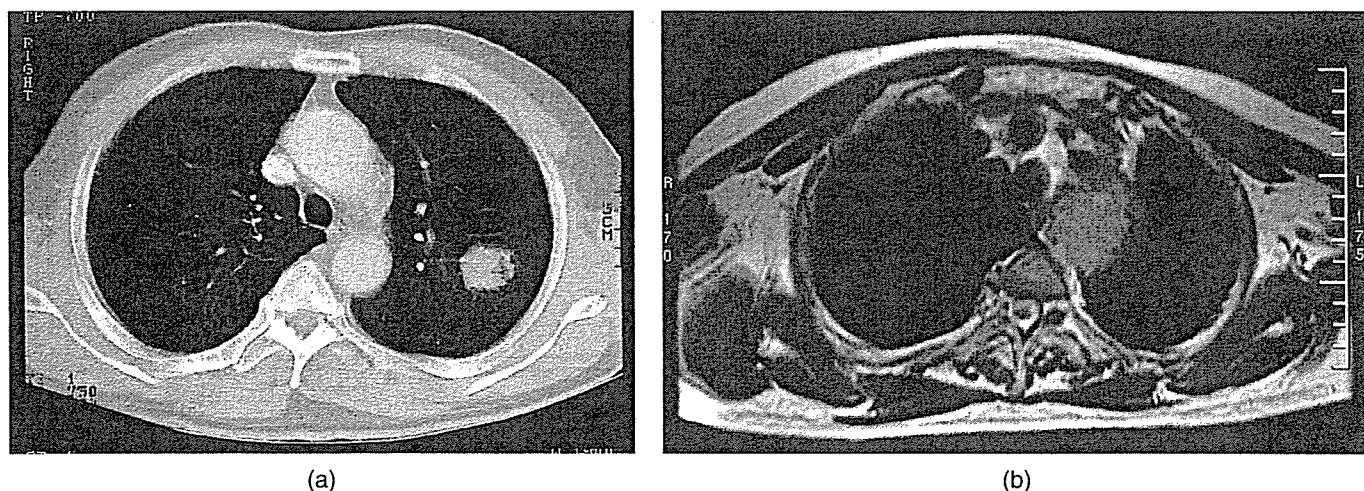

Figure 2. Findings on Chest CT (a) at diagnosis in July 1994 in Case 1. Findings on MRI (b) at relapse in 2004. There is mediastinal lymph node enlargement; size, 40 × 50 mm.

(CEA) were 360 pg/ml (normal range <46 pg/ml), 14.9 ng/ml and 1.4 ng/ml, respectively. The performance status on the Eastern Cooperative Oncology Group (ECOG) scale was zero, because he complained only of hoarseness and the serum lactate dehydrogenase (LDH) was normal. The standard staging procedures and upper gastro-intestinal screening by endoscopy revealed no evidence of metastases. Because of the poor pulmonary function of the patient and high metastatic potential of the disease, no surgery or chest irradiation was planned at this time. He was started on combination chemotherapy with irinotecan (CPT-11) at 60 mg/m^2 on day 1 and etoposide at 80 mg/m^2 on days 1–3, along with granulocyte-colony stimulating factor support on days 4–17 for one cycle, however, he developed severe neutropenia. The tumor regrew within 6 weeks of the treatment-free interval given to allow for his bone marrow recovery. He received CPT-11 at the dose of 50 mg/m^2 alone bi-weekly and enjoyed prolonged partial response (PR). In March 2005, multiple bone metastases were observed, along with left cervical adenopathy. Aspiration biopsy of the cervical lymph nodes revealed the typical histologic features of SCLC (Fig. 1c). Brain metastasis

occurred in July 2005, and in September 2005, the serum NSE level rose to 245 ng/ml. He died of cancer in October 2005.

CASE 2

In April 1987, a 72-year-old man visited our hospital with a month's history of productive cough and blood-streaked sputum. He had smoked one packet of cigarettes a day for 52 years; however, he stopped smoking at the first diagnosis of lung cancer. A CXR showed a right upper lobe mass, which was confirmed on chest CT (Fig. 3a). Fiberoptic bronchoscopy with tumor biopsy confirmed the diagnosis of SCLC (Fig. 4a and b). The patient was determined to have stage IB (T2N0M0) SCLC. Chemotherapy was administered with cyclophosphamide, doxorubicin and vincristine alternating with cisplatin-etoposide, for six cycles. Thereafter, sequential chest radiotherapy was administered.

In September 1998, when he was 82 years old and 11.4 years had passed since the initial treatment of SCLC, the patient complained of shortness of breath on walking even as little
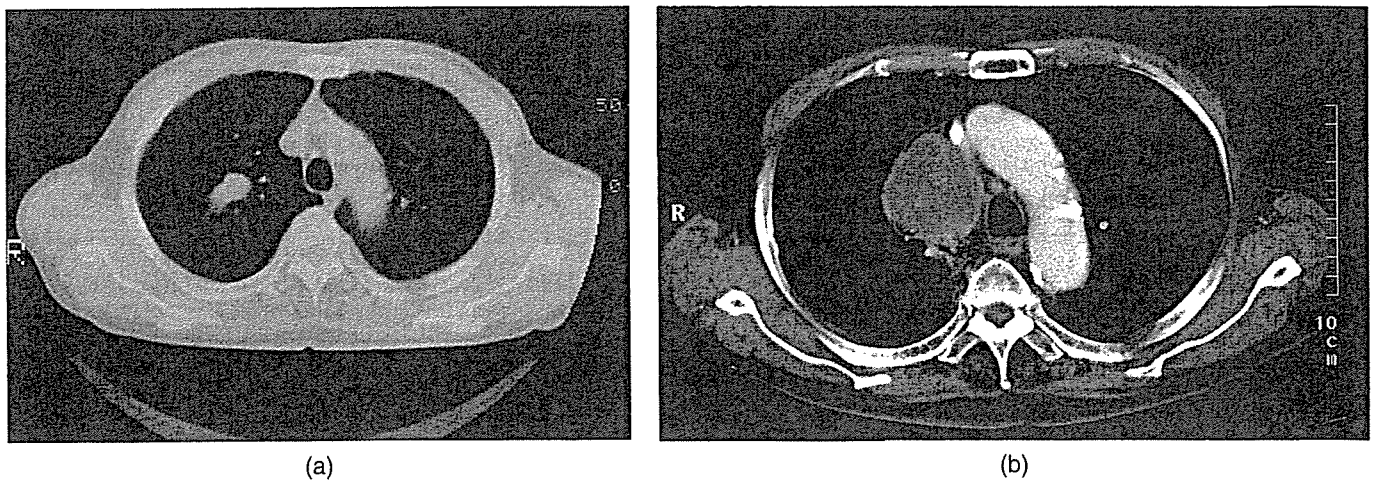
Figure 3. Findings on Chest CT (a) in Case 2 at diagnosis in April 1987. A mass measuring 31 × 13 mm in size in the right upper lobe. Chest CT (b) findings at relapse in 1998.
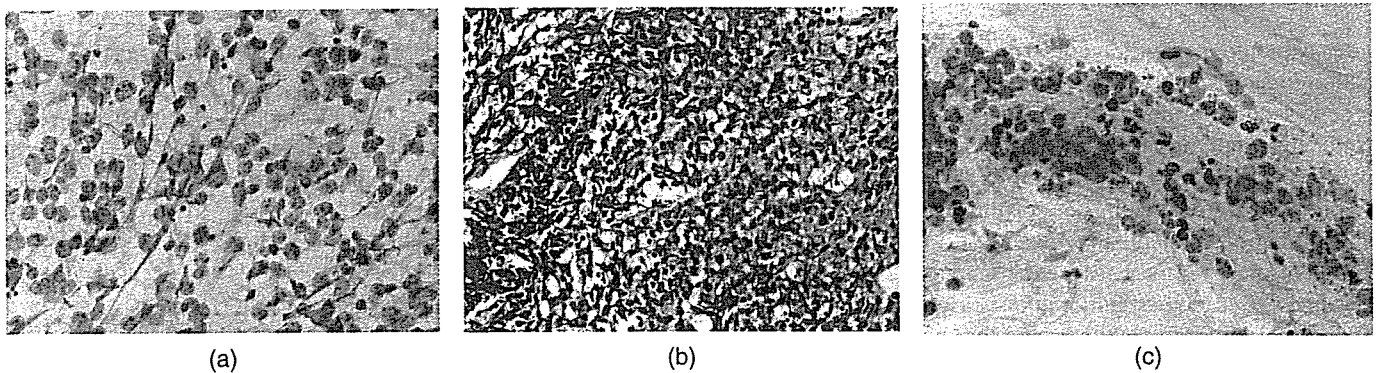

Figure 4. Findings on transbronchial biopsy [cytology (a), and histology (b)] in Case 2 at diagnosis. Sputum cytology (c) at relapse in 1998.

as one block, and hemoptysis. His performance status on the ECOG scale was 3. The serum levels of LDH, NSE and CEA were all within normal range. The sputum cytology result was consistent with the diagnosis of SCLC (Fig. 4c). Chest CT revealed multi-stage mediastinal lymphadenopathy, especially on the ipsilateral side (Fig. 3b). There was no evidence of metastasis elsewhere, as confirmed by brain CT. Because of his poor performance status, the patient received two cycles of monotherapy with oral etoposide (50 mg/body/day for 14 days), with no shrinkage of the tumor. He died of worsened SCLC on 2 May 1999.

Table 1 shows a review of adequately documented cases of recurrence and/or second SCLC after 10 years of disease-free survival. All the patients received systemic combination chemotherapy followed by thoracic irradiation.

DISCUSSION

Jacobs et al. (8) stated that there were continued relapses of disease until 39 months. Jacoulet et al. (5) reported that the risk of recurrence was <30% beyond 3 years and <10% beyond 5 years. In the treatment of SCLC, 5-year disease-free survival

has usually been considered as a benchmark of cure (9,10). However, Niiranen (11) described a case with relapse at the primary site, in the central nervous system and in the skin 11 years after the diagnosis of SCLC.

Brigham et al. (12) estimated that the clinical doubling time of SCLC ranged from 25 to 160 days (median, 77 days; log mean, 81 days; arithmetic mean, 91 days) on the basis of chest radiographic findings. He suggested that highly effective therapy which reduces the residual tumor burden level to that approaching a single cell can be followed by disease-free intervals of more than 6 years before apparent clinical recurrence (>30 doublings). If the longer doubling time of 160 days were used for the calculation, potential relapse of SCLC may not be expected until 13 years after successful induction therapy with complete response as suggested by Al-Ajam et al. (10). It is usually difficult to ascertain whether a second SCLC is a late relapse of the first SCLC or a second primary tumor after a long disease-free survival. Some authors (9,13) suggested that the second diagnosis of SCLC after a long period of survival following the first diagnosis of SCLC should be considered as representing a second primary SCLC, whereas others (14,15) interpret it as representing a relapse of the first SCLC. The

Table 1. Patients of SCLC with 10 years or greater disease-free survival before the second diagnosis of SCLC

Author	Year of publication	Age/ Sex	Stage	Location of initial tumor	Initial therapy	DFI (years)	First relapse site	Treatment after relapse	Survival after relapse (months)
Niiranen[12]	1988	60/M	LD (I)	NR	RT (60 Gy)	11	Lung, Brain, Skin	NR	2 dead
Lassen[15]	1995	65/F	NR	NR	NR	10.9	Lung, Brain, Kidney	NR	2 dead
Johnson[16]	1995	69/M	LD	LLL	CT+RT	12.2	LLL, LH, L-pl, ML	NR	NR
Kitamoto[13]	2002	56/M	LD (IIIB)	LLL	CT+RT*	10.4	LUL, LH	CT+RT***	10 live
Al-Ajam[11]	2005	52/M	LD	RUL	CT+RT**	10	RUL, Brain	Whole brain RT, CT$	17 alive
Present case 1		61/M	LD (IA)	LUL	OP+CT#	10	ML	CT$$	14 dead
Present case 2		72/M	LD (IB)	RUL	CT +RT##	11.4	ML	CT$$$	8 dead

DFI, disease-free interval; NR, not reported; LLL, left lower lobe; LUL, left upper lobe; RUL, right upper lobe; LD, limited disease; ED, extensive disease; ML, mediastinal lymph node; L-pl, left pleural effusion; LH, left hilum lymph node; CT, chemotherapy; RT, chest irradiation; *chemotherapy with cisplatin, etoposide and doxorubicin, and concurrent chest irradiation at 40 Gy in 20 fractions; **CAV (cyclophosphamide + adriamycine + vincristine) and sequential chest irradiation, #Left upper lobectomy and adjuvant chemotherapy with PE (cisplatin + etoposide), ##chemotherapy with CAV alternating with PE and sequential chest irradiation (45 Gy twice daily), ***PE sequential RT, $PE, $$etoposide + CPT and CPT alone, $$$oral etoposide.

latter contention may be valid if the tumor arose at the same anatomic site as the initial SCLC, although the possibility of a new second primary tumor can still not be completely excluded. Kitamoto et al. (13) considered the second diagnosis of SCLC as a second malignancy, because the primary tumor was located in a different lobe of the lung in his patient. We believe that our patients may have had a relapse rather than a second primary tumor, because the second SCLC developed at the same site as the first tumor in one case, and in the ipsilateral mediastinal nodes in the other, and the specimens at diagnosis and at relapse showed an identical cytological or histological appearance in our patients (Figs 1 and 4).

Wistuba et al. (16) reported of observing genetic damage in the adjacent normal and hyperplastic bronchial epithelium in cases of SCLC. Tucker et al. (17) reported that continued smoking increased the risk of second primary cancers in patients treated for SCLC, and the cumulative risk of development of a second primary lung cancer made this cancer a common cause of death. Despite the decreasing incidence of recurrent SCLC with time, the longevity of long-term disease-free survivors continues to be compromised by increasing incidence of second primary smoking-related cancers. Since cigarette smoking cessation after successful therapy is associated with a decreased risk for a second smoking-related primary cancer, the simplest and most important intervention should be to encourage patients to quit smoking (18).

Although the standard therapy for late recurrent disease has not been established, retreatment with chemotherapy similar to the initial treatment (reinduction therapy) is reported to often achieve second responses up to 1 year or longer (19). Sekine et al. (20) also reported a relative good prognosis of patients after late relapse. The median survival time after relapse in their 13 patients was 7.4 months. This may be explained in part by good response to reinduction treatment in these patients or by very sluggish growth in these tumor cells.

Although only seven cases of late relapses after a 10-year disease-free survival have been reported until now, including our two patients, there is still a chance of such rare recurrence occurring beyond this interval. Therefore, careful follow-up is necessary to detect malignant lesions as early as possible in these long-term survivors.

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