Table 3 Clinico-pathological data of 54 lung cancer patients

EGFR gene status			
Factors	Mutation patients	Wild type patients	*P*-value
Mean age (years) 62.5 ± 11.5	26	28	
Pathological subtypes			
Adeno	25 (52.1%)	23 (47.9%)	0.1938
Non-adeno	1 (16.7%)	5 (83.3%)	
Gender			
Male	11 (44.0%)	14 (56.0%)	0.5952
Female	15 (51.7%)	14 (48.3%)	
Smoking status			
Never smoker	18 (64.3%)	10 (35.7%)	0.0168
Smoker	8 (30.8%)	18 (69.2%)	
Age			
<60	13 (61.9%)	8 (38.1%)	0.1626
>60	13 (39.4%)	20 (60.6%)	
Gefitinib Response			
PR	19 (30.8%)	6 (69.2%)	<0.0001
SD or PD	7 (27.8%)	22 (72.2%)	

PR Progressive disease, *SD* stable disease, *PD* progressive disease

in a multivariate analysis. FISH-positive results were associated with better response rate, the same as *EGFR* mutation in the univariate analysis, but were not associated with prolonged survival (Han et al. 2006).

Although many reports have identified more than 30 different mutation in the tyrosine kinase domains of *EGFR*, the vast majority of which can be grouped into three major types, including in-frame deletion at exon 19, single-nucleotide substitution at exon 18 or 21 and in-frame duplication at exon 20 (Paez et al. 2004; Lynch et al. 2004; Pao et al. 2004; Shigematsu et al. 2005). The L858R missence mutation in exon 21 and deletions in exon 19 have been proven to be activating mutations (Paez et al. 2004; Lynch et al. 2004; Pao et al. 2004). The L858R single-nucleotide substitution mutation located near the conserved Asp-Phe-Gly sequence, stabilizes the activation loop (A-loop) (Paez et al. 2004; Shigematsu et al. 2005). The deletions in exon 19 were located on the side of the alpha-C-helix in the N lobe, which controls the angle of the ATP-binding pocket. This mutation might result in similar conformational changes in *EGFR* that cause a shift in the helical axis that results in the narrowing of the ATP-binding cleft, which leads to increased gene expression and tyrosine kinase inhibitor sensitivity. In vitro analysis, Y845 position of *EGFR* was

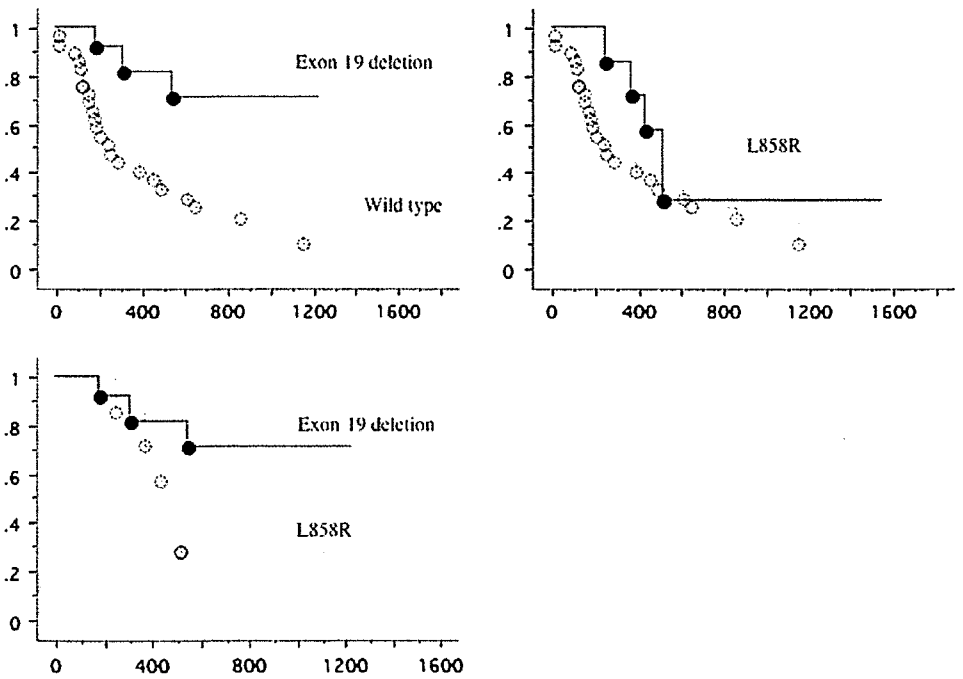

Fig. 5 The overall survival of 54 gefitinib-treated lung cancer patients was studied in reference to the *EGFR* mutation status. *Left upper* prognosis from patients with exon 19 deletion mutations ($n = 12$, 3 were dead) was significantly better than the patients without *EGFR* mutations (Log-rank test, $P = 0.0032$, Breslow–Gehan–Wilcoxon test; $P = 0.006$). *Right upper* prognosis from patients with L858R mutation ($n = 8$, 5 were dead) and patients without *EGFR* mutation was not significantly different (log-rank test, $P = 0.2823$, Breslow–Gehan–Wilcoxon test; $P = 0.142$). *Left lower* there was a tendency towards better prognosis in the patients with exon 19 deletions than in the patients with the L858R mutation (log-rank test, $P = 0.1032$, Breslow–Gehan–Wilcoxon test; $P = 0.1732$)

highly phosphorylated in the L858R mutant, but not in the wild type or the exon 19 deletion mutant, and hence appears to be unique in distinguishing the two types of *EGFR* mutant (Sordella et al. 2005). This might explain the difference in gefitinib response between tumors with L858R and those with deletions. Mitsudomi et al. (2005) noted a 62% (8 of 13) response rate in patients with *EGFR* point mutations compared with 100% (16 of 16) response rate in patients with *EGFR* exon 19 deletion ($P = 0.0019$). Two recent studies reported that patients with *EGFR* exon 19 deletion mutations had a longer median survival than the patients with *EGFR* L858R mutations, although these patients were treated with erlotinib or gefitinib (Jackman et al. 2006; Riely et al. 2006). The findings of the breakdown of *EGFR* mutations among the three exons were interesting, and all the mutations might not be equally correlated with sensitivity for gefitinib.

In summary, our results indicate that high *EGFR* gene amplification identified by FISH may not be an effective molecular predictive marker for gefitinib sensitivity in Japanese patients with NSCLC. Prospective data would be needed to determine if the treatment with gefitinib alters the natural history of patients with *EGFR* mutated Japanese NSCLC.

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References

Cappuzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, Haney J, Witta S, Danenberg K, Domenichini I, ludovini V, Magrini E, Gregorc V, Doglioni C, Sidoni A, Tonato M, Franklin WA, Crino L, Bunn Jr PA, Varella-Garcia M (2005) Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 97:643–655

Cappuzzo F, Ligorio C, Toschi L, Rossi E, Trisolini R, Paioli D, Magrini E, Finocchiaro G, Bartolini S, Cancellieri A, Hirsh FR, Crino L, Varella-Garcia M (2007) EGFR and HER2 gene copy number and response to first-line chemotherapy in patients with advanced non-small cell lung cancer (NSCLC). J Thorac Oncol 2:423–429

Endo K, Konishi A, Sasaki H, Takada M, Tanaka H, Okumura M, Kawahara M, Sugiura H, Kuwabara Y, Fukai I, Matsumura A, Yano M, Kobayashi Y, Mizuno K, Haneda H, Suzuki E, Iuchi K, Fujii Y (2005) Epidermal growth factor receptor gene mutation in non-small cell lung cancer using highly sensitive and fast TaqMan PCR assay. Lung Cancer 50:375–384

Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereuslova A, Dong RP, Baselga J (2003) Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol 21:2237–2246

Ginsberg RJ, Kris K, Armstrong G (1993) Cancer of the lung. In Principles and practice of oncology. 4th edn. Lippincott, Philadelphia, pp 673–682

Han SW, Kim TY, Jeon YK, Hwang PG, Im SA, Lee KH, Kim JH, Kim DW, Heo DS, Kim NK, Chung DH, Bang YJ (2006) Optimization of patient selection for gefitinib in non-small cell lung cancer by combined analysis of epidermal growth factor receptor mutation, K-ras mutation, and Akt phosphorylation. Clin Cancer Res 12:2538–2544

Hirsch FR, Varella-Garcia M, Bunn PA Jr, Di Maria MV, Veve R, Bremnes RM, Baron AE, Zeng C, Franklin WA (2003) Epidermal growth factor receptor in non-small-cell lung carcinoma: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 21:3798–3807

Jackman DM, Yeap BY, Sèquist LV, Lindeman N, Holmes AJ, Joshi VA, Bell DW, Huberman MS, Halmos B, Rabin MS, Haber DA, Lynch TJ, Meyerson M, Johnson BE, Janne PA (2006) Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients with gefitinib or erlotinib. Clin Cancer Res 12:3908–3914

Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T (2004) Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 64:8919–8923

Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, Schiller JH, Kelly K, Spiridonidis H, Sandler A, Albain KS, Cella D, Wolf MK, Averbuch SD, Ochs JJ, Kay AC (2003) Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non small cell lung cancer: randomized trial. JAMA 290:2149–2158

Krypuy M, Newnham GM, Thomas DM, Conron M, Dobrovic A (2006) High resolution melting analysis for the rapid and sensitive detection of mutations in clinical samples: KRAS codon 12 and 13 mutations in non-small cell lung cancer. BMC Cancer 6:295

Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Eng J Med 350:2129–2139

Marchetti A, Martella C, Felicioni L, Barassi F, Salvatore S, Chella A, Camplese PP, Iarussi T, Muchilli F, Mezzetti A, Cuccurullo F, Sacco R, Buttitta F (2005) EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Oncol 23:857–865

Miller VA, Kris MG, Shah N, Patel J, Azzoli C, Gomez J, Krug LM, Pao W, Rizvi N, Pizzo B, Tyson L, Venkatraman E, Ben-Porat L, Memoli N, Zakowski M, Rusch V, Heelan RT (2004) Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. J Clin Oncol 22:1103–1109

Mitsudomi T, Kosaka T, Endoh, Horio Y, Hida T, Mori S, Hatooka S, Shinoda M, Takahashi T, Yatabe Y (2005) Mutation of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. J Clin Oncol 23:2513–2520

Nicolson RI, Gee JM, Harper ME (2001) EGFR and cancer prognosis. Eur J Cancer 37:S9–S15

Onn A, Correa AM, Gilcrease M, Isobe T, Massarelli E, Bucane CD, O'Reilly MS, Hong WK, Fidler IJ, Putnum JB, Herbst RS (2004) Synchronous overexpression of epidermal growth factor receptor

and HER2-neu protein is a predictor of poor outcome in patients with stage I non-small cell lung cancer. Clin Cancer Res 10:136–143

Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Seller WR, Johnson BE, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304:1497–1500

Pao W, Miller V, Zakowski M Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kri M, Varmus H (2004) 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 USA 101:13306–13311

Riely GJ, Pao W, Pham DK, Li AR, Rizvi N, Venkatraman ES, Zakowski MF, Kris MG, Ladanyi M, Miller V (2006) Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res 12:839–844

Sasaki H, Endo K, Konishi A, Takada M, Kawahara M, Iuchi K, Matsumura A, Okumura M, Tanaka H, Kawaguchi T, Shimizu T, Takeuchi H, Yano M, Fukai I, Fujii Y (2005) EGFR mutation status in Japanese lung cancer patients: genotyping analysis using LighyCycler. Clin Cancer Res 11:2924–2929

Sasaki H, Shimizu S, Endo K, Takada M, Kawahara M, Tanaka H, Matsumura A, Iuchi K, Haneda H, Suzuki E, Kobayashi Y, Yano M, Fujii Y (2006) EGFR and erbB2 mutation status in Japanese lung cancer patients. Int J Cancer 118:180–184

Shigematsu H, Lin L, Takahashi T, Numura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD, Gazdar AF (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutation in lung cancers. J Natl Cancer Inst 97:339–346

Sone T, Kasahara K, Kimura H, Nishio K, Mizuguchi M, Nakatsumi Y, Shibata K, Waseda Y, Fujimura M, Nakao S (2007) Comparative analysis of epidermal growth factor receptor mutations and gene amplification as predictors of gefitinib efficacy in Japanese patients with nonsmall cell lung cancer. Cancer 109:1836–1844

Sordella R, Bell DW, Haber DA, Settleman J (2005) Gefitinib-sensitizing EGFR mutations in lung cancer activated anti-apoptotic pathways. Science 305:1163–1167

Takano T, Ohe Y, Sakamoto H, Tsuta K, Matsuno Y, Tateishi U, Yamamoto S, Nokihara H, Yamamoto N, Sekine I, Kunitoh H, Shibata T, Sakiyama T, Yoshida T, Tamura T (2005) Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. J Clin Oncol 23:6829–6837

Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K (2005) Gefitinib plus best supportive care in previously treated patients with refractory advance non-small-cell lung cancer: result from a randomized, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 366:1527–1537

Tokumo M, Toyooka S, Kiura K, Shigematsu H, Tomii K, Aoe M, Ichimura K, Tsuda T, Yano M, Tsukuda K, Tabata M, Ueoka H, Tanimoto M, Date H, Gazder AF, Shimizu N (2005) The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. Clin Cancer Res 11:1167–1173

Tracy S, Mukohara T, Hansen M, Meyerson M, Johnson BE, Janne PA (2004) Gefitinib induces apoptosis in the EGFR (L858R) non-small-cell lung cancer cell line H3255. Cancer Res 64:7241–7244

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

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

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

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

1. Introduction

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

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

2. Methods

2.1. Patient selection

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

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

2.2. Treatment and monitoring

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

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

2.3. Response criteria

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

2.4. Response evaluation and systemic chemotherapy

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

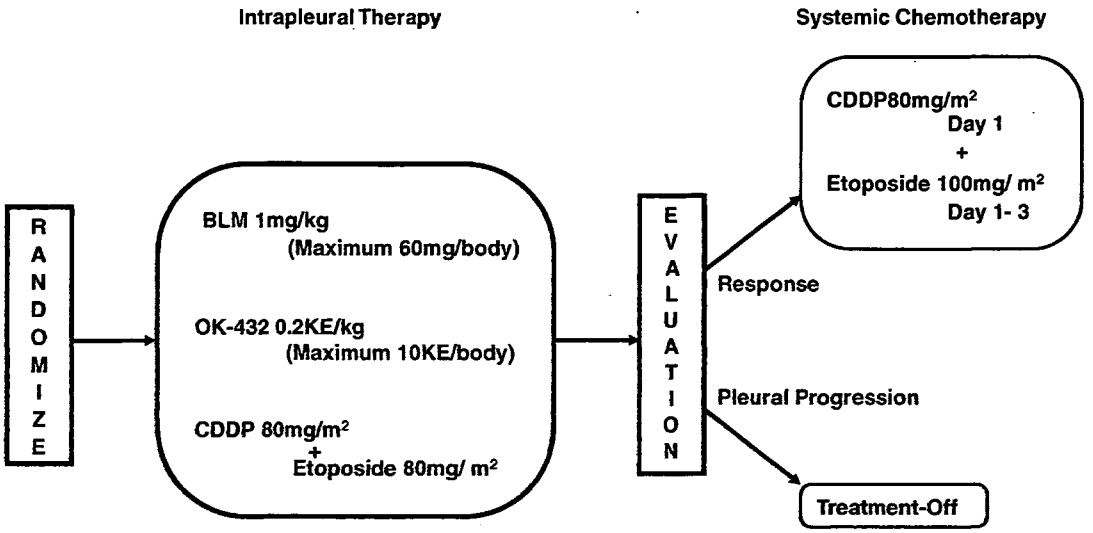

Fig. 1 Treatment schema.

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

2.5. Toxicity criteria and dose modification

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

2.6. Data management and statistical analysis

This study was designed as a multicenter randomized phase II trial among 21 participating centers in the Lung Cancer Study Group in the JCOG. Pleural progression-free survival (PPFS) was defined as the time from randomization to the first observation of pleural progression or death due to any cause. The primary endpoint of this study was 4-week PPFS rate. Assuming that the 4-week PPFS rate was at least 50% for these arms, the required number for each arm was 30 to select the better arm correctly with 90% probability if the better arm's 4-week PPFS rate was 70% or higher [15]. Planned accrual was set at 35 per arm. Secondary end-points were 8-, 12- and 24-week PPFS rates, overall survival (OS) and toxicity. The duration for OS was measured from the date of randomization to the date of death due to any cause or last follow-up. The mandated time to start treatment following randomization was within a week. Survival distribution was estimated by the Kaplan–Meier method, and confidence intervals were based on Greenwood's formula [16].

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

3. Results

3.1. Patients

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

3.2. Treatment compliance and toxicity

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

Table 1 Patient Characteristics

Characteristic	BLM	OK-432	PE
All patients	36	34	35
Eligible patients	35	33	34
Age (years)			
Median	64	60.5	61
Range	44–75	31–73	39–75
Sex			
Male	24	21	24
Female	12	13	11
PS (ECOG)[a]			
0	2	4	2
1	30	27	28
2	4	3	5
≥10% weight loss within 6 m			
No	33	27	31
Yes	3	7	4
Histology			
Adenocarcinoma	29	32	32
Squamous cell	4	1	3
Large cell	1	1	0
Other	1	0	0
TNM (N factor)			
N0	14	14	14
N1	2	0	2
N2	16	13	11
N3	3	7	8
Stage			
IIIB	23	17	25
IV	12	17	10

[a] At the time of reexpansion of the affected lung.

Table 2 Treatment compliance

Variable	BLM	OK-432	PE
Eligible patients	35	33	34
No therapy	1	2	1
End of study protocol	18	19	14
Progressive disease	14	11	16
Toxicity	1	0	1
Death	1	0	0
Patient refusal	0	1	1
Insufficient drainage	0	0	1

patients (5.7%) in the BLM arm had pneumonitis induced by BLM and one of them had treatment-related death. One patient in the PE group did not receive systemic chemotherapy due to elevation of serum creatinine. Other reasons for noncompletion of the protocol treatment were two patient refusals in each for the OK-432 and the PE arms, and one patient in the PE arm who could not receive sufficient drainage due to self-removal of the drain 48 h after intrapleural therapy.

Toxicities for intrapleural therapy in the three arms are listed in Table 3. Hematological toxic events were well tolerated in the three arms. Grade 4 nonhematological toxicity was not found in the three arms. Grade 2–3 chest pain occurred almost equally in the three arms. Grade 2–3 fever and nausea/vomiting occurred most frequently in the OK-432 arm (59.4%) and the PE arm (50.0%), respectively.

3.3. PPFS and OS

All eligible patients in the three arms were included in the survival analysis. PPFS and OS data are shown in Figs. 2 and 3, respectively. Median PPFS for the BLM arm was 20.9 weeks (95% confidence interval (CI), 4.7–25.9 weeks); for the OK-432 arm, 27.9 weeks (95% CI, 18.6–50.0 weeks); and for the PE arm, 18.4 weeks (95% CI, 4.4–41.4 weeks). The 4-week PPFS rate, which was the primary endpoint of this study, was 68.6% for the BLM arm (95% CI, 53.2–84.0%); 75.8% for the OK-432 arm (95% CI, 61.1–90.4%); and 70.6% for the PE arm (95% CI, 55.3–85.9%). The median survival time (MST) for the BLM arm was 32.1 weeks (95% CI, 21.6–37.9 weeks); 48.1 weeks for the OK-432 arm (95% CI, 26.7–58.4 weeks); and 45.7 weeks for the PE arm (95% CI, 34.4–57.1 weeks). The 48-week survival rate for the BLM arm was 29.9% (95% CI, 14.4–45.3%); 51.1% for the OK-432 arm (95% CI,

Table 3 Toxicity (JCOG grade) for Intrapleural Therapy

	BLM (n = 35)				OK-432 (n = 32)				PE (n = 34)			
	1	2	3	4	1	2	3	4	1	2	3	4
Leukocytes	3	3	0	1	1	0	1	0	8	3	2	1
Neutrophils	1	0	2	1	0	0	1	0	5	5	1	2
Hemoglobin	3	5	3	ND	3	6	1	ND	6	6	3	ND
Platelet	0	0	1	0	0	0	0	0	1	1	0	0
AST	8	0	0	0	15	2	0	0	6	0	0	0
ALT	11	0	0	0	14	7	0	0	10	2	0	0
Serum creatinine	1	0	0	0	0	0	0	0	4	1	0	0
Chest pain	10	5	4	0	15	8	1	0	13	6	1	0
Fever	12	13	0	0	6	18	1	0	9	7	2	0
Nausea/vomiting	7	3	0	ND	5	0	0	ND	10	13	4	ND

Abbreviation: ND, not defined.

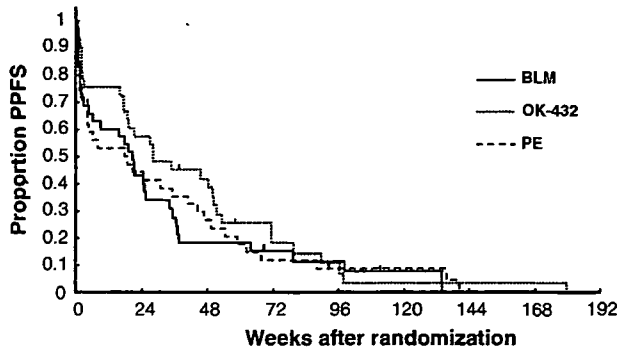

Fig. 2 Pleural progression-free survival (PPFS) in all eligible patients ($n = 102$).

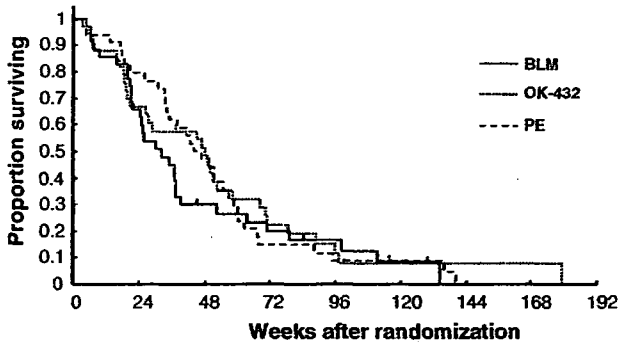

Fig. 3 Overall survival in all eligible patients ($n = 102$).

34.0–68.3%); and 47.1% for the PE arm (95% CI, 30.3–63.8%). Both the PPFS and OS for the OK-432 arm were superior to those for other two arms; however, the outcomes did not differ significantly between groups.

4. Discussion

To date, numerous chemical agents for treatment of MPE have been studied. These were antibiotics, antineoplastic agents, biological response modifiers (BRMs) and others that showed varied degrees of chemical sclerosis. Among them, BLM and talc are most frequently used for the management of MPE [5,7,17,18]. BLM is an antineoplastic antibiotic used in sclerotherapy with a success rate of 63–85% [7,8,18–21]. Talc applied as either slurry or poudrage is superior to other commonly used sclerosing agents with a success rate of 71–100% [5,7,22–24]. Because talc has not been available commercially in Japan and the use of talc was considered controversial at the beginning of this study because of severe complications, such as acute respiratory distress syndrome [25,26], we selected BLM as the sclerosing agent. A recent report demonstrated that the safety of talc pleurodesis and that acute respiratory distress syndrome can be avoided by using large-particle talc applied as thoracoscopic poudrage [27]. The thoracoscopic pleurodesis with talc is now considered to be the gold standard treatment for MPE [28,29].

OK-432 has been used as a BRM for gastric and lung cancer [9,10,30,31]. OK-432 has been reported to be effective in controlling MPE in two prospective randomized trials. One study reported a 73% success rate with OK-432 compared to 41% with mitomycin C treatment ($p = 0.03$) [11]. The other comparison found OK-432 70% effective compared to 46% in BLM subjects (statistical data not reported) [12]. OK-432 has been reported to induce various cytokines, such as tumor necrosis factor-α, interferon-γ, interleukin (IL)-1, IL-8 and IL-12 [32] and also to enhance cytotoxicity against tumor cells [33,34]. It is suggested that the main therapeutic effects of OK-432 for malignant effusion depend on increased expression of intercellular adhesion molecule-1 on tumor cells induced by interferon-γ [35].

Intrapleural combination chemotherapy is focused on achieving higher concentrations in the pleural cavity with less toxicity than systemic chemotherapy [36]. Two phase II studies with intrapleural cisplatin and cytarabine had success rates of 49% [2] and 73% [37]. Tohda et al. [13] reported that intrapleural instillation of cisplatin and etoposide for NSCLC with MPE resulted in a 46.2% overall response rate and the MST of 8 months was found to be improved, compared with previous reports for NSCLC with MPE of 3–6 months [11,18,38]. The reason for this was assumed to be that intrapleural combination chemotherapy of cisplatin and etoposide produced systemic as well as local effects. The overall response rates of intrapleural combination chemotherapy are variable and there are no prospective randomized studies compared modality of intrapleural combination chemotherapy with that of sclerotherapy.

There have been several special problems raised in the clinical trials for MPE, such as patient selection, response criteria, treatment procedures, short life expectancy, small sample sizes, and different endpoints [2–7,11,39]. To minimize the bias of patient selection, NSCLC patients with MPE who had received no prior therapy were entered into this study. Furthermore, justifiable and simplified response criteria and whether further treatment was required or not, as suggested by Ruckdeschel [18] and Rusch [40] were used and single intrapleural instillation of each agent was permitted to allow uniform estimation of responses. In many trials, successful pleurodesis was determined by assessing clinical and radiological findings. The positive response criteria have been defined generally as no pleural re-accumulation, 50% less effusion than that observed in the baseline radiograph taken immediately after the procedure, or no requirement for further thoracentesis. To determine the efficacy, we used the criterion that a decrease in effusion over one-quarter of the treated lung provides a stricter assessment of chemical pleurodesis that may relieve the symptoms of MPE. The position rotation after intrapleural instillation was recommended traditionally because it was thought to allow the agents to be distributed thoroughly throughout the entire pleural space. In contrast, studies using tetracycline and talc [41,42] demonstrated that rotation does not affect the overall intrapleural dispersion. It is unclear whether rotation is beneficial or not when applying the agents used in this study. Because a previous phase II study [13] showed that etoposide remains for a long period (β-phase half-life = 62.53 h) in intrapleural fluids, we applied the longer duration of clamping in the PE arm (72 h) than the other two arms (3 h) to provide enough exposure to the cancer cells. We found no major safety concerns such as excess pleural effusion as a result of the longer duration of clamping.

In this study, all three regimens were feasible. One treatment-related death occurred in the BLM arm 9 weeks after intrapleural instillation of BLM. Treatment compliance

rates for both intrapleural and systemic therapy was 50% (51 of the 102 eligible patients). This study lacks sufficient power to demonstrate differences between treatment arms; however, the OK-432 arm seemed to demonstrate modest benefit compared with the other two arms in terms of PPFS. It is assumed that the favorable efficacy in the OK-432 arm suggests that OK-432 has clinically meaningful activity for controlling MPE in NSCLC patients. NSCLC patients with MPE have been treated as patients with stage IV disease even when without metastasis, and systemic chemotherapy should be recommended when they have a good PS [43]. We prescribed systemic PE chemotherapy regimens, which were considered one of the standard regimens at the beginning of the study, following successful pleurodesis. However, we expect that platinum-based systemic combination chemotherapy regimens with several active new chemotherapeutic agents such as taxanes (paclitaxel and docetaxel), vinorelbine, gemcitabine and irrinotecan, which are the current standard treatment options for patients with advanced NSCLC, should enhance the survival benefit more than PE regimens.

This is the first fully reported randomized study that has evaluated the efficacy of intrapleural therapy for previously untreated patients with NSCLC and compliance with sequential systemic chemotherapy. As the results of this study demonstrate that intrapleural therapy with OK-432 shows a tendency to be more effective than BLM or PE in the management of MPE in NSCLC, in terms of PPFS, further studies are needed to compare OK-432 with talc.

Conflict of interest

None declared.

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References

[1] Figlin R, Mendoza E, Piantadosi S, Rusch V. Intrapleural chemotherapy without pleurodesis for malignant pleural effusions. LCSG Trial 861. Chest 1994;106:363S–6S.

[2] Rusch VW, Figlin R, Godwin D, Piantadosi S. Intrapleural cisplatin and cytarabine in the management of malignant pleural effusions: a Lung Cancer Study Group trial. J Clin Oncol 1991;9:313–9.

[3] Windsor PG, Como JA, Windsor KS. Sclerotherapy for malignant pleural effusions: alternatives to tetracycline. South Med J 1994;87:709–14.

[4] Walker-Renard PB, Vaughan LM, Sahn SA. Chemical pleurodesis for malignant pleural effusions. Ann Intern Med 1994;120:56–64.

[5] Grossi F, Pennucci MC, Tixi L, et al. Management of malignant pleural effusions. Drugs 1998;55:47–58.

[6] Ruckdeschel JC. Management of malignant pleural effusions. Semin Oncol 1995;22:58–63.

[7] Zimmer PW, Hill M, Casey K, et al. Prospective randomized trial of talc slurry vs bleomycin in pleurodesis for symptomatic malignant pleural effusions. Chest 1997;112:430–4.

[8] Sartori S, Tassinari D, Ceccotti P, et al. Prospective randomized trial of intrapleural bleomycin versus interferon alfa-2b via ultrasound-guided small-bore chest tube in the palliative treatment of malignant pleural effusions. J Clin Oncol 2004;22:1228–33.

[9] Kimura I, Ohnoshi T, Yasuhara S, et al. Immunochemotherapy in human lung cancer using the streptococcal agent OK-432. Cancer 1976;37:2201–3.

[10] Sakamoto J, Teramukai S, Watanabe Y, et al. Meta-analysis of adjuvant immunochemotherapy using OK-432 in patients with resected non-small-cell lung cancer. J Immunother 2001;24:250–6.

[11] Luh KT, Yang PC, Kuo SH, et al. Comparison of OK-432 and mitomycin C pleurodesis for malignant pleural effusion caused by lung cancer. A randomized trial. Cancer 1992;69:674–9.

[12] Saka H, Shimokata K, Watanabe A, Saito H, Minami H, Sakai S. Randomized comparison of OK-432 and Bleomycin in intrapleural therapy for malignant effusions. Proc Am Soc Clin Oncol 1994;450a [Abstr 1555].

[13] Tohda Y, Iwanaga T, Takada M, et al. Intrapleural administration of cisplatin and etoposide to treat malignant pleural effusions in patients with non-small cell lung cancer. Chemotherapy 1999;45:197–204.

[14] Tobinai K, Kohno A, Shimada Y, et al. Toxicity grading criteria of the Japan Clinical Oncology Group. The Clinical Trial Review Committee of the Japan Clinical Oncology Group. Jpn J Clin Oncol 1993;23:250–7.

[15] Simon R, Wittes RE, Ellenberg SS. Randomized phase II clinical trials. Cancer Treat Rep 1985;69:1375–81.

[16] Armitage P, Berry G. Survival analysis. In: Statistical Methods in Medical Research. Oxford, United Kingdom: Blackwell Scientific Publications; 1994.

[17] Ostrowski MJ. Intracavitary therapy with bleomycin for the treatment of malignant pleural effusions. J Surg Oncol Suppl 1989;1:7–13.

[18] Ruckdeschel JC, Moores D, Lee JY, et al. Intrapleural therapy for malignant pleural effusions. A randomized comparison of bleomycin and tetracycline. Chest 1991;100:1528–35.

[19] Paladine W, Cunningham TJ, Sponzo R, et al. Intracavitary bleomycin in the management of malignant effusions. Cancer 1976;38:1903–8.

[20] Bitran JD, Brown C, Desser RK, et al. Intracavitary bleomycin for the control of malignant effusions. J Surg Oncol 1981;16:273–7.

[21] Ostrowski MJ, Priestman TJ, Houston RF, Martin WM. A randomized trial of intracavitary bleomycin and *Corynebacterium parvum* in the control of malignant pleural effusions. Radiother Oncol 1989;14:19–26.

[22] Hamed H, Fentiman IS, Chaudary MA, Rubens RD. Comparison of intracavitary bleomycin and talc for control of pleural effusions secondary to carcinoma of the breast. Br J Surg 1989;76:1266–7.

[23] Hartman DL, Gaither JM, Kesler KA, et al. Comparison of insufflated talc under thoracoscopic guidance with standard tetracycline and bleomycin pleurodesis for control of malignant pleural effusions. J Thorac Cardiovasc Surg 1993;105:743–7 [Discussion747–8].

[24] Dresler CM, Olak J, Herndon II JE, et al. Phase III intergroup study of talc poudrage vs talc slurry sclerosis for malignant pleural effusion. Chest 2005;127:909–15.

[25] Rinaldo JE, Owens GR, Rogers RM. Adult respiratory distress syndrome following intrapleural instillation of talc. J Thorac Cardiovasc Surg 1983;85:523–6.

[26] Bouchama A, Chastre J, Gaudichet A, et al. Acute pneumonitis with bilateral pleural effusion after talc pleurodesis. Chest 1984;86:795–7.
[27] Janssen JP, Collier G, Astoul P, et al. Safety of pleurodesis with talc poudrage in malignant pleural effusion: a prospective cohort study. Lancet 2007;369:1535–9.
[28] Shaw P, Agarwal R. Pleurodesis for malignant pleural effusions. Cochrane Database Syst Rev (database online) 2004.
[29] Aelony Y. Talc pleurodesis and acute respiratory distress syndrome. Lancet 2007;369:1494–6.
[30] Yasue M, Murakami M, Nakazato H, et al. A controlled study of maintenance chemoimmunotherapy vs immunotherapy alone immediately following palliative gastrectomy and induction chemoimmunotherapy for advanced gastric cancer. Tokai cooperative study group for adjuvant chemoimmunotherapy of stomach cancer. Cancer Chemother Pharmacol 1981;7:5–10.
[31] Watanabe Y, Iwa T. Clinical value of immunotherapy with the streptococcal preparation OK-432 in non-small cell lung cancer. J Biol Response Mod 1987;6:169–80.
[32] Katano M, Morisaki T. The past, the present and future of the OK-432 therapy for patients with malignant effusions. Anticancer Res 1998;18:3917–25.
[33] Uchida A, Micksche M. Lysis of fresh human tumor cells by autologous peripheral blood lymphocytes and pleural effusion lymphocytes activated by OK432. J Natl Cancer Inst 1983;71:673–80.
[34] Wei Y, Zhao X, Kariya Y, et al. Induction of autologous tumor killing by heat treatment of fresh human tumor cells: involvement of gamma delta T cells and heat shock protein 70. Cancer Res 1996;56:1104–10.
[35] Kitsuki H, Katano M, Ikubo A, et al. Induction of inflammatory cytokines in effusion cavity by OK-432 injection therapy for patients with malignant effusion: role of interferon-gamma in enhancement of surface expression of ICAM-1 on tumor cells in vivo. Clin Immunol Immunopathol 1996;78:283–90.
[36] Markman M. Intracavitary chemotherapy. Curr Probl Cancer 1986;10:401–37.
[37] Aitini E, Cavazzini G, Pasquini E, et al. Treatment of primary or metastatic pleural effusion with intracavitary cytosine arabinoside and cisplatin. A phase II study. Acta Oncol 1994;33:191–4.
[38] Sugiura S, Ando Y, Minami H, et al. Prognostic value of pleural effusion in patients with non-small cell lung cancer. Clin Cancer Res 1997;3:47–50.
[39] Miles DW, Knight RK. Diagnosis and management of malignant pleural effusion. Cancer Treat Rev 1993;19:151–68.
[40] Rusch VW. The optimal treatment of malignant pleural effusions. A continuing dilemma. Chest 1991;100:1483–4.
[41] Dryzer SR, Allen ML, Strange C, et al. A comparison of rotation and nonrotation in tetracycline pleurodesis. Chest 1993;104:1763–6.
[42] Mager HJ, Maesen B, Verzijlbergen F, et al. Distribution of talc suspension during treatment of malignant pleural effusion with talc pleurodesis. Lung Cancer 2002;36:77–81.
[43] Pfister DG, Johnson DH, Azzoli CG, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. J Clin Oncol 2004;22:330–53.

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EGFR exon 20 insertion mutation in Japanese lung cancer

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Summary Mutations of the epidermal growth factor receptor (*EGFR*) gene have been reported in non-small cell lung cancer (NSCLC), especially in female, never smoker patients with adenocarcinoma. Some common somatic mutations in *EGFR*, including deletion mutations in exon 19 and leucine to arginine substitution at amino acid position 858 (L858R) in exon 21, have been examined for their ability to predict sensitivity to gefitinib or erlotinib. On the other hand, previous report has shown that the insertion mutation at exon 20 is related to gefitinib resistance. We investigated the exon 20 *EGFR* mutation statuses in 322 surgically treated non-small cell lung cancer cases. Two hundred and five adenocarcinoma cases were included. The presence or absence of *EGFR* mutations of kinase domains was analyzed by direct sequences. *EGFR* insertion mutations at exon 20 were found from 7 of 322 (2.17%) lung cancer patients. We also detected the 18 deletion type mutations in exon 19, and 25 L858R type mutations in exon 21. There was a tendency towards higher exon 20 insertion ratio in never smoker (never smoker 4.4% versus smoker 1.3%, $p = 0.0996$) and female (female 4.5% versus male 1.3%, $p = 0.0917$). Two exon 20 insertion cases were treated with gefitinib and failed to response.

EGFR insertion mutation in exon 20 could not be ignored from Japanese lung cancers.

1. Introduction

Lung cancer is a major cause of death from malignant diseases, due to its high incidence, malignant behavior and lack of major advancements in treatment strategy [1]. There are much accumulated evidences that epidermal growth fac-

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tor receptor (*EGFR*) and its family members are strongly implicated in the development and progression of numerous human tumors, including lung cancer [2,3]. The *EGFR* tyrosine kinase inhibitor, gefitinib, was approved in Japan for the treatment of non-small cell lung cancer (NSCLC) since 2002. Original two reports showed that *EGFR* mutations statuses at ATP binding pockets in NSCLC patients were correlated with the clinico-pathological features related to good response to gefitinib [4,5]. These *EGFR* mutations are predominantly found in Japanese lung cancer patients (about 25–40%) [4,6–9] when compared to USA patients (about 8–10%) [4,5,7,10] or European patients [7,11]. Actually, *EGFR* mutations in lung cancer have been correlated with clinical response to gefitinib therapy *in vivo* and *in vitro* [4,5,10]. Although many *EGFR* mutations have been reported, not all have been associated with responsiveness to gefitinib. The two most common *EGFR* mutations that have been identified, representing 85–90% of *EGFR* mutations, are the *EGFR* exon 19 deletion that eliminates a leucine–arginine–glutamate–alanine motif in the tyrosine kinase domain of *EGFR* and a thymine to guanine transversion that results in an arginine for leucine substitution at amino acid 858 (L858R). These two mutants responded significantly better for gefitinib therapy than other types of mutants [12,13]. However, Greulich et al. showed transformation by an exon 20 insertion, made cells resistant to gefitinib or erlotinib [14]. To determine the *EGFR* mutation status and correlation with clinico-pathological features in Japanese lung carcinoma, we investigated exon 20 insertion mutation status by direct sequences. The findings were compared to the clinico-pathological features of lung cancer.

2. Material and methods

2.1. Patients

The study group included 295 lung cancer patients who had undergone surgery at the Department of Surgery II, Nagoya City University Medical School between 1994 and 2005. We have also investigated *EGFR* mutation status for 27 lung cancer patients who had undergone surgery followed by treated with gefitinib at the National Hospital Organization, Kinki-chuo Chest Medical Center. Gefitinib was used after lung cancer recurrence, and clinical outcome was shown in reference [9]. The lung tumors were classified according to the general rule for clinical and pathological record of lung cancer in Japan [15]. All tumor samples were immediately frozen and stored at −80 °C until assayed. Written informed consent was obtained from the patients, and the institutional ethics committee of the Nagoya City University Medical School approved the study.

2.2. PCR assays for *EGFR* mutations

Genomic DNA was extracted using Wizard SV Genomic DNA Purification Systems (Promega) according to the manufacturers' instructions. The primers for exon 20 sequencing were designed with Primer Express 2.0 software (Applied Biosystems). The sequences of the primer sets used in the assay are: forward ACTTCACAGCCCTGCGTAAAC, and reverse: ATGGGACAGGCACTGATTTGT. The sequence results of exon 20 about 131 of 322 cases were already reported [4,16]. The cycling conditions were as follows: initial denaturation at 94 °C for 10 min, followed by 35 cycles at 94 °C for 30 s, 64 °C for 30 s, 72 °C for 60 s. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA). Amplified cDNAs were separated on 1% agarose gels, and the bands were visualized by ethidium bromide and photographed under ultraviolet transillumination. These samples were sequenced by ABI prism 3100 analyzer (Applied Biosystems Japan Ltd., Tokyo, Japan) and analyzed by BLAST and chromatograms by manual review form forward and reverse, both side.

2.3. Statistical analysis

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 means of 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. All analysis was done using the Stat-View software package (Abacus Concepts Inc. Berkeley, CA), and was considered significant when the *p*-value was less than 0.05.

3. Results

3.1. *EGFR* gene mutation status in Japanese lung cancer patients

The clinical and pathological characteristics of the 322 lung cancer patients are as follows: 234 (72.7%) were males and 88 were females. Two hundred and five (63.7%) were diagnosed as adenocarcinoma, and 117 were diagnosed as other types of carcinoma. Two hundred and thirty-one (71.7%) were smokers and 90 were non-smokers (one unknown). Of 295 lung cancer patients from Nagoya City University, 167 (56.6%) were stage I.

Most of the sequencing results about exon 18, 19 and 21 were already reported [4,16,17]. In exon 19, 18 patients had the deletion type mutation. In exon 18 or exon 21, 29 patients had the missense point mutations (2 G719S, 1 G719C, 25 L858R and 1 L861Q). Of these 47 patients, 17 were males and 30 were females. Thirty were non-smokers and 17 were smokers. Forty-three patients had adenocarcinoma, one had squamous cell carcinoma and three had adenosquamous cell carcinoma. Thus *EGFR* mutation status at exon 18, 19 or 21 was significantly correlated with gender ($p < 0.0001$), tobacco-smoking ($p < 0.0001$) and pathological subtypes (adenocarcinoma versus non-adenocarcinoma, $p < 0.0001$).

For exon 20, 7 patients had the insertion mutations (Table 1). These mutations were exclusively associated with other *EGFR* mutation. Three were males and four were females. Four were non-smokers and three were smokers. Six patients had adenocarcinoma and one had squamous

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Table 1 Clinico-pathological features of 322 lung cancer patients

Factors	*EGFR* exon 20 mutations		
	Mutation patients	Wild type patients	*p*-Value
Mean age (65.5 ± 9.3; years)	7	315	
Age			
≦60	1 (1.1%)	94 (98.9%)	0.6783
>60	6 (2.6%)	221 (97.4%)	
Gender			
Male	3 (1.3%)	231 (98.7%)	0.0917
Female	4 (4.5%)	84 (95.5%)	
Pathological subtypes			
Adeno	6 (3.0%)	197 (97.0%)	0.2666
Non-adeno	1 (0.8%)	118 (99.2%)	
Differentiation			
Well	4 (3.5%)	111 (96.5%)	0.4236
Moderately or poorly	2 (1.5%)	128 (98.5%)	
Lymph node metastasis			
N0	4 (1.9%)	205 (98.1%)	>0.9999
N+	2 (2.3%)	84 (97.7%)	
Smoking status			
Smoker	3 (1.3%)	228 (98.7%)	0.0996
Non-smoker	4 (4.5%)	86 (95.5%)	
Pathological stages			
I	4 (2.4%)	164 (97.6%)	0.7025
II–IV	2 (1.6%)	125 (98.4%)	

Adeno, adenocarcinoma; N+, lymph node metastasis positive.

cell carcinoma. Two were moderately differentiated, and four were well differentiated (one unclassified). There was a tendency towards higher exon 20 insertion mutation ratio in never smoker (never smoker 4.4% versus smoker 1.3%, $p=0.0996$) and female (female 4.5% versus male 1.3%, $p=0.0917$). Two female patients had 774_776 insertion NPH (2320-2328 insertion AACCCCCAC) mutations reported as D7 mutation by Shigematsu et al. (Fig. 1) [7]. A female patient had 770_772 insertion ASV (2308-2316 insertion GCCAGCGTG) mutation reported as D1 mutation by Shigematsu et al. [7]. A male patient had 771_773 insertion SVD (2311-19 insertion GCGTGGACA) mutation reported by Sonobe et al. [18]. A male patient had 772_773 insertion V (2312-14 insertion GGT) reported by Thomas et al. [19]. Two patients had 772_773insertion N (2312-14 insertion AAC) mutations (Fig. 1).

3.2. Relationship between clinical course of patients with lung cancer and *EGFR* mutations

The overall survival of 322 lung cancer patients with follow-up through December 30, 2006, was studied in reference to the *EGFR* mutation status. The prognosis from patients with exon 20 insertion mutation ($n=7$, 2 were dead) and the patient without exon 20 insertion mutation *EGFR* ($n=315$, 102 were dead) was not significantly different (Log-rank test, $p=0.7186$, Breslow–Gehan–Wilcoxon test, $p=0.8593$) (Fig. 2). Eighteen patients received adjuvant chemotherapy (five were with cisplatin base, seven were with calboplatin base and six were with Uracil-Ftegafur). Even if the 18 patients were excluded for survival analysis, the prognosis from patients with exon 20 mutation and without mutation was not significantly different ($p=0.7215$).

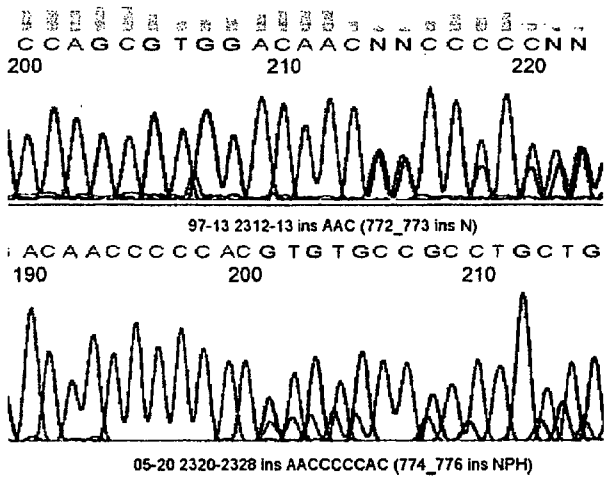

Fig. 1 Two patients had 774_776 insertion NPH (2320-2328 insertion AACCCCCAC) mutations reported as D7 mutation (upper). Two patients had 772_773insertion N (2312-14 insertion AAC) mutations (below).

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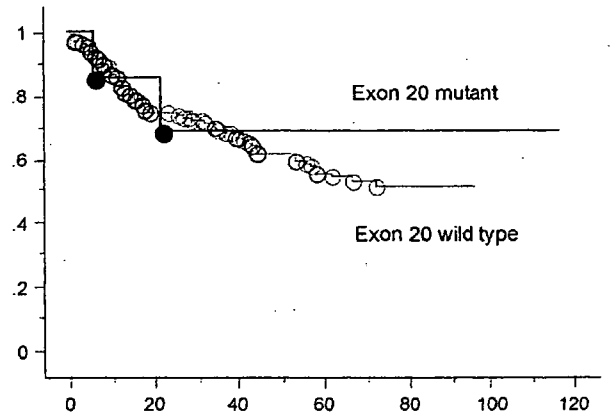

Fig. 2 The prognosis from patients with exon 20 insertion mutation ($n=7$, 2 were dead) and the patient without exon 20 insertion mutation *EGFR* ($n=315$, 92 were dead) was not significantly different (Log-rank test, $p=0.7186$, Breslow–Gehan–Wilcoxon test, $p=0.8593$).

3.3. Clinical course of two recurrent lung cancer patients treated with gefitinib

Case 1: 58-year-old adenocarcinoma woman with no history of smoking underwent surgery at Kinki-chuo Chest Medical Center. A molecular analysis revealed 772_773 insertion N (2312-14 insertion AAC) mutations at *EGFR* exon 20. Three years later, the recurrent lung cancer was treated with gemcitabine, vinorelbine and Uracil-Ftegafur in addition to radiotherapy. Because the treatment failed, gefitinib treatment was started at 2004. The patient died from progressive disease about 6 months after gefitinib administration. Case 2: 72-year-old adenocarcinoma man with no history of smoking underwent surgery at Nagoya City University Hospital. A molecular analysis revealed 772_773 insertion V (2312-14 insertion GGT) at *EGFR* exon 20 (Fig. 3), and wild type at *Kras* codon 12/13. Multiple lung metastasis were treated with Uracil-Ftegafur, however, the treatment failed. Gefitinib treatment was started at 2005. But the tumor size was increased (Fig. 3) and the treatment was quitted at 3 months.

4. Discussion

We obtained findings that exon 20 insertion type *EGFR* mutations tend to be higher in female gender and never smoker, as like as other *EGFR* mutation subtypes [8–14]. From the original three papers published by Lynch et al., Paez et al. and Pao et al., there was no *EGFR* exon 20 insertion subtypes. Shigematsu et al. reported that 12 of 617 (1.9%) had exon 20 insertion mutation, however, 356 of 617 patients were either from Japan or Taiwan [11]. Sonobe et al. reported that the 2 of 154 cases (1.3%) had *EGFR* exon 20 insertion mutations. These data suggested that *EGFR* mutations at exon 20 might be also higher in East Asian. More interestingly, patients with exon 20 mutation did not respond to gefitinib therapy.

Although many reports have identified more than 30 different mutations in the tyrosine kinase domains of *EGFR*, the vast majority of which can be grouped into three major types, including in-frame deletion at exon 19, single-nucleotide substitution at exon 18 or 21 and in-frame duplication at exon 20 [8–14]. To date, only the L858R missense mutation in exon 21 and deletions in exon 19 have been proven to be activating mutations [4,5,10,14]. On the

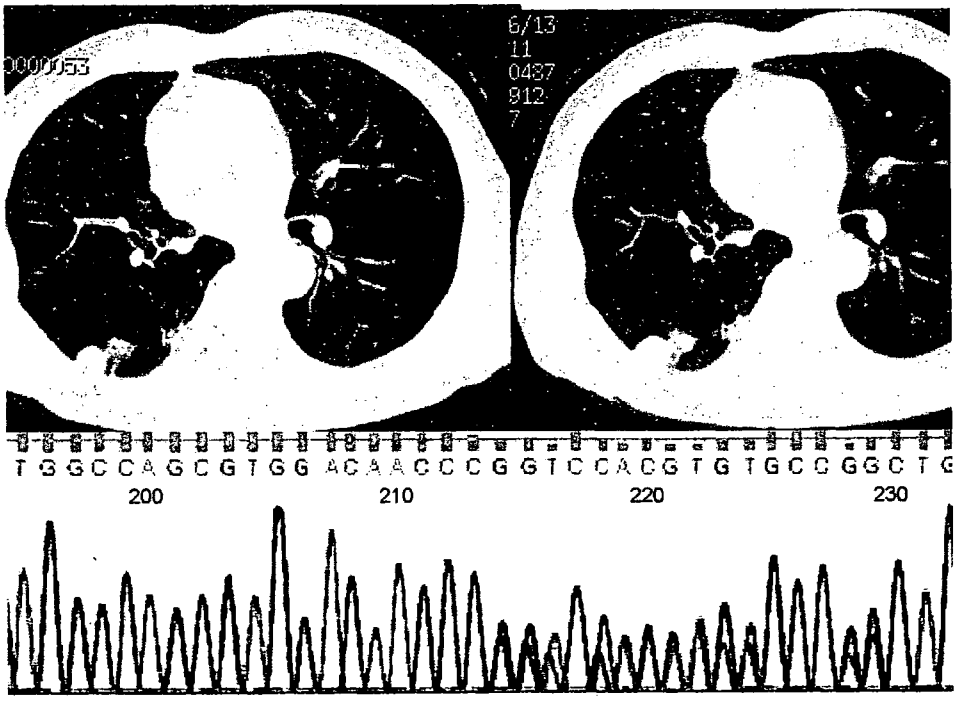

Fig. 3 CT examination before (left) and after (right) gefitinib therapy revealed increased tumor size. A molecular analysis revealed 772_773 insertion V (2312-14 insertion GGT) at *EGFR* exon 20 (below).

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other hands, Greulich et al. reported that transformation by the D770_N771 ins NPG (exon 20) *EGFR* insertion mutant was remarkably insensitive to gefitinib and erlotinib, as inhibition of colony growth in soft agar required exposure to 100-fold higher concentrations (>1 mM) of these agents than was required to inhibit colony formation by cells expressing the *EGFR* missense mutants or deletion mutant [14]. No significant inhibition of anchorage-independent growth of cells expressing D770_N771ins NPG *EGFR* was observed at 3 mM gefitinib or erlotinib [14]. Greulich et al. also reported that all three lung adenocarcinoma patients with known exon 20 insertion mutants of *EGFR* have failed to show a clinical response to treatment and have instead achieved only stable disease with erlotinib [14]. *In vitro* analysis, cells expressing the *EGFR* deletion and insertion mutants formed colonies in soft agar with a higher efficiency than that of cells expressing the missense mutants, comparable to the colony formation efficiency of cells expressing polyoma middle T antigen, suggested these mutants were oncogenic [14]. Interestingly, the irreversible *EGFR* inhibitor CL-387, 785 [20] is more effective than gefitinib or erlotinib for inhibition of colony formation by cells expressing the exon 20 insertion mutant [14]. CL-387, 785 had an even greater effect on colony formation by cells expressing L858R [14], and this compound was previously found to be active against *EGFR* containing the exon 20 point mutation T790M, associated with resistance to gefitinib and erlotinib [21]. Thus the distinct inhibitor sensitivity of various *EGFR* mutants argues that therapies may need to be targeted against specific mutant forms of a protein, whereas generalized inhibition of a particular oncogenic target may not be sufficient.

Conflict of interest

None declared.

Acknowledgements

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References

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

[2] Nicolson RI, Gee JM, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001;37:S9–15.

[3] Onn A, Correa AM, Gilcrease M, Isobe T, Massarelli E, Bucana CD, et al. Synchronous overexpression of epidermal growth factor receptor and HER2-neu protein is a predictor of poor outcome in patients with stage I non-small cell lung cancer. Clin Cancer Res 2004;10:136–43.

[4] Paez JG, Janne 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.

[5] 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. N Engl J Med 2004;350:2129–39.

[6] Kasaoka 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.

[7] Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Witsub II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutation in lung cancers. J Natl Cancer Inst 2005;97:339–46.

[8] Tokumo M, Toyooka S, Kiura K, Shigematsu H, Tomii K, Aoe M, et al. The relationship between epidermal growth factor receptor mutation and clinicopathological feature in non-small cell lung cancers. Clin Cancer Res 2005;11:1167–73.

[9] Endo K, Konishi A, Sasaki H, Takada M, Tanaka H, Okumura M, et al. Epidermal growth factor receptor gene mutation in non-small cell lung cancer using highly sensitive and fast TaqMan PCR assay. Lung Cancer 2005;50:375–84.

[10] Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, 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 USA 2004;101:13306–11.

[11] Marchetti A, Martella C, Felicioni L, Barassi F, Salvatore S, Chella A, et al. EGFR mutations in non-small cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Oncol 2005;23:857–65.

[12] Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, et al. Mutation of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small cell lung cancer with postoperative recurrence. J Clin Oncol 2005;23:2513–20.

[13] Moriguchi H, Kim T-Y, Sato C. Gefitinib for refractory advanced non-small cell lung cancer. Lancet 2006;367:299.

[14] Greulich H, Chen T-H, Feng W, Janne PA, Alvarez JV, Zappaterra M, et al. Oncogenic transformation by inhibitor sensitive and resistant EGFR mutants. PLos Med 2005;2:1167–76.

[15] Japan Lung Cancer Society. General rule for clinical and pathological record of lung cancer, vol. 5, 5th ed. The Japan Lung Cancer Society; 1999. p. 1–177.

[16] Sasaki H, Shimizu S, Endo K, Takada M, Kawahara M, Tanaka H, et al. EGFR and erbB2 mutation status in Japanese lung cancer patients. Int J Cancer 2006;118:180–4.

[17] 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 LighyCycler. Clin Cancer Res 2005;11:2924–9.

[18] Sonobe M, Manabe T, Wada H, Tanaka F. Mutations in the epidermal growth factor receptor gene are linked to smoking-independent, lung adenocarcinoma. Br J Cancer 2005;93:355–63.

[19] Thomas RK, Nickerson E, Simons JF, Janne PA, Tengs T, Yuza Y, et al. Sensitive mutation detection in heterogeneous cancer specimens by massively parallel picoliter reactor sequencing. Nat Med 2006;12:852–5.

[20] Discafani CM, Carroll MI, Floyd Jr MB, Hollander IJ, Husain Z, Johnson BD, et al. Irreversible inhibition of epidermal growth factor receptor tyrosine kinase with *in vivo* activity by *N*-[4-[(3-bromophenyl) amino]-6-quinazolinyl]-2-butynamide (CL-387, 785). Biochem Pharmacol 1999;57:917–25.

[21] Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small cell lung cancer to gefitinib. N Engl J Med 2005;352:786–92.

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CORRESPONDENCE

Prophylactic Cranial Irradiation in Small-Cell Lung Cancer

TO THE EDITOR: The trial reported by Slotman et al. (Aug. 16 issue)[1] showed a reduced incidence of symptomatic brain metastases and an improvement in overall survival with the addition of prophylactic cranial irradiation in patients with extensive-stage small-cell lung cancer. Brain imaging was not part of standard staging before randomization unless symptoms suggestive of metastasis were present. Published data suggest that up to 15% of patients have asymptomatic brain metastases, and the prognosis for these patients is similar to that for patients with symptomatic metastases.[2,3] Therefore, the benefit of prophylactic cranial irradiation may be less than that suggested because some patients probably had brain metastases at diagnosis.

The authors note that the high extracranial-progression rate should be given priority for future investigations. The role of thoracic radiation therapy in limited-stage small-cell lung cancer is well established.[4] Although systemic therapy is the primary treatment of extensive-stage disease, thoracic irradiation may provide an additional benefit. Jeremic et al. found that there was a 5.4% improvement in overall survival when thoracic irradiation was given after chemotherapy.[5] This type of aggressive local approach should be considered for future trials.

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1. Slotman B, Faivre-Finn C, Kramer G, et al. Prophylactic cranial irradiation in extensive small-cell lung cancer. N Engl J Med 2007;357:664-72.
2. Hochstenbag MMH, Twijnstra A, Wilmink JT, Wouters EF, ten Velde GP. Asymptomatic brain metastases (BM) in small cell lung cancer (SCLC): MR-imaging is useful at initial diagnosis. J Neurooncol 2000;48:243-8.
3. Seute T, Leffers P, Wilmink JT, ten Velde GP, Twijnstra A. Response of asymptomatic brain metastases from small-cell lung cancer to systemic first-line chemotherapy. J Clin Oncol 2006;24:2079-83.
4. Warde P, Payne D. Does thoracic irradiation improve survival and local control in limited-stage small-cell carcinoma of the lung? A meta-analysis. J Clin Oncol 1992;10:890-5.
5. Jeremic B, Shibamoto Y, Nikolic N, et al. Role of radiation therapy in the combined-modality treatment of patients with extensive disease small-cell lung cancer: a randomized study. J Clin Oncol 1999;17:2092-9.

TO THE EDITOR: Two pieces of essential information were not reported by Slotman et al. First, there are no data in their report regarding the use of standard chemotherapeutic regimens as induction therapy and whether these regimens, if used, were well balanced between the study groups. Second, the authors did not describe the tumor response to induction chemotherapy. Patients with a complete response are most likely to benefit from prophylactic cranial irradiation,[1,2] but the patients enrolled in this study were not stratified according to the response category at the time of randomization. We would also like to know whether patients with a partial response to the induction therapy, as well as those with a complete response, could benefit substantially from the use of prophylactic cranial irradiation.

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THIS WEEK'S LETTERS

1. Auperin A, Arriagada R, Pignon JP, et al. Prophylactic cranial irradiation for patients with small-cell lung cancer in complete remission. N Engl J Med 1999;341:476-84.
2. Meert AP, Paesmans M, Berghmans T, et al. Prophylactic cranial irradiation in small cell lung cancer: a systematic review of the literature with meta-analysis. BMC Cancer 2001;1:5.

TO THE EDITOR: Slotman and colleagues have contributed an important study. One weakness in the design was the heterogeneity introduced by allowing several radiotherapy regimens with a wide range of biologically equivalent doses — 25 to 39 Gy by the authors' calculations. Although assessment for a dose–response relationship was not part of the study design, did the authors detect such a relationship among these regimens? Also, would the authors comment on whether their findings would affect their management of extrapulmonary neuroendocrine primary cancers?

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THE AUTHORS REPLY: In our study, brain imaging was not mandatory for patients with extensive-stage small-cell lung cancer who did not have related symptoms, an approach that is in accordance with the prevailing guidelines.[1] Only 29% of randomized patients underwent brain imaging at diagnosis, and Dr. Shivnani suggests that this was a drawback in our study because some patients may have had asymptomatic brain metastases at randomization. However, the magnitude of the survival benefit with prophylactic cranial irradiation is such that it cannot be explained by an effect on existing metastases.[2] We concur that further evaluation of the role of chest radiotherapy is warranted, and such a trial is now in preparation.

Fujiwara et al. question the absence of detailed data on chemotherapy regimens used and also on potential imbalances of chemotherapy between the study groups. Most patients were treated with four to six cycles of cisplatin–etoposide, carboplatin–etoposide, cyclophosphamide–doxorubicin–etoposide, or carboplatin–paclitaxel. To reduce the risk of bias, randomization included stratification according to institution but not according to chemotherapy. Patients who had any response were eligible, since response evaluation in patients with extensive disease can be difficult (e.g., for bone metastases), and it is not standard practice. As we reported, 76% of patients had evidence of residual tumor at the primary site, and 71% had evidence of tumor at distant sites. Since a total of 87% of study patients had some residual disease, our study clearly shows the benefit of cranial irradiation after a partial response. A previous meta-analysis included patients with extensive disease who had had a complete response,[3] and the current data support the use of prophylactic cranial irradiation in all patients with small-cell lung cancer who have a response to chemotherapy.

In response to Drs. Khandelwal and Ghaemmaghami, we can confirm that no significant differences were observed in patient characteristics, the incidence of brain metastases, survival, or side effects between patients receiving 20 Gy in five fractions (62% of all patients) and those receiving treatment with the more fractionated schemes. Extrapulmonary small-cell cancer is most likely to benefit in the same way that small-cell lung cancer does. However, the role of prophylactic cranial irradiation for extrapulmonary neuroendocrine tumors or other neuroendocrine tumors of the lung is an unanswered question that needs to be investigated in new trials.

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1. Felip E. ESMO minimum clinical recommendations for diagnosis, treatment and follow-up of small-cell lung cancer (SCLC). Ann Oncol 2007;18:Suppl 2:ii32-ii32.
2. Postmus PE, Haaxma-Reiche H, Gregor A, et al. Brain-only metastases of small cell lung cancer: efficacy of whole brain radiotherapy: an EORTC phase II study. Radiother Oncol 1998;46:29-32.
3. Auperin A, Arriagada R, Pignon JP, et al. Prophylactic cranial irradiation for patients with small-cell lung cancer in complete remission. N Engl J Med 1999;341:476-84.

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LUNG CANCER

CASE REPORT

Isolated metastasis of lung cancer to the thyroid gland

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KEYWORDS
Lung cancer;
Thyroid;
Recurrence;
Small cell carcinoma;
Large cell carcinoma;
Metastasectomy;
Adjuvant chemotherapy

Summary A 67-year-old man with lung cancer developed an isolated metastasis to the thyroid gland. The patient had undergone a right upper lobectomy, followed by chemotherapy consisting of cisplatin and etoposide based on post-surgical diagnosis of small cell lung cancer. Four years later, he had an isolated metastasis to the thyroid gland. The patient underwent a metastasectomy and adjuvant chemotherapy including cisplatin and irinotecan. The cancer cells in resected thyroid tumor had large nuclei and cytoplasm, and expressed the neuroendocrine markers, CD56 and chromogranin A. Retrospectively, the primary lung cancer consisted of both small cell and large cell cancer, and the latter was consistent with the pathological finding of the thyroid tumor. This is the first report to document an isolated recurrence of the lung cancer to the thyroid.

1. Introduction

Distant metastasis in brain, bone, liver, lung and adrenal gland are often found in lung cancer patients following a surgical resection [1]. Although the thyroid gland has rich vasculature similar to the adrenal gland, metastatic thyroid tumors are rare. The incidence ranges from 3.9% to 24.2% based on autopsy studies [2,3], however, the clinically demonstrated incidence is only between 0.05% and 3.1% [4–6]. The primary cancers in these cases are commonly renal cell carcinoma, lung cancer and breast cancer [4,7–10]. Metastatic thyroid tumors are often accompanied with synchronous metastatic lesions to other organs [11]. Isolated metastatic disease to the thyroid gland is very rare and only one case where the primary lesion was the lung has been reported [12]. In that case, the thyroid tumor was found during diagnostic mediastinoscopy for squamous cell carcinoma of the lung and it was not a recurrent tumor. This study documents a patient developing an isolated recurrence of lung cancer to the thyroid gland.

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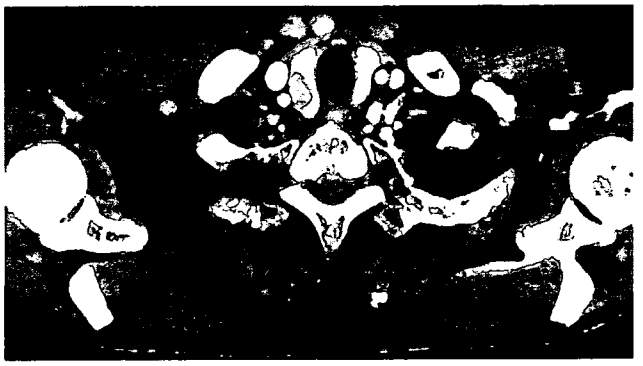

Fig. 1 CT scan of the right thyroid tumor.

2. Case

A 67-year-old man underwent a right upper lobectomy on 10 January 2001. The pathological diagnosis and stage were small cell lung cancer and T2N2M0 (stage IIIA), respectively. He underwent adjuvant chemotherapy consisting of cisplatin and etoposide, followed by prophylactic cranial irradiation. In September 2005, chest computed tomography (CT) scan demonstrated a tumor in the right thyroid measuring 8 mm × 12 mm (Fig. 1) and cytology observed in fine-needle aspiration was consistent with metastatic lung cancer. He presented no symptoms. Bronchoscopy, chest and abdominal CT scan, brain magnetic resonance imaging, bone scintigraphy and fluorine-18-2-fluoro-2-deoxy-D-glucose positron-emission tomography revealed no other metastatic lesions other than the right thyroid lobe. Serum levels of carcinoembryonic antigen, progastrin releasing peptide, neuron-specific enolase and cytokeratin 19 fragment were not elevated. The thyroid function was normal. The serum levels of calcitonin (28 pg/mL) and thyroglobulin (6.1 IU/mL) were not elevated.

The patient underwent a metastasectomy of the right thyroid lobe. The thyroid tumor was easily enucleated without adhesion of surrounding tissue. It was 15 mm in diameter and had a clear border to normal thyroid tissue. The cancer cells had large nuclei and cytoplasm (Fig. 2A) and immunohistochemical staining revealed the neuroendocrine markers CD56 (B) and chromogranin A (data not shown). In addition, they showed negatively staining for calcitonin and thyroglobulin (data not shown). The cells did not contain the characteristics of small cell cancer. The primary lung cancer consisted of small cells (C) and large cells (D), and the latter was consistent with the pathological findings of the thyroid tumor. The large cell component in the primary lesion was also positive for CD56 (data not shown). This case was thus considered to have combined small cell carcinoma [13] and its large cell component metastasized to the thyroid gland. The patient underwent a metastasectomy and adjuvant chemotherapy, consisting of cisplatin and irinotecan, and he has been disease-free for 16 months after undergoing treatment for the lesion.

3. Discussion

This is apparently the first documented case of a patient developing an isolated recurrence of the lung cancer in the thyroid gland. It is often difficult to differentiate tumor

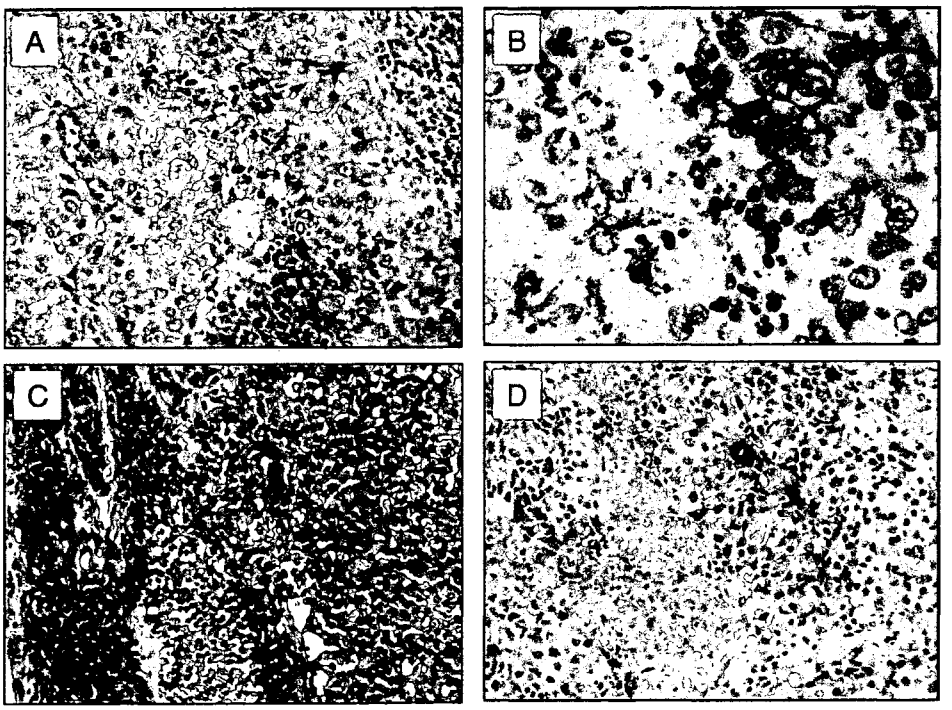

Fig. 2 The cancer cells in the thyroid tumor had large nuclei and cytoplasm seen with hematoxylin-eosin staining (A, 20×) and immunohistochemical staining identified the neuroendocrine marker, CD56 (B, 40×). The primary lung cancer consisted of both components of small cells (C, 20×) and large cells (D, 20×).

recurrence at a single site from a second primary cancer, especially after long-term complete remission. Although it is possible that the present case was a primary thyroid cancer, this tumor was determined to be an isolated metastasis to the thyroid gland because pathologically, it was very similar to the large cell component of the primary small cell lung cancer. It may therefore be possible to distinguish primary thyroid cancer with features of neuroendcrine origin, such medullary carcinoma, from metastatic lung cancer because immunoreactivity for calcitonin and thyroglobulin in the thyroid tumor and serum was negative [14]. In addition, the thyroid tumor was easily enucleated and had a clear border with the normal thyroid tissue. A rapid blood flow in the thyroid [11] may inhibit tumor cells from attaching to the thyroid, despite the rich vasculature. Because hematogenic metastases of lung cancer easily occur, other lesions may have already been occupied by cancer cells when the cancer cells attached to the thyroid. This may explain why isolated metastases are very rare.

Although the need for surgery for metastatic thyroid tumors has not yet been established, a metastasectomy is recommended to prolong survival in the absence of both locally recurrent disease and other metastatic disease [11,12]. As early as in 1960, Elliott and Frantz reported that a partial thyroidectomy resulted in a 3-year disease-free survival and a 5-year survival in a patient with thyroid metastases from primary kidney cancer that had been removed 14 years previously [15]. They described that the removal of either part or all of the metastatic thyroid tumor resulted in a good palliation in some cases. In the present case, metastasectomy and adjuvant chemotherapy were performed, and the patient has been disease-free for over 1 year. In conclusion, this report documents a patient developing an isolated recurrence of lung cancer to the thyroid gland. Although this relapse pattern very rarely occurs, physicians should be alert to this unusual metastatic site because a timely diagnosis could enhance patient survival.

Conflict of interest

None declared.

References

[1] Nesbitt JC, Putnam Jr JB, Walsh GL, Roth JA, Mountain CF. Survival in early-stage non-small cell lung cancer. Ann Thorac Surg 1995;60:466–72.
[2] Silverberg SG, Vidone RA. Metastatic tumors in the thyroid. Pacif Med Surg 1966;74:175–80.
[3] Mortensen JD, Woolner LB, Bennett WA. Secondary malignant tumors of the thyroid gland. Cancer 1956;9:306–9.
[4] Lin JD, Weng HF, Ho YS. Clinical and pathological characteristics of secondary thyroid cancer. Thyroid 1998;8:149–53.
[5] Shimaoka K, Sokal JE, Pickren JW. Metastatic neoplasms in the thyroid gland. Cancer 1962;15:557–65.
[6] Nakhjavani MK, Gharib H, Goellner JR, van Heerden JA. Metastasis to the thyroid gland. Cancer 1997;79:574–8.
[7] Trokoudes KM, Rosen IB, Strawbridge HT, Bain J. Carcinomatous pseudothyroiditis: a problem in differential diagnosis. Can Med Assoc J 1978;21(119):896–8.
[8] Edmonds CJ, Thompson BD. Hyperthyroidism induced by secondary carcinoma in the thyroid. Clin Endocrinol 1978;8:411–5.
[9] Eriksson M, Ajmani SK, Mallette LE. Hyperthyroidism from thyroid metastasis of pancreatic carcinoma. JAMA 1977;238:1276–8.
[10] Miyakawa M, Sato K, Hasegawa M, Nagai A, Sawada T, Tsushima T, et al. Severe thyrotoxicosis induced by thyroid metastasis of lung adenocarcinoma: a case report and review of the literature. Thyroid 2001;11:883–8.
[11] Cichon S, Anielski R, Konturek A, Barczynski M, Cichon W. Metastases to the thyroid gland: seventeen cases operated on in a single clinical center. Langenbecks Arch Surg 2006;391:581–7.
[12] Chen H, Nicol TL, Udelsman R. Clinically significant, isolated metastatic disease to the thyroid gland. World J Surg 1999;23:177–80.
[13] Brambilla E, Travis WD, Colby TV, Corrin B, Shimosato Y. The new World Health Organization classification of lung tumours. Eur Respir J 2001;18:1059–68.
[14] Matias-Guiu X, LaGuette J, Puras-Gil AM, Rosai J. Metastatic neuroendocrine tumors to the thyroid gland mimicking medullary carcinoma: a pathologic and immunohistochemical study of six cases. Am J Surg Pathol 1997;21:754–62.
[15] Elliott RHE, Frantz VK. Metastatic carcinoma masquerading as primary thyroid cancer: a report of authors' 14 cases. Ann Surg 1960;151:551–61.