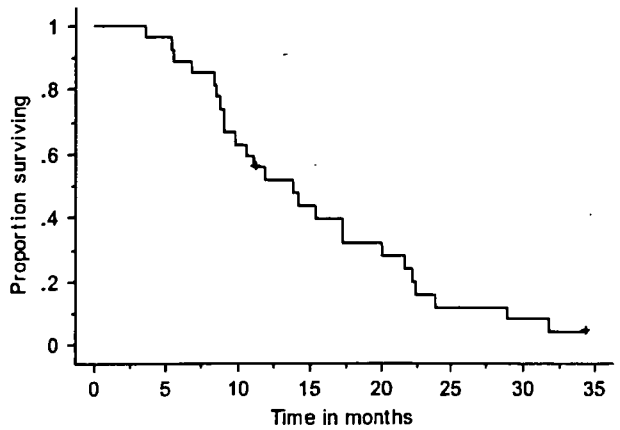


**Fig. 1** Overall survival curve for the 27 patients

**Table 2** Toxicity profiles for all cycles

| Toxicity | No. of patients (%) | | | |
|---|---|---|---|---|
| | Grades | | | |
| | 1 | 2 | 3 | 4 |
| Leucopenia | 0 | 5 (19%) | 16 (59%) | 6 (22%) |
| Neutropenia | 1 (4%) | 0 | 2 (7%) | 24 (89%) |
| Febrile neutropenia | | | 8 (30%) | |
| Anemia | 2 (7%) | 15 (56%) | 8 (30%) | 2 (7%) |
| Thrombocytopenia | 10 (37%) | 7 (26%) | 5 (19%) | 1 (4%) |
| Nausea/vomiting | 10 (37%) | 6 (22%) | 4 (15%) | 0 |
| Diarrhea | 6 (22%) | 5 (19%) | 1 (4%) | 0 |
| Nephrotoxicity | 3 (11%) | 1 (4%) | 0 | 0 |
| Hepatotoxicity | 2 (7%) | 2 (7%) | 0 | 0 |
| Constipation | 8 (30%) | 1 (4%) | 0 | 0 |
| Alopecia | 11 (41%) | 14 (52%) | – | – |
| Peripheral neuropathy | 3 (11%) | 1 (4%) | 0 | 0 |

*DI* dose intensity, *PI* cisplatin and irinotecan, *CDDP* cisplatin, *CPT* irinotecan, *ACE* doxorubicin, cyclophosphamide and etoposide, *DXR* doxorubicin, *CPA* cyclophosphamide, *ETP* etoposide

4 leucopenia: 37 versus 81%, grade 3 and 4 anemia: 19 versus 37%) (Table 3).

### Non-hematological toxicity

Severe diarrhea was rare (Table 2); only one patient (4%) developed grade 3 diarrhea and none had no grade 4 diarrhea. The PI regimen produced diarrhea more frequently than the ACE regimen (all grades; 41 vs. 15%) (Table 3). Other toxicities were also generally mild and there were no treatment-related deaths.

### Treatment delivery and dose intensity

In total, 126 cycles were administered. In the first, third and fifth cycles (total 68 cycles), 27, 24 and 17 cycles of PI were administered, while 27, 22 and 9 cycles of ACE were administered in the second, fourth and sixth (total 58 cycles). Median number of cycles of therapy was five ranging from two to six. Dose modification and/or treatment omission was undertaken in 30 (44%) of the 68 PI cycles and 31 (53%) of the 58 ACE cycles. However, the dose intensity of each drug was favorable (Table 4) and the mean percentages of the delivered doses relative to the projected doses of PI and ACE were 84.6 and 91.1%, respectively. The dose intensity correlated with neither objective response ($P = 0.7062$) nor overall survival ($P = 0.3132$).

## Discussion

In this study, we obtained the following results: (1) PI–ACE alternating chemotherapy showed a promising antitumor activity with a response rate of 93% and median survival time of 12.9 months, comparable to those of PE chemotherapy (response rate of 44–68% and median survival time of 9.4–10.2 months) and PI chemotherapy (response rate of 48–84% and median survival time of 9.3–12.8 months) [5, 11], (2) toxicity was moderate and the main toxicity was myelosuppression, and (3) this regimen produced a favorable dose intensity.

In evaluating the efficacy of alternating chemotherapy, cross-resistance of the two regimens is an important consideration. Fukuoka et al. [3] reported a three-arm phase III trial of CAV, PE and CAV/PE in patients with SCLC. In their trial, only one (8%) of 13 patients responded to CAV after failing to respond to the PE regimen, which might suggest the CAV and PE regimens to be cross-resistant. However, nine (23%) of 39 patients who failed to respond to the initial CAV regimen responded to PE when they were crossed over, and in another study, patients who relapsed after receiving platinum plus etoposide chemotherapy responded to subsequent PI chemotherapy [6]. Thus, we initially considered that these findings appeared to point away from cross-resistance between PI and ACE regimens, and designed to investigate the combination chemotherapy of PI alternating with ACE. In the current trial, however, we could not evaluate whether PI and ACE were cross-resistant because the two regimens were rapidly alternated with a very short interval. Therefore, we could not assess the degree of cross-resistance between the PI and ACE regimens, which is one of the major limitations in our study.

Our favorable efficacy data might also be explained by the fact that combined use of topoisomerase I and II inhibitors has been demonstrated to be complementary in both preclinical and clinical studies [12, 13]; it was previously shown that development of cellular resistance to topoisomerase II inhibitors conferred an increased sensitivity to

**Table 3** Toxicity profiles for all cycles stratified by treatment regimens

| Toxicity | Cisplatin and irinotecan (PI $n = 27$) | | | | Doxorubicin, cyclophosphamide and etoposide (ACE $n = 27$) | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of patients (%) | | | | No. of patients (%) | | | |
| | Grades | | | | | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Leucopenia | 2 (17%) | 12 (44%) | 10 (37%) | 0 | 0 | 5 (19%) | 16 (59%) | 6 (22%) |
| Neutropenia | 2 (17%) | 0 | 11 (41%) | 11 (41%) | 1 (8%) | 2 (17%) | 1 (8%) | 23 (85%) |
| Anemia | 3 (25%) | 17 (63%) | 5 (19%) | | 3 (25%) | 14 (52%) | 8 (30%) | 2 (7%) |
| Thrombocytopenia | 11 (41%) | 2 (7%) | 3 (11%) | 0 | 12 (44%) | 7 (26%) | 3 (11%) | 1 (4%) |
| Diarrhea | 6 (22%) | 4 (15%) | 1 (4%) | 0 | 2 (7%) | 2 (7%) | 0 | 0 |
| Nephrotoxicity | 2 (7%) | 1 (4%) | 0 | 0 | 1 (4%) | 0 | 0 | 0 |
| Hepatotoxicity | 2 (7%) | 2 (7%) | 0 | 0 | 1 (4%) | 0 | 0 | 0 |

*DI* dose intensity, *PI* cisplatin and irinotecan, *CDDP* cisplatin, *CPT* irinotecan, *ACE* doxorubicin, cyclophosphamide and etoposide, *DXR* doxorubicin, *CPA* cyclophosphamide, *ETP* etoposide

**Table 4** Dose intensity of each drug

| Drug | Projected DI (mg/m$^2$ week) | Mean actual DI (mg/m$^2$/week) (range) | Mean percentage of projected DI (range) |
|---|---|---|---|
| PI | – | – | 0.846 (0.608–1.201) |
| CDDP | 15 | 14.1 (10.4–19.6) | 0.943 (0.695–1.310) |
| CPT | 45 | 33.7 (13.8–51.8) | 0.749 (0.306-1.151) |
| ACE | – | – | 0.911 (0.565–1.246) |
| DXR | 12.5 | 11.3 (6.8–15.7) | 0.902 (0.542–1.256) |
| CPA | 187.5 | 173.4 (115.7–235.2) | 0.925 (0.617–1.254) |
| ETP | 60 | 54.4 (13.6–75.5) | 0.906 (0.227–1.258) |

topoisomerase I inhibitors [12]. The reverse effect, in which resistance to a topoisomerase I inhibitor enhanced sensitivity to topoisomerase II inhibitors, has also been reported [13]. In a clinical trial of topoisomerase I and II inhibitors, Masuda et al. [14] evaluated combination chemotherapy with irinotecan and etoposide in patients with relapsed SCLC, and the response rate of 71% far exceeded the response rates of 40–50% previously reported with PE for replased SCLC [15]. Our regimen was also designed to administer topoisomerase I (irinotecan) and II (etoposide) inhibitors alternately. This might have produced the favorable efficacy seen with our alternating chemotherapy in spite of the small sample size and the wide confidence intervals of response rate.

As to the toxicity profile, one of the advantages of our trial was that the incidence of diarrhea, a dose-limiting toxicity of irinotecan, was lower with this regimen than with the PI combination used in the aforementioned phase III trial (44 vs. 70%) [5]. On the contrary, there was a higher incidence of neutropenia in our trial than in the PI combination trial (96 vs. 62%) [5], leading to a higher incidence of febrile neutropenia (30%). However, toxicities were reversible with appropriate supportive care including antibiotics and G-CSF and there were no treatment-related mortalities. Thus, our combination chemotherapy appeared to be well-tolerated.

In conclusion, PI–ACE alternating chemotherapy showed promising antitumor activity, with moderate toxicities, in patients with ED-SCLC.

**Acknowledgments** We wish to thank Drs. Akihiro Bessho and Naoyuki Nogami (National Hospital Organization Shikoku Cancer Center), Kenichi Gemba (Okayama Rousai Hospital), Tomonori Moritaka and Takushi Kitajima (Ehime Prefectural Central Hospital), Tadashi Maeda, Keisuke Aoe and Hideki Katayama (National Hospital Organization National Sanyo Hospital), Junichiro Hiyama (Kure Kyosai Hospital) and Hiroaki Miyamoto (Okayama City Hospital) for their support and the provision of data for our study.

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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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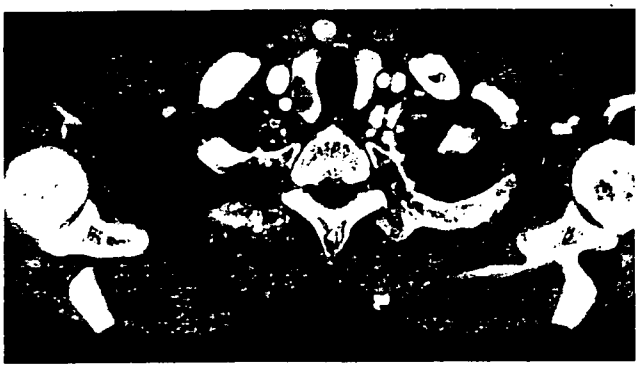

Fig. 1 CT scan of the right thyroid tumor.

## 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

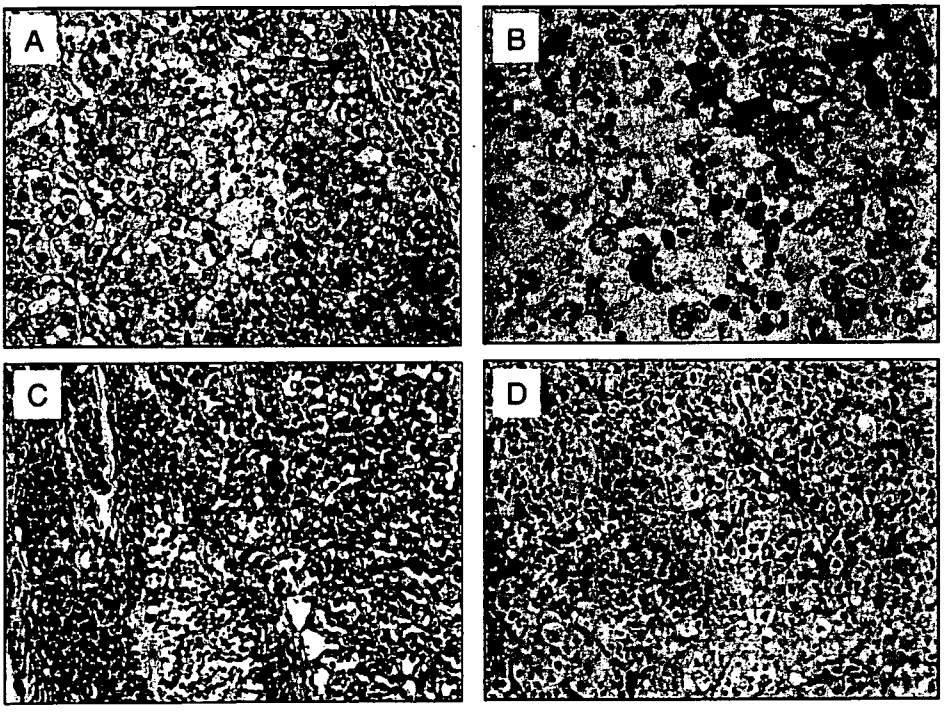


Fig. 2 The cancer cells in the thyroid tumor had large nuclei and cytoplasm seen with hematoxylin-eosin staining (A, 20×) and immunohistochemical staining identified the neuroendocrine marker, CD56 (B, 40×). The primary lung cancer consisted of both components of small cells (C, 20×) and large cells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

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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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**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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ORIGINAL PAPER

# Epidermal growth factor receptor gene amplification and gefitinib sensitivity in patients with recurrent lung cancer

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**Abstract** To evaluate the epidermal growth factor receptor (EGFR) protein expression, gene mutations and amplification as predictors of clinical outcome in patients with non-small-cell lung cancer (NSCLC) receiving gefitinib, we have performed fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC). We investigated the *EGFR* amplification and EGFR protein expression statuses in 27 surgically treated non-small-cell lung cancer (NSCLC) cases. These patients experienced relapse after surgery and received gefitinib 250 mg/day. The presence or absence of *EGFR* mutations of kinase domains was analyzed by genotyping analysis and sequences, and already reported. *EGFR* mutations were found from 15/27 lung cancer patients. *EGFR* mutation status was significantly correlated with better prognosis (log-rank test $P = 0.0023$). Smoking status (never smoker vs. smoker, $P = 0.0032$), and pathological subtypes (adenocarcinoma vs. non-adenocarcinoma, $P = 0.0011$), but not *EGFR* amplification ($P = 0.1278$), were correlated with survival of lung cancers. EGFR IHC results were correlated with FISH results ($P = 0.0125$), but not correlated with prognosis ($P = 0.7921$). Thus, the *EGFR* gene amplification or protein expression is not a predictor of gefitinib efficacy in Japanese patients with NSCLC. We have also evaluated the *EGFR* mutation status and clinico-pathological features for 27 NSCLC patients who had undergone surgery followed by treatment with gefitinib at the National Hospital Organization, Kinki-chuo Chest Medical Center. The *EGFR* mutation status, especially exon19 mutation was correlated with good response to gefitinib than exon 21 point mutation.

**Keywords** *EGFR* · Lung cancer · Mutations · Amplification · Exon19

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## 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 (Ginsberg et al. 1993). There are much accumulated evidences that epidermal growth factor receptor (*EGFR*) and its family member are strongly implicated in the development and progression of numerous human tumors, including lung cancer (Nicolson et al. 2001; Onn et al. 2004). The *EGFR* tyrosine kinase inhibitor, gefitinib, was approved in Japan for the treatment of non-small-cell lung cancer (NSCLC) since 2002. Phase II and III trials have shown partial responses in 8–12% of unselected patients with progressive NSCLC after chemotherapy (Kris et al. 2003; Thatcher et al. 2005), especially higher response in never smokers, females and Asian ethnicity (more than 20%) (Fukuoka et al. 2003; Miller et al. 2004). Two original reports showed that *EGFR* mutations status at ATP binding pockets in NSCLC patients was correlated with the clinico-pathological features related

to good response to gefitinib (Paez et al. 2004; Lynch et al. 2004). These *EGFR* mutations were predominantly found in Japanese lung cancer patients (about 25–40%) (Paez et al. 2004; Kosaka et al. 2004; Shigematsu et al. 2005; Tokumo et al. 2005; Endo et al. 2005) when compared to USA patients (about 8–10%) (Paez et al. 2004; Lynch et al. 2004; Shigematsu et al. 2005; Pao et al. 2004) or European patients (Shigematsu et al. 2005; Marchetti et al. 2005). Actually, *EGFR* mutations in lung cancer have been correlated with clinical response to gefitinib therapy (Paez et al. 2004; Lynch et al. 2004; Pao et al. 2004; Mitsudomi et al. 2005). On the other hands, Cappuzzo et al. (2005) reported that *EGFR* amplification by fluorescence in situ hybridization (FISH) and high *EGFR* protein expression has been associated with responsiveness to gefitinib. Takano et al. (2005) showed that both *EGFR* gene mutation and increased copy numbers predicted gefitinib sensitivity in patients with recurrent NSCLC. However, this Japanese report is based on polymerase chain reaction (PCR) assay.

To determine the *EGFR* amplification and *EGFR* mutation statuses and correlation with clinico-pathological features in Japanese gefitinib-treated lung carcinoma, we retrospectively performed FISH and immunohistochemistry. The findings were compared to the clinico-pathologic features of lung cancer.

## Materials and methods

### Patients and samples

This was a retrospective study and the study group included 27 lung cancer patients who were treated with gefitinib for their recurrent diseases after they had undergone surgery at the Department of Surgery II, Nagoya City University Medical School. Written informed consent was obtained and the institutional ethics committee of the Nagoya City University Medical School approved the study. We have also investigated *EGFR* mutation status for 27 NSCLC patients who were treated with gefitinib for their recurrent diseases after they had undergone surgery at the National Hospital Organization, Kinki-chuo Chest Medical Center (Endo et al. 2005). The lung tumors were classified according to the general rule for clinical and pathological record of lung cancer in Japan. All tumor samples were immediately frozen and stored at −80°C until assayed. The clinical and pathological characteristics of the 27 lung cancer patients are as follows; 14 (67.7%) were male and 13 were female. Twenty-two (63%) were diagnosed as adenocarcinoma, and five were diagnosed as other types of carcinoma. Fourteen (52%) were never smokers and 13 were smokers.

### PCR assays for *EGFR* and *K-ras* mutations

Genomic DNA was extracted using Wizard SV Genomic DNA purification Systems (Promega) according to the manufacturers' instructions. The primers and TaqMan MGB probe were designed with Primer Express 2.0 software (Applied Biosystems). The sequences of 13 allele-specific probes and primer sets used in the TaqMan PCR assay are already shown (Endo et al. 2005). The results of TaqMan PCR assays were already reported (Endo et al. 2005). *K-ras* codon 12/13 mutation status was investigated by direct sequencing using the primers reported by Krypuy et al. (2006). Total RNA was extracted from the lung cancer tissues using Isogen kit (Nippon gene, Tokyo, Japan) according to the manufacturers' instructions. RNA (1 μg) was reverse transcribed by Superscript II enzyme (Gibco BRL, Gaithersburg, MD, USA) with 0.5 μg oligo $(dT)_{12-16}$ (Amersham Pharmacia Biotech Inc. Piscataway, NJ, USA). The direct sequencing for *EGFR* genes was performed from genomic DNA (Paez et al. 2004) or cDNA (Sasaki et al. 2006). Some cases were genotyped using LightCycler (Sasaki et al. 2005) and confirmed.

### FISH analysis

Tumor specimens were obtained at surgical operation and embedded in paraffin. Serial sections (6 μm) containing representative malignant cell were stained with hematoxylin and eosin. Gene copy number per cell was investigated by FISH using the LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis, Abbott laboratories, IL, USA) according to a published protocol (Hirsch et al. 2003). Sections were incubated at 56°C overnight, deparaffinized and dehydrated. After incubation in 2× saline sodium citrate buffer (2× SSC; pH 7.0) at 75°C for 15–25 min, sections were digested with protein K (0.25 mg/ml in 2× SSC; pH 7.0) at 37°C for 15–25 min, rinsed in 2× SSC at room temperature for 5 min, and dehydrated using ethanol in a series of increasing concentrations. The EGFR/CEP 7 probe set was applied per the manufacture's instructions onto the selected area based on the presence of tumor foci on each slide. The slides were incubated at 80°C for 8–10 min for codenaturation of chromosomal and probe DNA and then placed in a humidified chamber at 37°C for 20–24 h to allow hybridization to occur. Post hybridization washes were performed in 1.5 M urea and 0.1× SSC at 45°C for 30 min and in 2× SSC for 2 min at room temperature. Pathologist who was blinded to the patients' clinical characteristics and all other molecular variables performed FISH analysis independently. Patients were classified according to the Cappuzzo et al. (2005) criteria with ascending number of copies of the *EGFR* gene

per cell and the frequency of tumor cells with specific number of copies of the *EGFR* gene and chromosome 7 centromere: high polysomy (≥4 copies in ≥40% of cells) and gene amplification (defined by presence of tight *EGFR* gene clusters and a ratio of *EGFR* gene to chromosome of ≥2 or ≥copies of *EGFR* per cell in ≥10% of analyzed cells) were considered as FISH positive. Disomy (≤2 copies in >90% of cells); low trisomy (≤2 copies in ≥40% of cells, 3 copies in 10–40% of cells, 4 ≥copies in <10% of cells); high trisomy (≤2 copies in ≥40% of cells, 3 copies in ≥40% of cells, ≥4 copies in <10% of cells) and low polysomy (≥4 copies in 10–40% of cells) were considered as FISH negative.

### Immunohistochemistry

EGFR protein expression was evaluated by immunohistochemistry using the mouse anti-human EGFR, clone 2-18C9 monoclonal antibody (Dako NorthAmerica, Inc., Via Real, Carpinteria, CA, USA). Four micrometer sections were made from paraffin tissue blocks from lung tumors. The slides were treated with xylenes, and then dehydrated in alcohol. After treated with proteinase K for 5 min, endogenous peroxidase was blocked with Peroxidase ($H_2O_2$) Block. After washed with Wash Buffer (Dako NorthAmerica Inc., USA), the slides were incubated with the monoclonal antibody against EGFR (ready-to use) for 30 min at room temperature. Labeled Polymer, HRP (30 min) and 3,3-diaminobenzidine (DAB) substrate (10 min) were used to visualize the antibody binding, and the sections were counterstained with hematoxylin. The intensity score was defined according to Cappuzzo et al. (2005); 1 = barely detectable, 2 = readily appreciable brown staining, 3 = dark brown staining, 4 = very strong staining. The total score was calculated by multiplying the intensity score and the fraction score (positive cells; 0–100%). Scores of 201–400 were considered positive.

### Statistical methods

Statistical analyses were done using the Mann–Whitney *U* test for unpaired samples and Wilcoxon's signed rank test for paired samples. Linear relationships between the variables were determined by means of simple linear regression. Correlation coefficients were determined by rank correlation using Spearman's test and $\chi^2$ 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 analyses were done using the Stat-View software package (Abacus Concepts Inc. Berkeley, CA, USA), and were considered significant when the *P*-value was less than 0.05.

## Results

### *EGFR* gene copy number and clinical outcome

First we assessed *EGFR* copy number by FISH according to Cappuzzo et al. criteria (2005). High polysomy for the *EGFR* gene was present in 44.4% ($n = 12$), and low polysomy in 11.1% ($n = 3$) (Fig. 1). However no association was observed between gene amplification and clinical characteristics (Table 1). Smoking status (never smoker vs. smoker, $P = 0.1283$), pathological subtypes (adenocarcinoma vs. non-adenocarcinoma, $P = 0.6280$), or gender (male vs. female, $P = 0.2519$) did not correlate with the *EGFR* amplification status. FISH positive results were obtained in 40% of the patients with *EGFR* mutations. Three other patients with *EGFR* mutations had low polysomy.

A partial response (PR) was achieved in 14 patients, 5 patients had stable disease (SD), and 8 had progressive disease (PD). *EGFR* amplification status was not associated with gefitinib response ($P = 0.7036$). *EGFR* amplification status was not significantly correlated with prognosis (log-rank test, $P = 0.1278$; Breslow–Gehan–Wilcoxon test, $P = 0.0528$) (Fig. 2).

### EGFR protein expression and clinical outcome

EGFR protein expression was evaluated by immunohistochemisry (Fig. 3) and the outcome of patients according to the protein score is shown in Fig. 2. Patients with EGFR immunohistochemistry positive ($n = 13$) did not have any advantage for outcomes after treated with gefitinib therapy ($P = 0.7921$).

### *EGFR* gene mutation status in Japanese lung cancer patients

Among 27 patients, 15 had *EGFR* mutations, including four deletion 1a type mutations (2235–2249 del GGAATTAA GAGAAGC), two other types of exon 19 deletion mutations and six L858R mutations. Interestingly, exon 20 insertion mutant patients experienced progressive disease (manuscript submitted). We also compared associations between *EGFR* mutation status, FISH status, and protein expression in each tumor with patient's outcome. Summarized data are shown in Table 2. The overall survival of 27 gefitinib treated-lung cancer patients from Nagoya City University, with follow-up through 30 April 2007, was studied in reference to *EGFR* mutation status. *EGFR* mutations were not associated with FISH+ status, and high protein expression (wild type; 57.1% vs. $P > 0.9999$). Gene mutations were statistically significantly associated with better response ($P = 0.0018$) and longer survival. Patients

**Table 1** Clinico-pathological data of 27 lung cancer patients

| EGFR gene status | | | |
|---|---|---|---|
| Factors | FISH positive patients | FISH negative patients | *P* value |
| Mean age (years) 64.0 ± 11.9 | 12 | 15 | |
| Pathological subtypes | | | |
| Adeno | 9 (40.9%) | 13 (59.1%) | 0.6260 |
| Non-adeno | 3 (60.0%) | 2 (40.0%) | |
| Gender | | | |
| Male | 8 (57.1%) | 6 (42.9%) | 0.2519 |
| Female | 4 (30.8%) | 9 (69.2%) | |
| Smoking status | | | |
| Never smoker | 4 (28.6%) | 10 (71.4%) | 0.1283 |
| Smoker | 8 (61.5%) | 5 (38.5%) | |
| Differentiation | | | |
| Well | 6 (35.3%) | 11 (64.7%) | 0.2566 |
| Moderately or poorly or Others | 6 (60.0%) | 4 (40.0%) | |
| Gefitinib response | | | |
| Responder | 7 (50.0%) | 7 (50.0%) | 0.7036 |
| Non-responder | 5 (38.5%) | 8 (61.5%) | |
| EGFR mutations | | | |
| Wild type | 6 (50.0%) | 6 (50.0%) | 0.8052 |
| Mutant | 6 (40.0%) | 9 (60.0%) | |
| IHC | | | |
| Positive | 9 (69.2%) | 4 (30.8%) | 0.0213 |
| Negative | 3 (21.4%) | 11 (78.6%) | |

*IHC* immunohistochemistry, *Adeno* adenocarcinoma

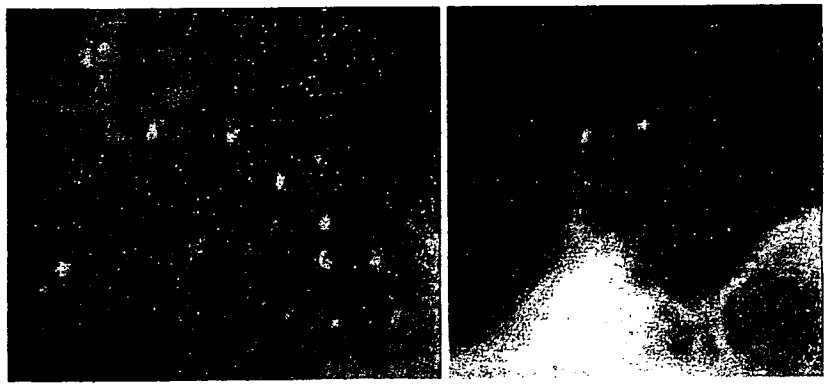

**Fig. 1** FISH analysis for lung cancer tissues. *Left* high polysomy case (4 copy numbers in cells >40%), *right* disomy case

with *EGFR* mutations were significantly better in prognosis than the patients with wild type (log-rank test $P = 0.0023$, Breslow–Gehan–Wilcoxon test, $P = 0.0012$) (Fig. 4). Smoking status (never smoker vs. smoker, log-rank test $P = 0.0032$; Breslow–Gehan–Wilcoxon test, $P = 0.0012$), and pathological subtypes (adenocarcinoma vs. non-adenocarcinoma, log-rank test $P = 0.0011$, Breslow–Gehan–Wilcoxon test, $P = 0.0019$), but neither gender (male vs. female, log-rank test $P = 0.0709$, Breslow–Gehan–Wilcoxon test, $P = 0.0353$), nor response (log-rank test $P = 0.2465$, Breslow–Gehan–Wilcoxon test, $P = 0.0588$) were correlated with better prognosis. Using the Cox hazard regression model, *EGFR* mutations ($P = 0.0208$) and smoking status ($P = 0.0218$) were independent prognostic factors, but not pathological subtypes (0.1121). In this analysis, only one *K-ras* codon 12 mutation was found among 27 patients. This patient was wild type for *EGFR* and did not respond to gefitinib therapy.

We have sequenced 27 NSCLC patients who had undergone surgery followed by treatment with gefitinib at the National Hospital Organization, Kinki-chuo Chest Medical Center and already reported. We have added these data

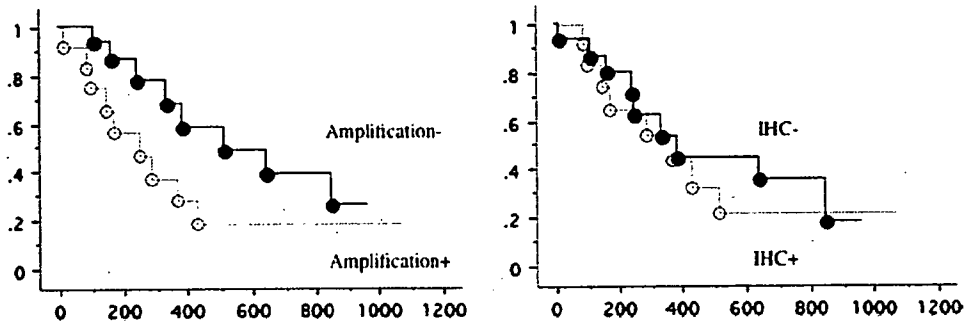


**Fig. 2** The overall survival of 27 gefitinib untreated lung cancer patients was studied in reference to the *EGFR* amplification status (*left*) and EGFR protein expression (*right*). Prognosis from patients with EGFR amplification ($n = 12$, 9 were dead) and without *EGFR* amplification ($n = 15$, 8 were dead) was not significantly different (log-rank test, $P = 0.1278$; Breslow–Gehan–Wilcoxon test, $P = 0.0528$). Prognosis from patients with positive EGFR expression ($n = 13$, 8 were dead) and without negative EGFR expression ($n = 14$, 9 were dead) was not significantly different (log-rank test, $P = 0.7921$; Breslow–Gehan–Wilcoxon test; $P = 0.9105$)

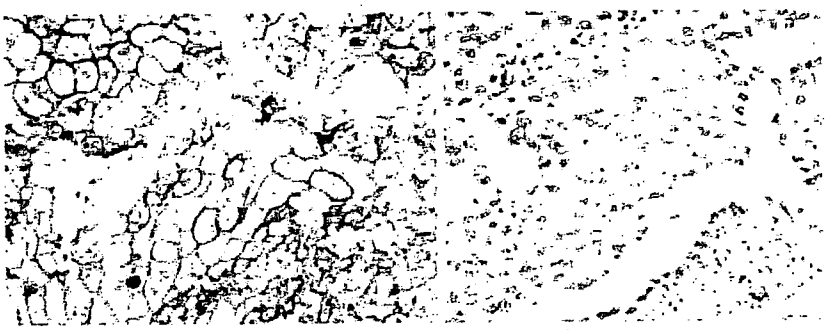

**Fig. 3** EGFR protein expression by immunohistochemistry. *Left* positive case, *right* negative case

(Table 3). Ten patients had *EGFR* mutations, including two L858R, one deletion type 1a, and one G719S at exon 18. Three patients had deletion 1b type mutation (2236–2250 del GAATTAAGAGAAGCA). Of 54 patients, 25 were male and 29 female. Twenty-eight were never smokers and 26 were smokers. Forty-eight patients had adenocarcinoma, four had squamous cell carcinoma and one had adenosquamous cell carcinoma. *EGFR* mutation status was significantly correlated with better prognosis (log-rank test $P = 0.0128$, Breslow–Gehan–Wilcoxon test $P = 0.0051$). Patients with *EGFR* mutation at exon 19 deletion 1 types had significantly better prognosis than wild type patients ($P = 0.0032$). However, the prognosis of patients with L858R mutation and wild type was not significantly different ($P = 0.2823$) (Fig. 5).

## Discussion

We obtained the findings that the *EGFR* amplification, detected by FISH according to Cappuzzo et al. criteria, was not associated with the response to gefitinib. *EGFR* mutations, smoking history, and pathological subtype of lung cancers were correlated with survival of gefitinib-treated patients. This was in agreement with the recent reports that *EGFR* gene mutations are prognostic factor for gefitinib therapy (Takano et al. 2005; Mitsudomi et al. 2005; Sone et al. 2007). In addition, our analysis also suggested that the deletion type *EGFR* mutation might be more correlated with the survival for gefitinib-treated patients.

Some limitations to the study must be taken into consideration. Our finding is so far based on a single retrospective study with a relatively small number of patients, and the data need to be verified in a large cohort of patients and prospectively. The *EGFR* status was determined on the tumor tissue at the time of primary diagnosis, and possible changes after chemotherapy were not determined in this study (Cappuzzo et al. 2007).

Previous report suggested that NSCLC patients with resected tumors carrying high *EGFR* gene copy number have a tendency to a shorter survival (Hirsch et al. 2003). This might affect the controversial results of Cappuzo et al. (2005) In our analysis, FISH positive population did not correlate with the gender, smoking status and pathological subtypes. The presence of *EGFR* gene amplification did not reach statistical significance. An interesting finding was the association between *EGFR* mutations and increased gene copy number, a phenomenon that was recently described in the human lung cancer cell line H3255 (Tracy et al. 2004) and is probably relevant to gefitinib sensitivity. In fact,

**Table 2** EGFR mutation and amplification statuses in 27 gefitinib treated patients

| Age | Gender | Smoking | Pathology | EGFR mutation | EGFR amplification | IHC score | Survival (day) |
|---|---|---|---|---|---|---|---|
| 71 | F | 0 | Adeno | Della | High polysomy | 220 | 1,080 (A) |
| 72 | M | 600 | Adeno | L858R | Low polysomy | 240 | 885 (A) |
| 76 | M | 800 | Adeno | WT | High polysomy | 90 | 248 (D) |
| 72 | M | 0 | Adeno | exon 20 ins V | Disomy | 80 | 660 (A) |
| 70 | M | 1,000 | Adeno | L858R | Disomy | 210 | 515 (D) |
| 61 | F | 0 | Adeno | WT | Disomy | 160 | 854 (D) |
| 51 | M | 500 | Adeno | Della | High polysomy | 220 | 286 (D) |
| 76 | F | 0 | Adeno | WT | Disomy | 30 | 640 (D) |
| 57 | M | 20 | Adeno | WT | High polysomy | 210 | 101 (D) |
| 77 | M | 1,200 | Adeno | WT | Disomy | 0 | 168 (D) |
| 38 | M | 300 | Adeno | L858R | High polysomy | 210 | 430 (D) |
| 73 | F | 0 | Adeno | G719S | Disomy | 180 | 339 (D) |
| 42 | F | 0 | Adeno | Del4 | High polysomy | 100 | 700 (A) |
| 76 | F | 920 | SCC | WT | High polysomy | 220 | 145 (D) |
| 56 | F | 0 | Adeno | L858R | High polysomy | 200 | 368 (D) |
| 56 | M | 1,200 | Adeno | WT | High polysomy | 200 | 85 (D) |
| 78 | M | 1200 | SCC | WT | High polysomy | 250 | 174 (D) |
| 42 | M | 400 | SCC | WT | Disomy | 120 | 110 (D) |
| 67 | M | 800 | Adeno | WT | Disomy | 80 | 384 (D) |
| 63 | M | 600 | Adsq | WT | High polysomy | 90 | 11 (D) |
| 47 | F | 0 | Adeno | Del5 | Disomy | 210 | 945 (A) |
| 62 | F | 0 | Adeno | L858R | Disomy | 80 | 245 (D) |
| 71 | F | 0 | Adeno | L861Q | Low polysomy | 210 | 210 (A) |
| 61 | F | 0 | Adeno | Della | Low polysomy | 120 | 180 (A) |
| 64 | F | 0 | Adeno | WT | Disomy | 180 | 230 (A) |
| 72 | M | 0 | Adeno | L858R | High polysomy | 210 | 110 (A) |
| 77 | F | 0 | Adsq | Della | Disomy | 60 | 210 (A) |

*F* Female, *M* male, *Adeno* adenocarcinoma, *SCC* squamous cell carcinoma, *Adsq* adenosquamous cell carcinoma, *WT* wild type, *IHC* immunohistochemistry, *A* alive, *D* death

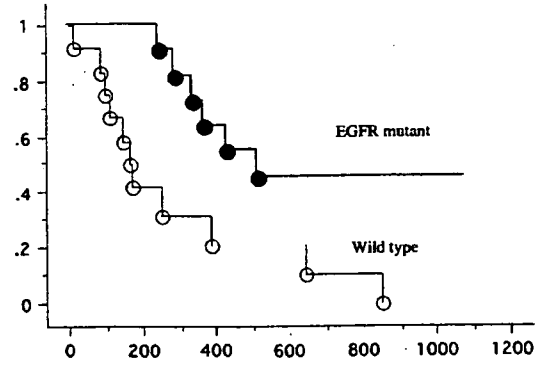


**Fig. 4** The overall survival of 27 gefitinib-treated lung cancer patients was studied in reference to the *EGFR* mutation status. Prognosis from patients with EGFR mutations ($n = 15$, 6 were dead) was significantly better than the patients without *EGFR* mutations ($n = 12$, 11 were dead) (log-rank test, $P = 0.0023$, Breslow–Gehan–Wilcoxon test; $P = 0.0012$)

among the 15 patients with *EGFR* mutations who responded to gefitinib therapy, six were also FISH positive (high polysomy) and three were low polysomy. However, between the two non-responding patients with *EGFR* mutations, both were FISH negative. Sone et al. (2007) reported that the *EGFR* mutations and not the gene amplifications were the predictors of gefitinib efficacy in Japanese lung cancers. They evaluated the biopsy specimens and 5/59 samples were small and inadequate for FISH analysis. Another possible explanation for the discrepancies between the findings from the studies described by Cappuzo et al. and our findings is the difference in *EGFR* mutation statuses according to ethnicity. Han et al. (2006) investigated *EGFR* gene mutations, gene amplification, *K-ras* mutation, and Akt phosphorylation in tumor samples from East-Asian patients with NSCLC and demonstrated that *EGFR* mutation was an independent predictor of response and survival

**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

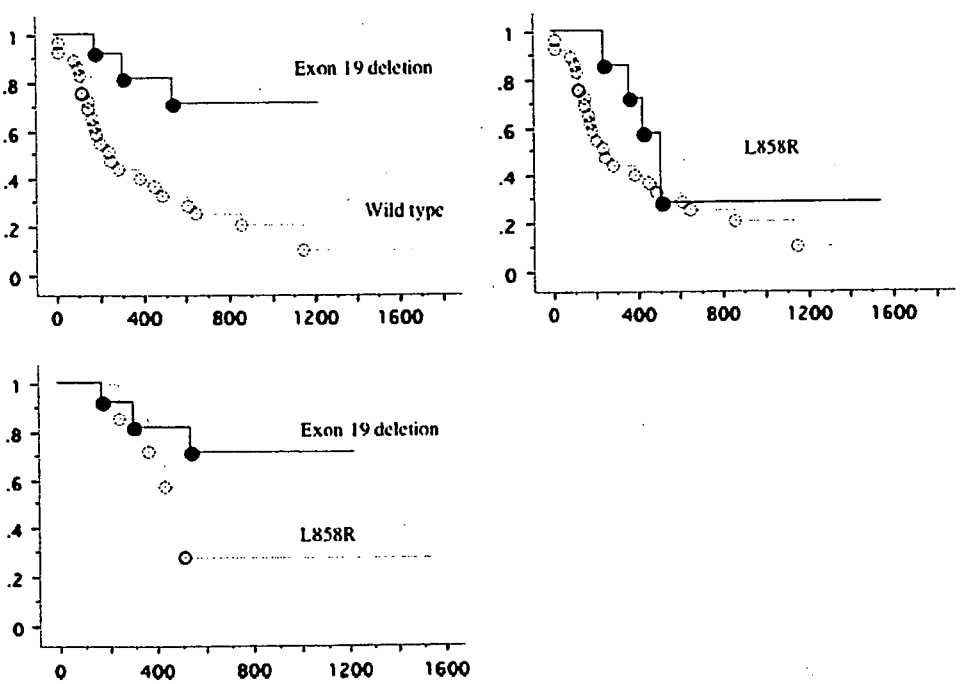


**Fig. 5** The overall survival of 54 gefitinib-treated lung cancer patients was studied in reference to the *EGFR* mutation status. *Left upper* prognosis from patients with exon 19 deletion mutations ($n = 12$, 3 were dead) was significantly better than the patients without *EGFR* mutations (Log-rank 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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# 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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Non-small cell lung cancer;
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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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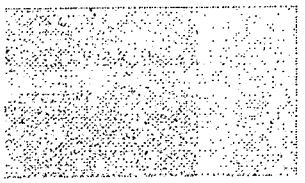

for the PE arm. However, the outcomes did not differ significantly between groups. Toxicity was tolerable in all arms except for one treatment-related death due to interstitial pneumonia induced by BLM. We will select intrapleural treatment using OK-432 in the management of MPE in NSCLC for further investigation 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 $\geq$9.5 g/dL, and platelet count $\geq$100,000 $\mu L^{-1}$), and liver (total bilirubin $\leq$1.5 mg/dL and transaminase levels $\leq$ twice the upper limit of the normal value) and renal (BUN $\leq$25 mg/dL, serum creatinine $\leq$1.2 mg/dL, and creatinine clearance $\geq$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

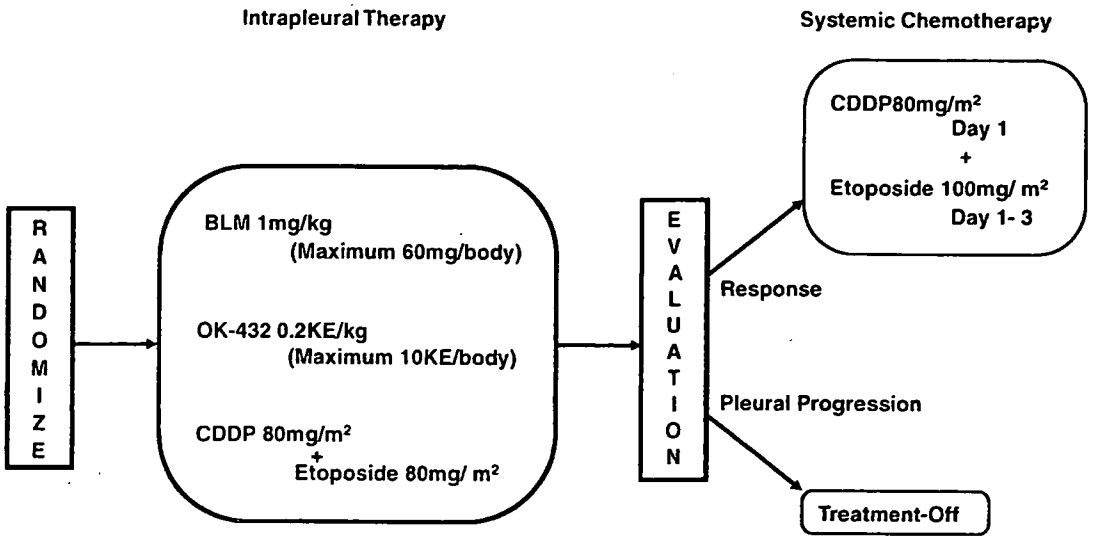


Fig. 1 Treatment schema.

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 $\mu L^{-1}$ or the platelet count was less than 75,000 $\mu L^{-1}$. 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