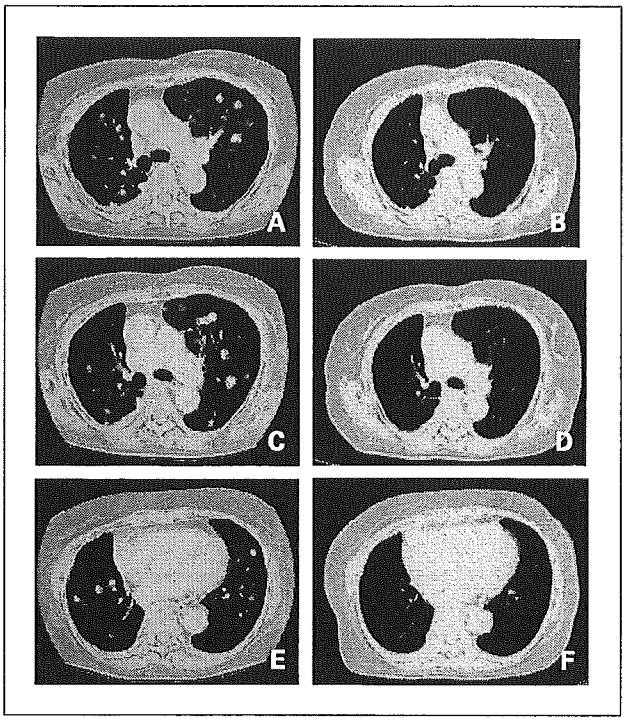


Fig. 1. Example of response to gefitinib in a representative patient with recurring NSCLC after three lines of chemotherapy. Computed tomography slices before gefitinib treatment (*A*, *C*, and *E*) and after 8 weeks of gefitinib treatment (*B*, *D*, and *F*).

female with relapsed lung adenocarcinoma underwent an upper left lobe lobectomy in 2002; the tumor contained an *EGFR* mutation (delE746-A750). One and a half years later, in 2004, the patient developed severe neurologic symptoms with impairment of walking, eating, and speaking and required a gastric feeding tube. The brain computed tomography showed multiple cystic, rim-enhancing supratentorial masses of various sizes (Fig. 2*A*). Brain biopsy was not done. Dexamethasone was given, without improvement, and brain irradiation was not indicated. One month later, gefitinib was given through the gastric feeding tube, and a rapid recovery of neurologic functions was observed, accompanied by a regression of the brain metastases (Fig. 2*B*). The patient is still in remission. A third patient, an 84-year-old North American female with lung adenocarcinoma underwent a lobectomy in 2003; the tumor contained an *EGFR* mutation (delE746-A750). The patient relapsed with bone and lung metastases; one cycle of chemotherapy was given, but she suffered a pulmonary embolism with a myocardial infarction. She recovered but did not receive additional chemotherapy. She developed a cardiac tamponade with clear evidence of progression of her lung metastases. Seven months later, in 2004, she started gefitinib treatment, and 3 weeks later she was clinically improved. New bone metastases were detected after 1 year and the patient died . Finally, a 42-year-old Japanese female with lung adenocarcinoma underwent a left upper lobectomy in 2001; the tumor contained not only a delE746-A750 mutation but also >20 *EGFR* gene copies by fluorescence *in situ* hybridization, elevated *EGFR* (47.3) and *caveolin-1* (0.9) mRNA expression, and increased number of CA repeats (20 of 21; Fig. 3). The patient developed brain metastases 9 months later, in 2002 and received stereotactic radiosurgery. Multiple lung metastases developed after 2 months, and six cycles of cisplatin/gemcitabine/vinorelbine were given. Eight months later, in 2003, the patient initiated gefitinib treatment. Before treatment, her carcinoembryonic antigen level was 257.2 ng/mL (normal level, <5 ng/mL). After 6 months of gefitinib treatment, her carcinoembryonic antigen level was 2.2 ng/mL. A complete remission of the lung metastases has been attained.

Median survival for patients carrying *EGFR* mutations was not reached, whereas it was 9.9 months (95% CI, 6.8-12.9) for those patients carrying wild-type *EGFR* ($P = 0.001$; Fig. 4).

Table 4 shows the characteristics of the 28 patients in whom we assessed CA repeats, *EGFR* and *caveolin-1* mRNA expression, and *EGFR* gene copy numbers. All patients with *EGFR* mutations also had increased numbers of CA repeats (≥19). The highly gefitinib-sensitive PC9 lung adenocarcinoma cell line, which harbored the deletion delE746-A750, also displayed increased numbers of CA repeats (20 of 20). There were no differences in median mRNA levels of *EGFR* or *caveolin-1* according to EGFR mutation status. Increased *EGFR* gene copy numbers were observed more frequently in patients with EGFR mutations. Gene amplification ranged widely from low to high levels, and in some patients,

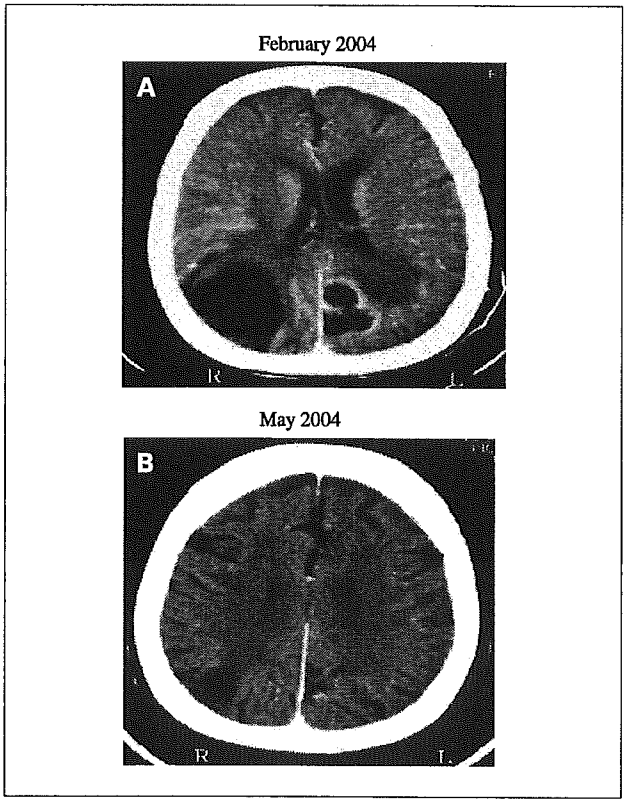


Fig. 2. Example of response to gefitinib in a lung adenocarcinoma patient with brain metastases. Computed tomography before gefitinib treatment (*A*) and after 8 weeks of gefitinib treatment (*B*). *A*, enlarged ventricles were observed in the pretreatment computed tomography. *B*, after treatment, with the disappearance of the periventricular brain metastases, ventricles were less visible.

amplification was seen in only 25% of the tumor cells examined. In this subset of 28 patients, the response rate for patients with increased gene copy numbers was 45%, in contrast with 89% for patients with *EGFR* mutations (P = 0.02). The response rate was 100% in patients with both *EGFR* mutations and gene amplification. Table 5 illustrates the levels of *EGFR* and *caveolin-1* mRNA according to *EGFR* mutation status and further broken down by gene copy numbers and number of CA repeats. The highest levels of *EGFR* mRNA were observed in the group of patients with both *EGFR* mutations and increased *EGFR* copy numbers. Patients with both *EGFR* mutations and low levels of *EGFR* or *caveolin-1* mRNA had a median survival of 13 months, whereas median survival has not been reached for those patients with *EGFR* mutations and high levels of *EGFR* or *caveolin-1* mRNA (data not shown).

Discussion

In the present study, we have observed that *EGFR* mutations are a strong predictor of gefitinib response in chemoresistant NSCLC patients. Sixteen of 17 patients (94.1%) with *EGFR* mutations attained an objective response, in contrast with only 6 of 51 patients (12.6%) with wild-type *EGFR* (P < 0.0001). These results mirror accumulated data from three studies (10–12) in which 25 of 31 (81%) NSCLC patients with *EGFR* mutations attained an objective response, whereas none of 29 nonresponders had mutations. Furthermore, it has recently been shown that in 16 gefitinib-treated Taiwanese NSCLC patients, seven of nine responders had *EGFR* mutations (13). The delE746-A750 in the PC-9 cell line found in the present study has also been observed in a separate study (29), in which it also conferred hypersensitivity to gefitinib. *EGFR* mutations found in previous studies have mostly been heterozygous (10–12); however, Paez et al. (11) reported one homozygous mutation at exon 19 and Pao et al. (12) found homozygous mutations in two of seven gefitinib-treated patients, leading them to speculate that homozygosity may be the result of the selective amplification of the mutant gene or that mutations in general may be homozygous with the wild-type sequence originating from contaminating "normal" DNA. In the study by Huang et al. (13), 4 of 10 mutations were homozygous, and in the present study, 6 of 17 mutations were also homozygous. Contaminating "normal" cells with wild-type EGFR seems the most likely explanation for apparently heterozygous mutations, because even with microdissection, nonneoplastic tissue contamination cannot be completely ruled out. However, amplification of mutant *EGFR* could account for detection of only mutant sequences.

In the original studies (10, 11), only one mutation per tumor was detected. However, Pao et al. (12) found a tumor sample with two mutations, from a female never smoker with adenocarcinoma, treated with erlotinib for 13 months, and surviving 22 months. Furthermore, in the study by Huang et al. (13), two patients had two mutations in their tumors; one responded and one did not. In our study, one patient had two mutations: a 67-year-old Hong Kong Chinese female never smoker with adenocarcinoma. She attained a partial response and is still alive at 22 months (January 2005). It is not possible to draw definite conclusions from only four patients, and more data regarding the potential predictive value of two mutations in the same tumor is needed.

In the present study, 6 of 51 patients with wild-type *EGFR* attained partial response to gefitinib. There were no differences in baseline clinical characteristics between responders with EGFR mutations and responders with wild-type *EGFR* (Table 2). However, only 16% of responders with wild-type *EGFR* remain alive at the time of submitting this article, in contrast with 81% of responders harboring *EGFR* mutations. In the series reported by Lynch et al. (10), one of nine gefitinib-sensitive patients did not have *EGFR* mutations. Along the same lines, Pao et al. (12) reported that 5 of 17 patients with partial response or clinical improvement to gefitinib or erlotinib had wild-type *EGFR* in exons 18 to 24.

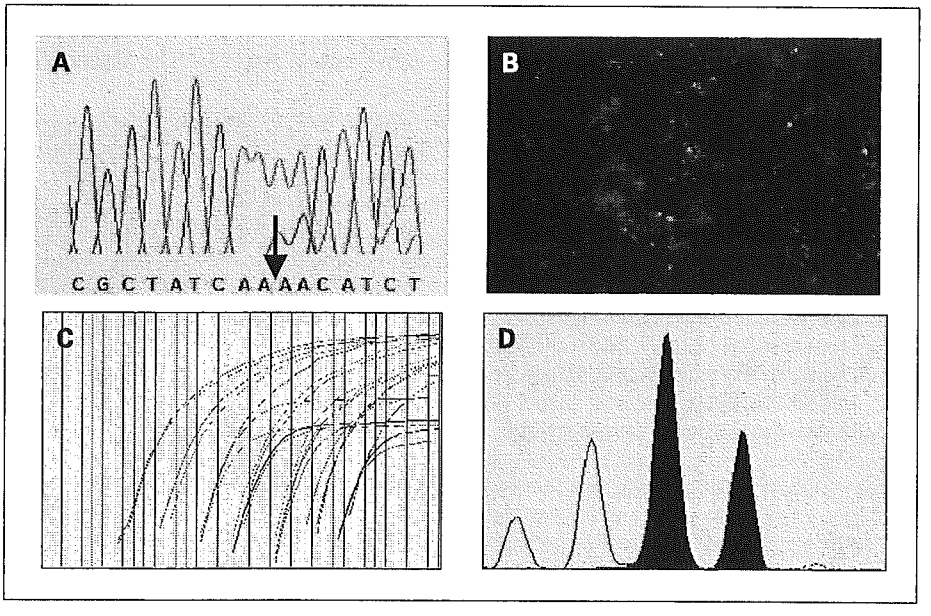
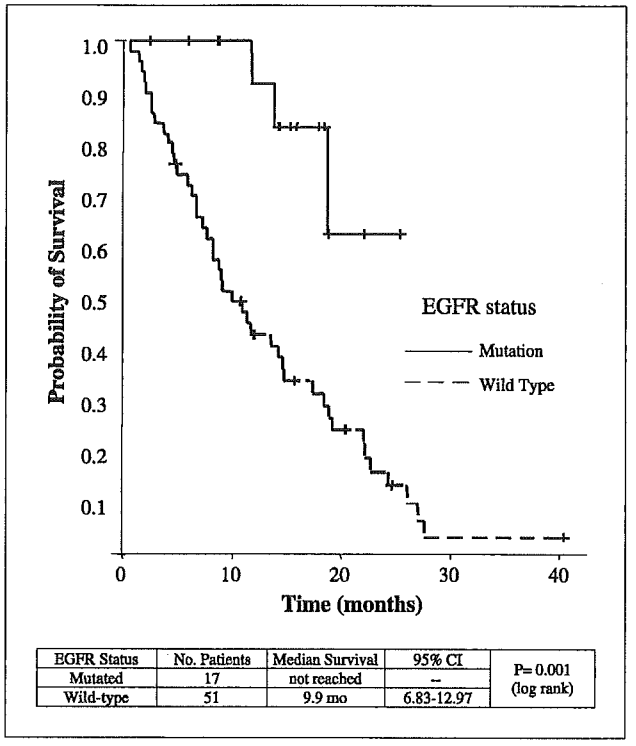


Fig. 3. Gefitinib responder showing, clockwise from top left: (*A*) an *EGFR* mutation (del E746-A750); (*B*) a high level of gene amplification (spots); (*C*) high *EGFR* and *caveolin-1* mRNA levels (superimposed one on the other); (*D*) and increased numbers of CA repeats. *C*, cDNAs for the gene of interest and an internal reference gene (*β-actin*) were quantified using a fluorescence-based real-time detection method. For each sample, parallel triplex Taqman PCR reactions were performed for the gene of interest and the *β-actin* reference gene to normalize for input cDNA. The expression of individual *EGFR* and *caveolin-1* was calculated using a relative quantification algorithm. In this patient, the *EGFR* mRNA level was 47 and the *caveolin-1* mRNA level was 0.98. *D*, number of CA repeats, determined by GeneScan analysis software (Applera). The number of CA repeats is determined by the mobility in the chromatogram. The shaded peaks represent the intensities of the two alleles. The left peak represents 20 CA repeats and the right peak represents 21 CA repeats. At submission, this patient has been in complete remission for 18.9 months.



EGFR Status	No. Patients	Median Survival	95% CI	P= 0.001 (log rank)
Mutated	17	not reached	–	
Wild-type	51	9.9 mo	6.83-12.97	

Fig. 4. Survival from the start of gefitinib treatment according to EGFR mutation status.

Mutations in these responders may not have been detected because they were below the detection rate of sequencing assays (30), or increased *EGFR* gene copy numbers in these responders may have conferred enhanced gefitinib sensitivity (12) in the absence of *EGFR* mutations. NSCLC cell lines with wild-type *EGFR* but with high levels of *EGFR*, *ErbB2*, or *ERbB3* mRNA have shown intermediate sensitivity to gefitinib and erlotinib (31).

The small number of patients examined in the present study limits the conclusions that can be drawn as to the role of CA repeats, *EGFR* and *caveolin-1* mRNA expression, and *EGFR* gene copy numbers. However, interethnic differences in the number of CA repeats warrant further investigation in Asian lung cancer patients, in whom increased numbers of CA repeats may be more frequently associated with the presence of *EGFR* mutations (19, 20). Amador et al. (32) found that head and neck cell lines with decreased numbers of CA repeats had higher expression of *EGFR* mRNA and were more sensitive to the inhibitory effects of erlotinib. In addition, in 19 gefitinib-treated colorectal cancer patients (32), 84% of those with decreased numbers of CA repeats developed skin toxicity, a feature related to the antitumor activity of EGFR inhibitors (33), compared with only 33% of those with increased numbers of CA repeats (P = 0.04; ref. 32).

In surgically resected NSCLC patients (13, 34), *EGFR* mutations were associated with well and moderately differentiated adenocarcinomas and smoking status but not with female gender. Dramatic clinical response to gefitinib is observed in only 10% to 19% of chemorefractory advanced NSCLC. Kris et al. (5) showed that female gender predicted response to gefitinib, whereas the number of prior chemotherapy regimens did not influence response. In our study, the number of prior chemotherapy regimens increased the probability of response in tumors containing *EGFR* mutations.

The strong correlation we observed between EGFR mutations and improved response and survival leads us to recommend the assessment of *EGFR* mutations in lung adenocarcinoma

Table 4. Patient characteristics of a subgroup of 28 patients according to *EGFR* mutation status, number of CA repeats in intron 1, *EGFR* and *caveolin-1* mRNA levels, and *EGFR* gene copy numbers

	Wild-type *EGFR*, *n* (%)	Mutated *EGFR*, *n* (%)	*P*
No. patients	19	9	
Age (y)			
<65	10 (52)	6 (66.6)	NS
≥65	9 (48)	3 (33.3)	
Sex			
Male	15 (79)	4 (45)	NS
Female	4 (21)	5 (55)	
Ethnicity			
Caucasian	8 (42)	4 (45)	NS
Asian	11 (58)	5 (55)	
Histology			
Adenocarcinoma	16 (85)	9 (100)	NS
Large cell carcinoma	1 (5)	0	
Squamous cell carcinoma	2 (10)	0	
Smoking status			
Smoker	15 (79)	3 (33.3)	0.035
Nonsmoker	4 (21)	6 (66.6)	
Response to gefitinib			
Yes	2 (11)	8 (88)	<0.0001
No	16 (84)	1 (12)	
Nonevaluable	1 (5)	—	
Duration of gefitinib response (wk)			
Median (range)	6.93 (0.2-27.6)	7.73 (1.05-15.63)	NS
CA repeats in intron 1			
<19	3 (20.5)*	0 (-)*	NS
≥19	11 (79.5)*	7 (100)*	
EGFR mRNA levels			
No. patients	15	8	0.087
Median (range)	2.61 (0.42-23.09)*	5.04 (1.79-47.37)*	
Caveolin-1 mRNA levels			
No. patients	14	8	NS
Median (range)	0.71 (0.06-2.16)*	0.55 (0.19-1.07)*	
EGFR gene copy numbers			
Increased	4 (21)	5 (55)	0.087
Normal	15 (79)	4 (45)	

Abbreviation: NS, not significant.
*Disparity between some figures is due to the lower availability of tumor tissue in some patients.

Table 5. *EGFR* and *caveolin-1* mRNA levels in patients with wild-type and mutated *EGFR*, further broken down according to number of CA repeats in intron 1 and *EGFR* gene copy numbers

	Wild-type *EGFR*				Mutated *EGFR*			
	EGFR mRNA		*Caveolin-1* mRNA		*EGFR* mRNA		*Caveolin-1* mRNA	
	No. patients	Median (range)	No. patients	Median (range)	No. patients	Median (range)	No. patients	Median (range)
CA repeats								
<19	2	14.24 (5.38-23.09)	1	2.16 (2.16-2.16)	0		0	
≥19	10	2.94 (1.54-6.44)	10	0.75 (0.19-1.47)	6	13.21 (3.0-47.37)	6	0.41 (0.19-1.07)
EGFR gene copy numbers								
Increased	4	5.12 (2.61-23.09)	3	1.23 (0.19-2.16)	5	20.31 (3.0-47.37)	5	0.61 (0.19-1.07)
Normal	11	2.04 (0.42-6.43)	11	0.57 (0.06-1.47)	3	3.01 (1.79-3.97)	3	0.46 (0.30-0.63)

patients to customize treatment. NSCLC cell lines containing *EGFR* mutations are chemoresistant but highly sensitive to gefitinib (17, 35). In the present study, we detected an unprecedented median survival in patients with *EGFR* mutations. The Spanish Lung Cancer Group is currently screening for *EGFR* mutations in metastatic lung adenocarcinomas to identify patients who could benefit from treatment with tyrosine kinase inhibitors.

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Case report

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Malignant schwannoma of the upper mediastinum originating from the vagus nerve

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Abstract

Background: Malignant schwannoma of the upper mediastinum originating from the vagus nerve is extremely rare.

Case presentation: A 46-year-old female was admitted for a left cervical mass which was associated with both hoarseness and Horner's syndrome. Chest computed tomography showed a mass extending from the left upper mediastinum to the left supraclavicular area. A fine needle aspiration cytological examination suggested primary lung cancer stage IIIB large cell carcinoma. After administering induction chemo-radiotherapy, a complete surgical resection was performed. The tumor was found to involve both the left vagus nerve and the left sympathetic nerve. Histological examination of the resected specimen revealed the tumor to be malignant schwannoma.

Conclusion: Despite incorrect preoperative diagnosis, the multimodality treatment administered in this case, including induction chemo-radiotherapy and surgery, proved to be effective.

Background

According to a collected series of 2399 cases of mediastinal tumors reported in the literature [1], 496 cases (20.7%) were of neurogenic tumors, and most of them occurred in the posterior mediastinum. Neurogenic tumors can be divided into two groups depending on their origin: those that arise from the nerve sheath and those that arise from nerve cells. The majority of the tumors of nerve sheath origin in adults are either benign schwannomas or neurofibromas, and they usually arise from either an intercostal nerve or a sympathetic nerve. Intrathoracic schwannoma originating from the vagus nerve, is extremely rare.

Case presentation

A 46-year-old female with symptoms of hoarseness and Horner's syndrome presented with a left cervical mass that was diagnosed to be undifferentiated carcinoma based on the findings of aspiration cytology (Figure 1). The patient's chest computed tomography (CT) findings showed a mass measuring 5.0 cm in size spreading from the left upper mediastinum to the left supraclavicular area, which pressed against both the trachea and

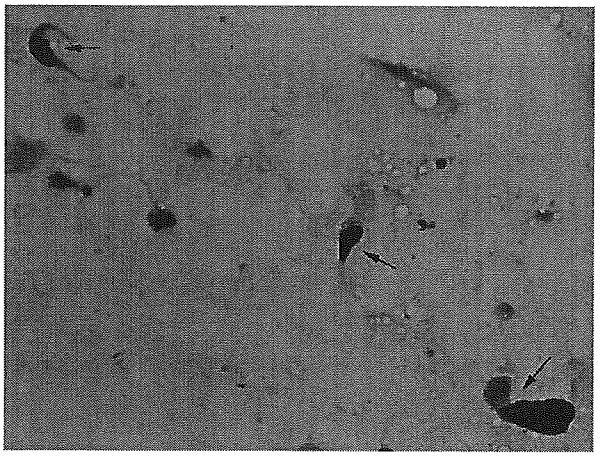

Figure 1
Aspiration cytology of the tumor before treatment showing scattered atypical spindle cells (arrows) (Giemsa staining, high power view x400).

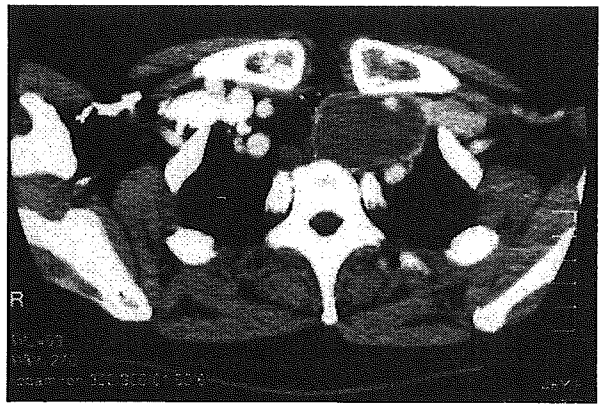

Figure 2
Chest CT showed a mass, measuring 5.0 cm in size in the upper mediastinum.

esophagus and it seemed to involve the left common carotid artery (Figure 2). Based on these findings, and cytology findings a clinical diagnosis of stage IIIB (T4N3M0) non-small cell lung cancer (NSCLC) originating from the apex of the left lung involving both the mediastinum and the supraclavicular lymph nodes was made [2]. The patient received concurrent chemo-radiotherapy (cisplatin 80 mg/m^2 for days 8 and 36 + UFT 400 mg/m^2, both on days 1–14 and on days 29–42 plus radiotherapy, 2 Gy/day on days 1–20 for a total of 40 Gy) [3]. After this treatment regimen, the tumor size decreased by 35.0%. Thereafter, the patient underwent a surgical resection though a median sternotomy with a combined resection of the left clavicle. During the operation, an encapsulated tumor was detected in the mediastinum. Although the tumor was easily ablated from the left common carotid artery, it involved both the left vagus and the sympathetic nerves. As a result, both nerves had to be sacrificed in order to achieve a complete resection of the tumor. Grossly, the tumor was in continuity of the vagus nerve was whitish in color and oval shaped measuring 5 × 3 cm in diameter (Figure 3). Both cytological and histological examinations revealed 1) Continuity between the vagus nerve and tumor was seen, while the no continuity between the tumor and the sympathetic nerve was found. 2) The findings of aspiration cytology of the tumor diagnosing it to be undifferentiated carcinoma before the treatment included an atypical spindle cell. (Figure 1). 3) The predominantly tumor consisted of necrotic tissue and a few viable atypical spindle cells (Figure 4A) which were positive for S-100 protein (Figure 4B). As a result, the tumor was considered to arise from the left vagus nerve while invading the left sympathetic ganglion, and was therefore diagnosed it to be a malignant schwannoma. At present, the patient has survived for about 2 years since operation without any recurrence.

Discussion

Tumors of vagus nerve origin are observed in about 2% of all neurogenic tumors of the mediastinum [4], however, no instace of malignant schwannoma was reported in this review. To our knowledge, only a few such cases have been previously reported [5-7]. As a result, malignant schwannoma originating from the vagus nerve is therefore considered to be extremely rare.

Malignant peripheral nerve sheath tumors (MPNST) including malignant schwannoma are the malignant variants of schwannomas and neurofibromas. Although the 5-year survival rates have been reported to be up to 75 % in MPNST's patients, MPNST often advance locally and can also occasionally metastasize to the lung or other organs [8]. Therefore, in addition to a complete surgical resection, adjuvant therapy is usually advocated. However, in an adjuvant setting, the efficacy of chemotherapy or radiotherapy appears to provide little additional benefit [9,10]. We previously reported concurrent chemo-radiotherapy with UFT plus cisplatin as an induction treatment followed by a surgical resection for patients with marginally resectable stage IIIB NSCLC to be both a feasible and promising treatment [3]. Since we preoperatively considered the disease to be marginally resectable stage IIIB NSCLC, we performed concurrent induction chemo-radiotherapy followed by surgery. As a result, this

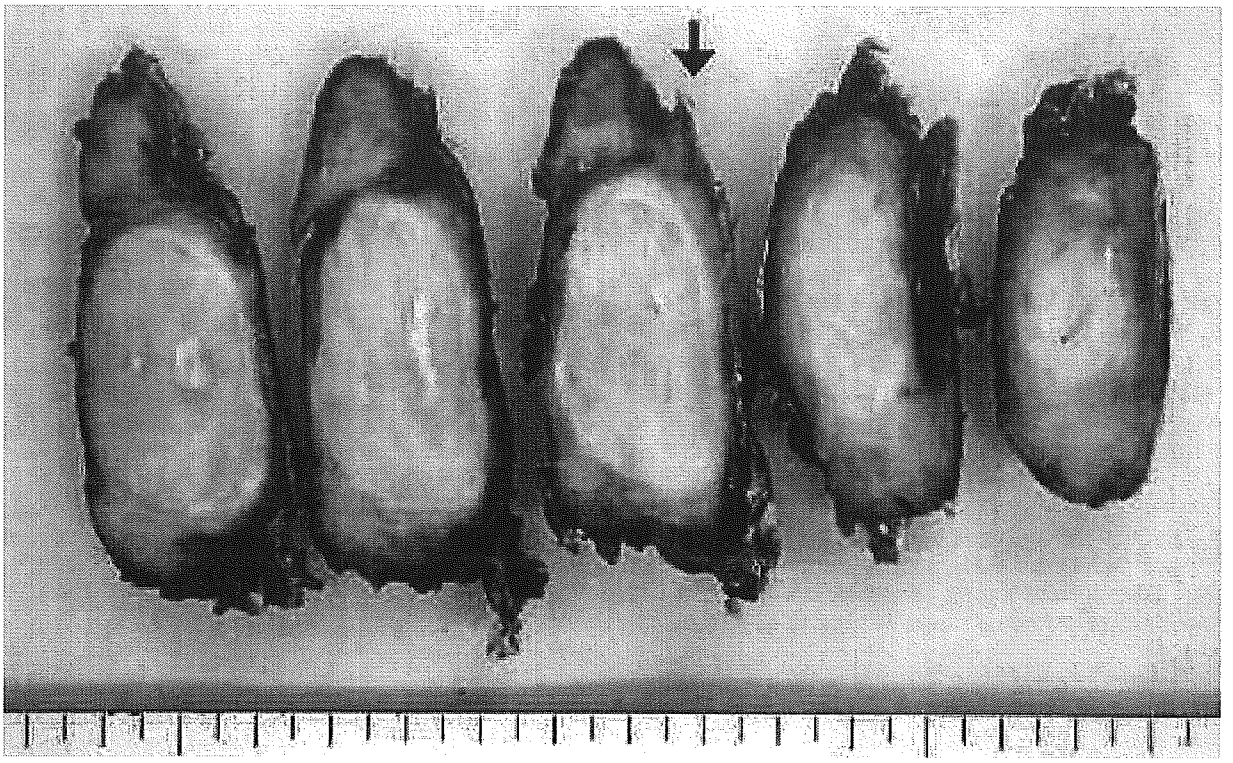

Figure 3
Macroscopic findings of the tumor. The tumor showing the continuity of the vagus nerve (arrow head) was whitish in color and oval shaped while measuring 5 × 3 cm in diameter.

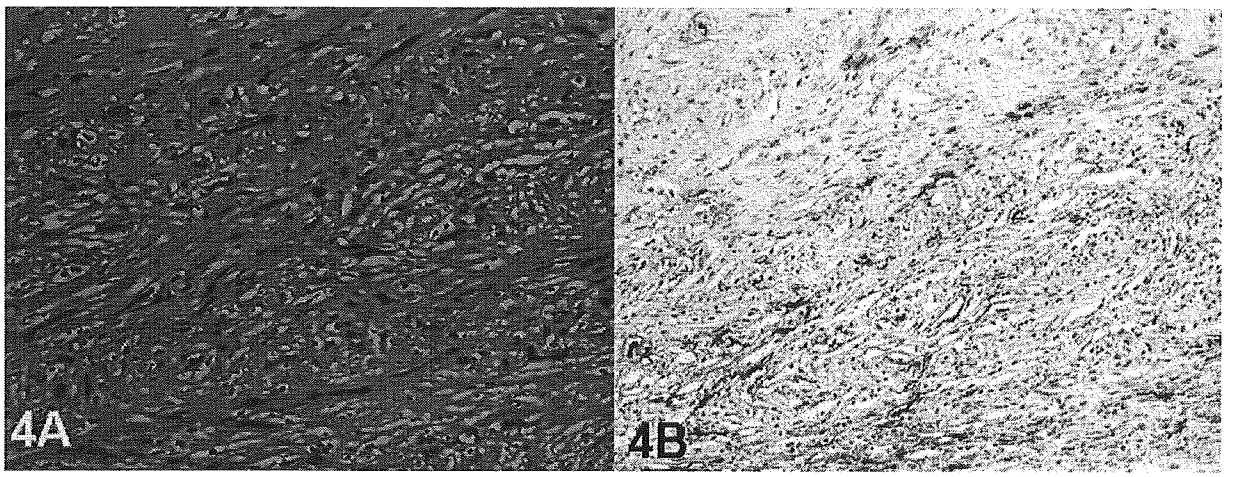

Figure 4
Histological findings of the tumor. 4A) The tumor was found to consist of a few of viable atypical spindle cells with hyperchromatic nuclei (Hematoxilin-eosin staining x200) 4B). Tumor cells were positive for S-100 protein (immunohistochemical staining, original magnification: X200).

multimodality treatment proved to be effective and the patient is now doing well without any recurrence.

We initially misdiagnosed this patient's disease to be non-small cell lung cancer. The reason for this was partly due to the cytological findings which indicated undifferentiated carcinoma. In general, an exact diagnosis cannot always be made based on the findings of aspiration cytology alone. The second reason for a misdiagnosis in this case was due to the patient's symptoms which included hoarseness and Horner's syndrome. Apical lung cancer involving both the vagus and the sympathetic nerve is occasionally observed. However, to the best of our knowledge, the present case is considered to be the first case demonstrating malignant schwannoma of the vagus nerve involving the sympathetic nerve.

Conclusion

Malignant schwannoma of the upper mediastinum arising from the vagus nerve is rare. The multimodality treatment administered in this case, including induction chemo-radiotherapy and surgery, proved to be effective.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

FS: conceived of the study, participated in its design and coordination and drafted the manuscript.

RM and TO: carried out the literature search and helped in drafting the manuscript.

HW: participated in the design of the study and helped in drafting the manuscript.

KN: performed histological examination and provided photographs.

YI: shaped the idea for the study, coordinated the study and edited the manuscript.

All authors read and approved the final manuscript.

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Written consent was obtained from the patient for the publication of this case.

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Case report

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Long-term survival after an aggressive surgical resection and chemotherapy for stage IV pulmonary giant cell carcinoma

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Abstract

Background: Pulmonary giant cell carcinoma is one of the rare histological subtypes with pleomorphic, sarcomatoid or sarcomatous elements. The prognosis of patients with this tumor tends to be poor, because surgery, irradiation and chemotherapy are not usually effective.

Case presentation: We herein report a patient with pulmonary giant cell carcinoma with stage IV disease in whom aggressive multi-modality therapy resulted in a long-term survival. A 51-year-old male underwent an emergent operation with a partial resection of small intestinal metastases due to bleeding from the tumor. The patient also underwent a left pneumonectomy due to hemothorax as a result of the rapid growth of the primary tumor. Thereafter, two different regimens of chemotherapy and a partial resection for other site of small intestinal metastases and a splenectomy for splenic metastases were performed. The patient is presently doing well without any evidence of recurrence for 3 years after the initial operation.

Conclusion: This is a first report of a rare case with stage IV pulmonary giant cell carcinoma who has survived long-term after undergoing aggressive surgical treatment and chemotherapy.

Background

The recent World Health Organization (WHO) classification of lung tumors has unified the heterogeneous group of non-small cell lung carcinomas that contains sarcoma or sarcoma-like components under the designation of "carcinomas with pleomorphic, sarcomatoid or sarcomatous elements" [1]. This group includes different entities, such as pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma and pulmonary blastoma. In general, these tumors are rare, comprising approximately from 0.1–0.4% of all lung malignancies [2-4]. The patients with these tumors tend to demonstrate a despondent clinical course and the prognosis for them is gernally poor [5], because surgery, irradiation and chemotherapy are ineffective. We experienced a pulmonary giant cell carcinoma patient with stage IV disease in whom aggressive multi-modality therapy consisting of surgical resections for the primary lesion and multi-organ metastases and also chemotherapy which together resulted in a long-term survival.

Case presentation

A 51-year-old male was admitted in June 2001, due to hemosputum, cough, hemo-stool and an abnormal shadow on a chest roentgenogram. Laboratory results showed severe anemia with hemoglobin of 4.0 g/dl (13.6 < normal range < 16.8 g/dl) and hematocrit of 16.0 % (40 < normal range < 48 %). The patient's chest X-ray demonstrated a huge mass lesion in the left upper lung field (Figure 1). Computed tomography (CT) of the chest showed a mass shadow, measuring 7.0 × 7.0 cm in size in the left upper lobe (S^{1+2}) without any invasion of the surrounding tissue such as the vessels, plexus or thoracic wall and with no mediastinal lymph node swelling. Abdominal CT revealed a huge mass, measuring 12.7 × 7.5 cm in size in the small intestine. Prior to performing any treatment for the presumed lung cancer, we tried to stop the continuous bleeding from tumor in the small intestine. As a result, we performed an emergency operation. The tumor was observed in the jejunum at a location about 30 cm from the ligament of Treitz on the anal side and a 25 cm length of the jejunum, including the tumor, was thus resected. Six days later, the patient experienced sudden chest pain, dyspnoea and hemoptysis. The patient's chest X ray showed the left lung mass shadow to have rapidly increased in size, while the broncho-fiberscopy findings showed bleeding from the left upper bronchus and an obstruction of the left lower bronchus due to coagulation. Hemothorax due to a rupture of the lung induced by the rapid growth of the tumor was found after an emergency thoracotmy. The tumor was so large that it was difficult to approach the interlobular pulmonary artery. Therefore, a left pneumonectomy with mediastinal lymph nodal dissection was performed. Thereafter, intraoperative intrapleural hypotonic cisplatin treatment [6] was performed because some tumor cells were suspected to exist in the pleural cavity due to the rupture of the tumor. A histological examination revealed pure giant cell carcinoma containing no sarcomatoid component, similar to that found in the small intestine (Figure 2). As a result, we diagnosed the patient to have stage IV disease (pathological stage T2N0M1) according to the TNM classification [1]. The patient had an uneventful recovery without any complications. However, about 4 months after the first operation, the patient was diagnosed to have a recurrence at another site in the small intestine and spleen by abdominal CT. The patient received 2 cycles of chemotherapy (cisplatin 40 mg/m^2 + gemcitabine 800 mg/m^2+ vinorelbine 20 mg/m^2), at days 1 and 8, and thereafter every 4 weeks). The splenic metastases increased in size while the size of the tumor in the small intestine decreased. At this time, no recurrence site except for those in the small intestine and spleen were found, therefore, to avoid the risk of bleeding either from tumors in the small intestine or a rupture of spleen in the future, surgical treatment consisting of a partial resection of the small intestine and a splenectomy was performed. The intestinal tumor was found in the jejunum at a location about 10 cm from the ligament of Treitz on the anal side and a total 20 cm length of the jejunum, including the tumor, was resected. A pathological examination revealed a proliferation of pure giant cell carcinoma with extensive necrosis both in the small intestine and the spleen, thus suggesting the chemotherapy to be effective in the both organs. Thereafter, the patient received 2 additional cycles of this triplet chemotherapy. The patient experienced neither any hematological nor severe non-hematological adverse events. About 6 months later, metastases in multiple abdominal lymph nodes were found (Figure 3A). The patient was started on chemotherapy (carboplatin AUC = 2 + paclitaxel 60 mg/m^2, on days 1 and 8, and thereafter every 3 weeks). After receiving a total of 10 cycles of chemotherapy on an outpatient basis, abdominal CT showed the chemotherapeutic effect to be a complete response (Figure 3B), without any severe hematological or non-hematological adverse events. At present, the patient has survived for about 3-years since the first operation and a complete response has been maintained for 15 months.

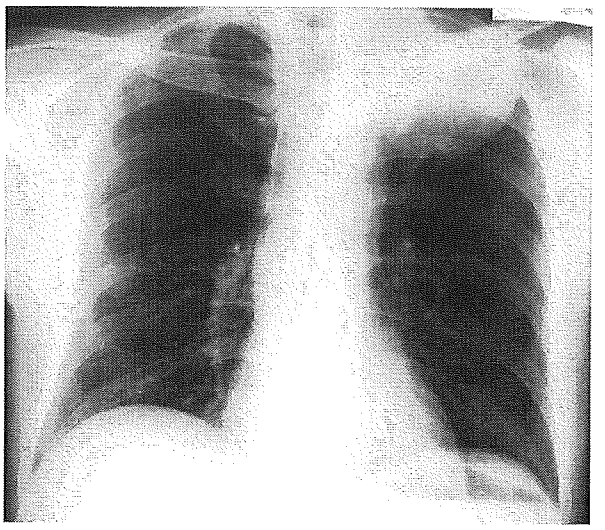

Figure 1
Posterior-anterior view of a chest X-ray film demonstrated a huge mass shadow in the left upper lung field.

Discussion

According to the treatment guidelines for unresectable non-small cell lung cancer of American Society of Clinical Oncology (ASCO)[7], chemotherapy prolongs survival and is the most appropriate treatment for stage IV patients with a good performance status. Although both resections of primary lung cancer and either brain or adrenal

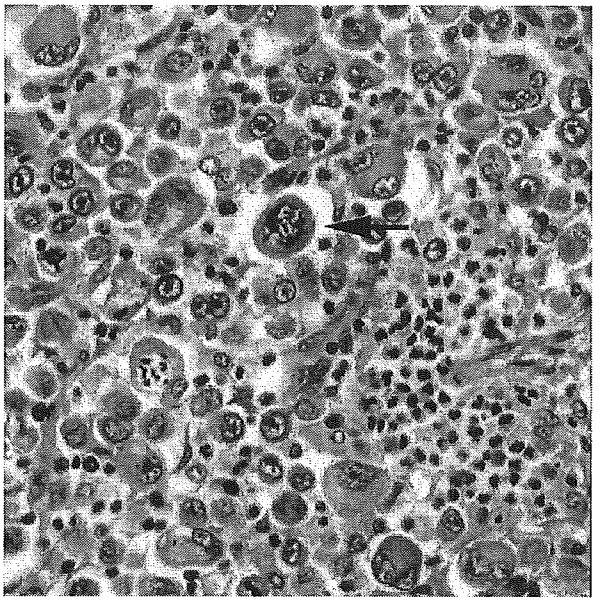

Figure 2
Pathological findings of the left lung. The section consists of a diffuse proliferation of atypical, giant and bizarre cells (arrowhead). No sarcomatoid component is seen.

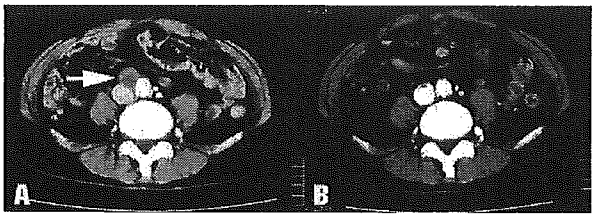

Figure 3
Computerised tomographic scan before and after treatment. A) Abdominal CT showed multiple lymph node swelling, suggesting the presence of metastases (arrowhead). B) Abdominal CT showed the lymph nodes metastases to have completely disappeared.

metastases are occasionally recommended in highly selected patients, a surgical resection of other metastasized sites is hardly ever performed. Therefore, the present patient is an extremely rare case because he underwent an emergency surgical resection of small intestinal metastases and a primary tumor due to bleeding from both tumors, as well as a surgical resection of other metastases in the small intestine and spleen in order to avoid a risk of bleeding from the recurrent site in the future.

Fishback *et al*, reported the overall survival of total 78 patients with pleomorphic (spindle/ giant cell) carcinoma (stage I-IV), among whom 57 patients received a surgical resection, to be poor with a median survival time of 10 months and a survival rate of 10% at 5 years [8]. According to Chang *et al*, the mean survival time of resected pleomorphic carcinoma patients was 5 months while the median survival time of pleomorphic carcinoma patients treated with concurrent or sequential chemo-radiotherapy was 2.7 months [9]. To our knowledge, a case of a long-term survivor with stage IV pleomorphic (spindle/ giant cell) carcinoma has never been previously reported. The tumor histology of the present case was very rare, pure giant cell carcinoma, which belongs to the category of carcinomas with pleomorphic, sarcomatoid or sarcomatous elements according to new WHO classification, and the prognosis is estimated to be poor. Although pleomorphic carcinoma has been reported to usually be resistant to chemotherapy, we first chose chemotherapy including cisplatin, gemcitabine and vinorelbine, which has been shown to demonstrate the highest response rate in advanced non-small cell lung cancer based on our experience. In our prior phase II trial using this combination chemotherapy in 79 advanced non-small cell lung cancer patients, the response rate was 56% and the 1-year survival rate was 75% while the toxicity levels were acceptable [10]. After recurrence, we chose chemotherapy with carboplatin and paclitaxel, which is most frequently used for the treatment of advanced non-small cell lung cancer. Since the standard treatment method using carboplatin and paclitaxel in Japan is the administration of AUC of 6 and 200 mg/m^2, respectively once every 3 weeks [11], the administered regimen (carboplatin AUC = 2 and paclitaxel 60 mg/m^2, on days 1 and 8, and thereafter every 3 weeks) in this patient was unusual and the dose intensity was relatively small. However, this regimen nevertheless effectively treated his disease and he was also able to work normally during the treatment process. At present, the patient has survived for 3 years since the first operation and has remained healthy without any signs of recurrence for 15 months after the last treatment.

Conclusion

This is a first report of a rare case with stage IV pulmonary giant cell carcinoma who has survived long-term after undergoing aggressive surgical treatment and chemotherapy.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

FS: Conceived the study, participated in its design and coordination and drafted the manuscript.

RM and TO: carried out the literature search and helped in drafting the manuscript

JI, TN and HW: participated in the study design and helped with preparation of the manuscript

YI: Shaped the idea for the manuscript, coordinated the study and edited the manuscript.

All authors conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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We thank Mr. Brian Quinn for critical comments on the manuscript.

Written consent was obtained from the patient for the publication of this case.

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Mutations in the tyrosine kinase domain of the EGFR gene associated with gefitinib response in non-small-cell lung cancer

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KEYWORDS
EGFR mutations;
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Summary The potential relevance of epidermal growth factor receptor (EGFR) mutations to non-small-cell lung cancer treatment has recently been identified. We have examined the presence of EGFR mutations in Japanese and Spanish gefitinib-treated non-small-cell lung cancer patients. A total of 34 gefitinib-treated patients were screened, 18 from Japan and 16 from Spain. Laser capture microdissection was performed for the accurate procurement of tumor cells. EGFR exons 18, 19 and 21 were amplified from genomic DNA by means of PCR, and the samples were then subjected to bi-directional automatic sequencing. EGFR somatic mutations in the tyrosine kinase domain were found in 8 of 34 patients (23.5%). Gefitinib response was observed in 7 of 8 patients (87.5%) with EGFR mutations and in 3 of 24 (12.5%) with wild-type EGFR ($P=0.0003$). Five deletion mutations were clustered in the region spanning codons 746 to 750 (ELREA) within exon 19. Three additional tumors had amino acid substitutions within exon 18, at codons 718 and 719. Logistic regression analysis showed that response was primarily linked to the presence of EGFR mutations and secondarily linked to female gender, non-smoker status and a greater number of prior chemotherapy regimens. The presence of EGFR mutations is a major determinant of gefitinib response, and EGFR tyrosine kinase inhibitors should be tested in clinical trials of first-line treatment of lung adenocarcinomas harboring EGFR mutations.

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1. Introduction

Epidermal growth factor receptor (EGFR) is surfacing as an important target for therapy. EGFR stimulates cell growth and differentiation after binding of specific ligands, acting as a membrane-bound receptor with intrinsic tyrosine kinase (TK) activity in the intracellular domain. Overexpression of EGFR has been shown to transform NIH 3T3 cells in an EGF-dependent manner [1]. Dimerization with other erbB receptors and the activation of the kinase domain are essential for phosphorylation of a variety of intracellular protein cascades. EGFR is amplified in 9% of NSCLC cases. Therefore, it is broadly accepted that overexpression of EGFR in NSCLC is commonly regulated on the transcriptional level [1]. The Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL) 1 trial investigated the efficacy of oral gefitinib in advanced NSCLC patients who had previously received one or two chemotherapy regimens. The response rate was higher for Japanese than for non-Japanese patients (27.5% versus 10.4%; odds ratio = 3.27; $P = 0.0023$). The response rate was also 2.5 times higher in women than in men and 3.5 times higher in adenocarcinoma than in other histologies [2]. The IDEAL 2 study was carried out only in patients in the United States previously treated with two or more regimens containing cisplatin or carboplatin plus docetaxel. The overall response rate was 10%, with a median survival of 6 months and one-year survival of 25%. Response rate was 19% for women and 3% for men, 13% for adenocarcinoma and 4% for other histologies [3]. In addition to the IDEAL 1 and IDEAL 2 trials, 122 patients were treated on the Expanded Access Program, a compassionate use program, at the Memorial Sloan-Kettering Cancer Center. Results confirmed the better response in women (19%) than in men (8%) ($P = 0.14$) and in adenocarcinoma (19%) than in other histologies (0%) ($P = 0.004$). Response was also higher in never-smokers (36%) than in former or current smokers (8%) ($P = 0.001$) [4].

The Iressa NSCLC Trial Assessing Combination Treatment (INTACT) 1 investigated the efficacy of gefitinib versus placebo in combination with cisplatin plus gemcitabine in chemotherapy-naïve patients from Europe (74%), North America (12.7%), Asia (5.3%), South America (4.1%) and South Africa (1.6%). No differences were observed in response, time to progression or median survival [5]. In the INTACT 2 trial, where 80% of the patients were from the United States, patients were randomized to receive paclitaxel plus carboplatin with or without gefitinib. There was no survival advantage in any of the subgroups when gefitinib was added to chemotherapy, but there was a trend toward improved survival in the subgroup of patients with adenocarcinoma who had received chemotherapy for more than 90 days [6].

Personalized treatment can be based on the kinases that are mutationally altered in individual tumors. TKs are central regulators of signaling pathways that control differentiation, transcription, cell cycle progression, apoptosis, motility and invasion. TK mutations have been described in PI3KCA, which encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase (PI3K) [7,8]. Mutations have also been described in B-raf, the most common of which is a substitution mutation changing valine 599 to glutamic acid (V599E), which dramatically enhances B-raf activity [9]. Recently, EGFR TK mutations have been described and linked to gefitinib sensitivity in NSCLC patients [10,11]. Paez et al. [10] observed mutations in the EGFR TK domain only in responders. These mutations were more frequent in women (9/45, 20%) than in men (7/74, 9%) ($P = 0.009$), in adenocarcinoma (15/70, 21%) than in other histological subtypes (1/49, 2%) ($P = 0.001$), in non-smokers (13/37, 54%) than in smokers (6/62, 11%) ($P = 0.0009$), and in Japanese (15/58, 26%) than in non-Japanese subjects (1/61, 2%) ($P = 0.001$). In addition, no mutations were observed in four patients who progressed on gefitinib, while all five tumors from gefitinib responders harbored EGFR mutations ($P = 0.0027$). Lynch et al. [11] also identified mutations in the EGFR TK domain in eight of nine patients with gefitinib-responsive NSCLC, compared with none of the seven non-responders. In both studies, the majority of mutations was clustered in exons 18, 19 and 21 and were either in-frame deletion or heterozygous missense mutations around the adenosine triphosphate (ATP) binding pocket [12]. Substitution mutations changing leucine 858 to arginine (L858R), guanine 719 to serine (G719S), and leucine 861 to glutamine (L861Q) lay in the activation and glycine-rich P loops, which are important for autoregulation, while multiple deletion mutations clustered in the region spanning codons 746 to 750 (ELREA), around the active site of the kinase [10,11]. Mutant EGFR has also been found in gefitinib-sensitive cell lines [10,11].

These studies demonstrate that mutations around the EGFR TK domain enhance ligand-inducing EGFR autophosphorylation and confer increased sensitivity to gefitinib, suggesting that gefitinib may be highly effective for treating NSCLC patients with somatic EGFR mutations. We have studied EGFR TK mutations in NSCLC patients from Japan and Spain who were treated with gefitinib

after second- or third-line chemotherapy and correlated results with response.

2. Patients and methods

2.1. Patients

Paraffin-embedded tumor tissue was obtained from a total of 34 NSCLC patients who had been treated with gefitinib as part of Expanded Access programs after at least second- or third-line chemotherapy failure. Eighteen samples, including 17 resected primary tumors and one bronchial biopsy of a metastatic tumor, were from the National Kyushu Cancer Center in Fukuoka, Japan, and 16 samples, including eight resected primary tumors and eight bronchial biopsies of metastatic tumors, were from the Catalan Institute of Oncology in Badalona, Spain. Patients were selected retrospectively for the present study based on the availability of tumor specimens. Acquisition of tumor specimens and examination of clinical records were approved by the ethical committees of both institutions. All patients gave their signed informed consent for genetic assessment. Patients were divided into smokers and non-smokers; non-smokers were defined as those who had smoked less than 100 cigarettes in their lifetimes. Tumor response was defined according to the Response Evaluation Criteria in Solid Tumors (RECIST) [13]. Follow-up was calculated from the start of gefitinib treatment; median follow-up was 8 months (range, 1.05–27.6 months).

2.2. Methods

In order to obtain relatively pure cell samples for DNA extraction, laser capture microdissection was used (Palm, Oberlensheim, Germany). For isolation of DNA from deparaffinated, microdissected tissue, the material was incubated with proteinase K, and DNA was extracted with phenol-chloroform and ethanol precipitation. Primers and cycling conditions for PCR amplification and direct sequencing for exons 18, 19 and 21 of the EGFR gene (GenBank accession number: X00588) were modified from those previously described [10,11]. Primer pairs and PCR conditions utilized are available in the supplementary appendix. Sequencing was performed using forward and reverse primers with the ABI Prism 3100 DNA Analyzer (Perkin-Elmer, Applied Biosystems). Electropherograms were analyzed for the presence of mutations using Seqscape v2.1.1 software in combination with Factura to mark heterozygous positions. Results were confirmed by independent reruns of the same samples.

2.3. Statistical analyses

The primary objective of this study was to compare clinical characteristics, response rates, and survival in patients with and without mutations in the EGFR TK domain treated with gefitinib. Differences in response rates and clinical characteristics between patients with and without somatic EGFR mutations were analyzed using the chi-square, Fisher's exact test, and Student's *t*-test. Time to death was calculated from the start of gefitinib treatment. The Kaplan–Meier method and log-rank test were used to examine survival differences according to response, EGFR mutation status and nationality. Equation of logistic regression models without constant was fit to examine the relationship between the odds of response and each covariate in the whole group and in different sub-groups after adjusting for the following factors: EGFR mutation status, age, gender, smoking status, nationality and number of prior chemotherapy regimens. In the EGFR mutation-response interaction analysis, we used multiple approaches to evaluate consistency of results, including crude and adjusted analyses. The SPSS 11.5 statistical software package was used for all calculations.

3. Results

Patient characteristics are shown in Table 1, broken down by the presence or absence of EGFR mutations. Eight of the 34 patients harbored mutations. All mutations observed were somatic and were found exclusively in adenocarcinomas and more frequently in non-smokers than in smokers. Gefitinib response was observed in 7 of 8 patients (87.5%) with EGFR mutations and in 3 of 24 (12.5%) with wild-type EGFR ($P = 0.0003$). Two patients with wild-type EGFR were not evaluable for response. Although mutations were not observed in non-responders, one was found in a Spanish patient with stable disease who had two primary lung cancers. The mutation (W731Stop) was found in the resected specimen of the primary lung adenocarcinoma but not in a second squamous cell carcinoma. Exon sequencing of genomic DNA revealed missense and deletion EGFR mutations in 7 of 9 Japanese responders (Table 2), all within exons 18 and 19. We detected four deletion mutations clustered in the region spanning codons 746 to 750 (ELREA) within exon 19 (Fig. 1). Three of these mutations were

Table 1 Patient characteristics according to EGFR mutations

	EGFR mutation	Wild-type EGFR	*P*
No.	8	26	
Age (years)			
≤60	6 (75)	14 (53.8)	0.42
>60	2 (25)	12 (46.2)	
Sex			
Male	4 (50)	19 (73.1)	0.38
Female	4 (50)	7 (26.9)	
Ethnicity			
Caucasian	1 (12.5)	14 (53.8)	0.05
Asian	7 (87.5)	12 (46.2)	
Histology			
Adenocarcinoma	8 (100)	20 (76.9)	0.001[a]
Large cell carcinoma	0 (0)	4 (15.4)	
Squamous cell carcinoma	0 (0)	2 (7.7)	
Smokers	3 (37.5)	21 (80.8)	0.03
No. of prior regimens	2 (0–6)	2 (0–6)	0.65
Response to gefitinib			
Yes	7 (87.5)	3 (12.5)	0.0003
No	1 (12.5)	21 (87.5)	
NE		2	
Skin toxicity			0.47
None	2 (25)	12 (46.2)	
G1	2 (25)	7 (26.9)	
G2	4 (50)	6 (23.1)	
G3	0 (0)	1 (3.8)	
Duration of gefitinib, weeks (range)	31.2 (4–62.4)	11.2 (0.8–119.8)	0.35

NE, non-evaluable; CR, complete response; PR, partial response; SD, stable disease.
[a] Adenocarcinomas with EGFR mutations compared to adenocarcinomas with wild-type EGFR.

Table 2 Patient characteristics and EGFR mutations for gefitinib responders

Country	Sex	Age[a]	Histology	No. of prior regimens	Smoking status	Duration of therapy	Overall survival (months)	EGFR mutations
Japan	F	71	adeno	1	No	5.6	5.6+	719 (GGC → GC)
Japan	F	68	adeno	2	No	17.7	17.7+	No
Japan[b]	F	60	adeno	3	No	3.5	6.9+	718 (CTG → CCG)
Japan	F	53	LCC	3	No	16.9	17.8+	719 (GGC → GCC)
Japan	F	42	adeno	2	No	10.6	10.6+	In-frame deletion (746–750)
Japan	M	50	adeno	0	No	10.4	10.4+	In-frame deletion (747–751) insertion of A
Japan	M	76	adeno	3	Yes	2.2	2.2+	No
Japan	M	52	adeno	1	Yes	1.1	1.1+	In-frame deletion (745–750)
Japan	F	54	adeno	2	No	15.6	18.7	In-frame deletion (746–750)
Spain	M	63	adeno	3	Yes	10.1	10.1+	In-frame deletion (746–751) insertion of F

[a] Age at start of gefitinib treatment.
[b] This patient has no measurable disease and suffered an increase of CEA levels adeno, adenocarcinoma; LCC, large cell carcinoma.

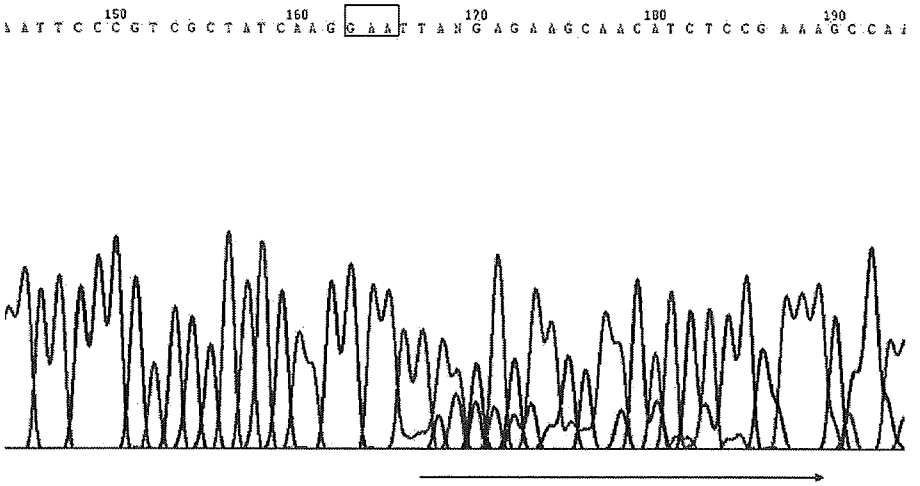

Fig. 1 Partial electropherogram after direct DNA sequencing of PCR products for heterozygous deletion at codons 747–751. Loss of amino acids L R E A T of exon 19 is indicated by the arrow.

homozygous in-frame deletion (745–750), and one was a heterozygous in-frame deletion (747–751) and insertion of a phenylalanine residue. The only Spanish gefitinib responder also harbored a heterozygous in-frame deletion (746–751) and insertion of alanine residue. Three additional tumors had amino acid substitutions within exon 18, at codons 718 (Fig. 2) and 719 (Fig. 3).

Table 3 shows the adjusted odds ratio for the joint effect on response of different variates and covariates. Response was primarily linked to the presence of EGFR mutations. The odds ratio was slightly lower in males than in females and increased 23-fold in non-smokers. In addition, patients who had received more prior chemotherapy lines showed a 11-fold increased probability of response. Other variates are shown in Table 3.

Median survival for all patients, calculated from the start of gefitinib treatment, was 14.1 months (95% CI, 7.8–20.5). Median survival was 15.6 months (95% CI, 0–38.1) for the Japanese patients

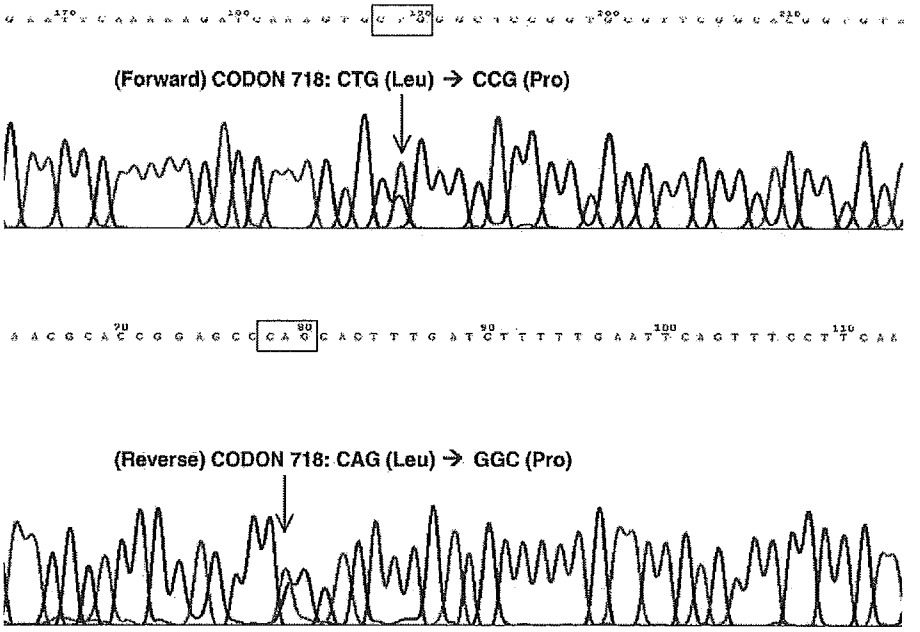

Fig. 2 Partial electropherogram after direct and reverse DNA sequencing of PCR products for a heterozygous missense mutation (T to C) at codon 718 of exon 18 yielding a leucine (Leu) to proline (Pro) amino acid change.

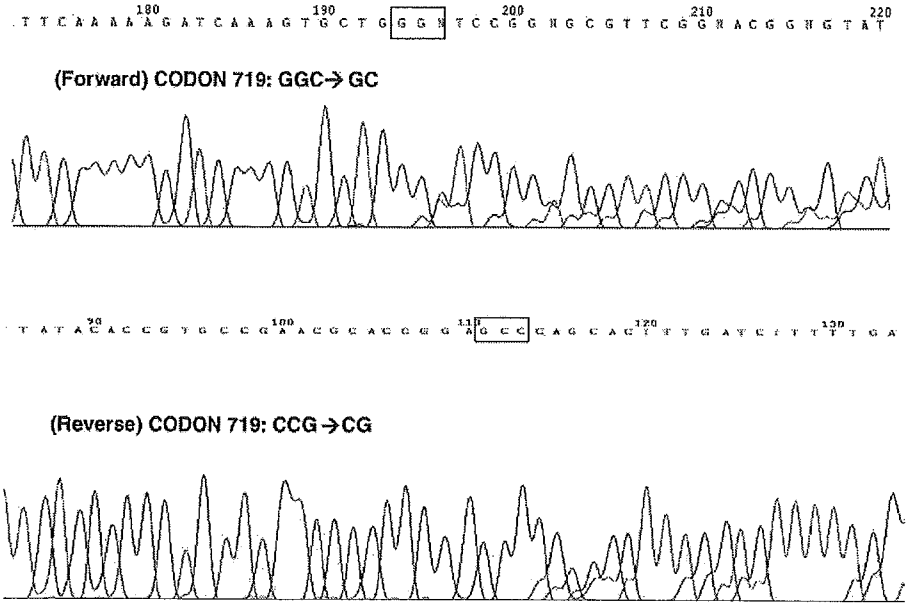

Fig. 3 Partial electropherogram after direct and reverse DNA sequencing of PCR products showing a nucleotide deletion (G) at codon 719 that disrupts the wild-type reading frame of exon 18.

Table 3 Adjusted odds ratios (OR) for the joint effect on response of different covariates

Variates/covariates	OR (95% CI)	*P*
EGFR mutations	7 (1.1–56)	0.05
EGFR mutations by sex (male)	6.6 (1.3–33.3)	0.02
EGFR mutations by smoking status (non-smoker)	23.3 (3.1–166.7)	0.002
EGFR mutations by no. prior chemotherapy regimens	11.6 (2.12–62.5)	0.005
EGFR mutations by ethnicity	4 (1.05–15.6)	0.04
EGFR mutations by age	7 (4.8–1000)	0.002
Sex (male)	0.23 (0.08–0.69)	0.009
Smoking status (smoker)	0.15 (0.04–0.5)	0.002
No. of prior chemotherapy regimens	0.7 (0.5–0.9)	0.04
Ethnicity (Asian)	7 (1.7–100)	0.01
Age	1.03 (0.9–1.1)	0.07

Odds ratio was calculated according to the logistic regression model without constant.

Table 4 Patient characteristics of 12 Spanish patients with stable disease after gefitinib treatment

Sex	Age[a]	Histology	No. of prior regimens	Smoking status	Duration of therapy	Overall survival (months)	EGFR mutations
M	62	adeno	3	Yes	3.2	3.2+	No
F	42	adeno	2	Yes	2.5	2.5+	No
M	52	adeno	3	Yes	20.4	20.4+	No
M	40	LCC	3	Yes	19.1	19.1	No
F	51	adeno	1	Yes	12.8	23.7+	No
M	49	adeno	2	Yes	27.6	27.6	No
M	50	adeno	2	Yes	14.1	14.1	No
M	70	LCC	2	Yes	11.3	11.3	No
M	56	adeno	5	Yes	9.6	11.6	No
M	52	SCC	2	Yes	6.9	18.9	No
M	65	adeno	2	Yes	1.4	2.1	No
M	56	adeno	6	Yes	6.0	7.6	W731Stop

[a] Age at start of gefitinib treatment adeno, adenocarcinoma; LCC, large cell carcinoma; SCC, squamous cell carcinoma.

Table 5 EGFR mutations according to patient characteristics in Japanese patients

	EGFR mutation	Wild-type EGFR	*P*
Age, years			
≤60	6 (66.7)	3 (33.3)	0.05
>60	1 (12.5)	7 (87.5)	
Sex			
Male	2 (25)	6 (75)	0.33
Female	5 (44.4)	4 (55.6)	
Histology			
Adenocarcinoma	6 (40)	9 (60)	0.9[a]
Large cell carcinoma	1 (100)	0 (0)	
Squamous cell carcinoma	0 (0)	1 (100)	
Smoking status			
Smoker	1 (12.5)	7 (87.5)	0.05
Non-smoker	6 (66.7)	3 (33.3)	

[a] Adenocarcinomas with EGFR mutations compared to adenocarcinomas with wild-type EGFR.

and 11.3 months (95% CI, 5.4–17.2) for the Spanish patients ($P = 0.8$). Median survival for responders has not been reached, while for non-responders, it is 6.9 months (95% CI, 0–13.9). Twelve Spanish patients had stable disease after gefitinib treatment (Table 4). Median survival for these patients was 14.1 months (95% CI, 7.9–20.4).

Since almost 90% of mutations were in the Japanese patients, further analyses were performed on this group. EGFR mutations were found in 62% in Japanese female non-smokers with adenocarcinoma. In Japanese patients, mutations were more frequently observed in patients younger than 60 years ($P = 0.05$), in non-smokers ($P = 0.05$), in women and in adenocarcinoma (Table 5). Median survival for the seven Japanese patients with EGFR mutations was 15.6 months (95% CI, 0–53) and for the 10 Japanese patients with wild-type EGFR, it was 2.3 months ($P = 0.04$) (Fig. 4).

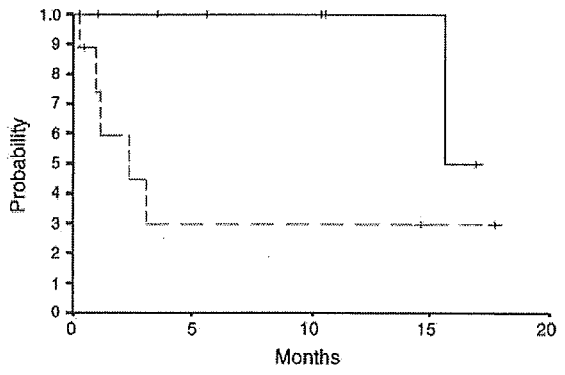

Fig. 4 Survival in Japanese patients according to the presence of EGFR mutations. The solid line represents the seven patients with mutations, and the broken line represents the 10 patients with wild-type EGFR.

4. Discussion

In the present study, we have observed that EGFR mutations are a strong predictor of gefitinib response in chemoresistant NSCLC patients. Seven of eight patients (87.5%) with EGFR mutations attained an objective response, in contrast with only three of 24 patients (12.5%) with wild-type EGFR ($P < 0.0003$). These results mirror accumulated data from three studies [10,11,14], in which 25 of 31 (81%) NSCLC patients with EGFR mutations attained objective response, while none of 29 non-responders had mutations. In addition, recent work [15] has demonstrated that NSCLC cell lines containing EGFR mutations are chemoresistant but highly sensitive to gefitinib. In our study, an increased number of prior chemotherapy regimens reduced the chances of response to gefitinib in general but increased chances of response in the presence of EGFR mutations.

To date, it has not been clearly demonstrated that gene mutations in general can be predictive markers of response. For example, K-ras gene mutations in cell lines derived from NSCLC patients were associated with shorter survival when treated with chemotherapy [16], but this was not confirmed in studies where K-ras mutations were analyzed in archival pathology blocks [17]. In human tumor cell lines, the presence of ras mutations enhances gemcitabine sensitivity in comparison to tumor cells with wild-type ras alleles [18]. A single nucleotide

polymorphism in exon 4 (arginine allele), together with wild-type p53, has been associated with favorable response to chemoradiotherapy of squamous cell carcinoma [19].

It seems that in non-smokers and some adenocarcinoma patients, the EGFR signaling pathway can be selectively activated, as has been observed in gefitinib-sensitive human NSCLC cell lines, like PC9 [20] and others harboring EGFR mutations [10,11]. Homo- and/or heterodimerization of EGFR activates several intracellular signal transducing elements, such as phospholipase Cγ, PI3K, protein kinase B/AKT (Akt), a small G-protein (Ras), the Ras GTPase-activating protein extracellular signal-regulated kinase (ERK) 1/2, Src family kinases, and signal transducers and activators of transcription (STATs). Experimentally, tobacco carcinogen exposure (NNK) has been shown to promote the activation of PI3K/Akt pathway [21], and it is thus plausible that the PI3K signaling pathway is activated in heavy smokers through the stimulation of other TKs [22]. In MDA-468 human breast cancer cells overexpressing EGFR, the concomitant amplification of the PI3K/Akt pathway as a consequence of loss of PTEN increased Akt activity and led to resistance to EGFR TK inhibitors; the re-introduction of PTEN restored sensitivity to EGFR TK inhibitors [23].

Although these results may be biased due to the small number of patients studied and because they were selected on the basis of availability of tumor tissue, they are in line with those reported [10,11,14]. The discovery of EGFR mutations in the TK domain that predict response to gefitinib is a breakthrough in the implementation of predictive markers for selecting treatment. Screening for EGFR mutations may be particularly useful in non-smokers, patients younger than 60 years, females and patients with adenocarcinoma, especially Japanese patients. The low response rate to gefitinib observed in Spanish patients may be explained by the low frequency of EGFR mutation in these patients. However, stable disease was observed in a meaningful number of gefitinib-treated Spanish patients, resulting in a median survival that was close to that of Japanese responders. Further research should be carried out in Caucasians to elucidate the predictive markers for the significant number of patients who can obtain a clinical benefit from gefitinib. A polymorphic CA repeat located at the 5′-regulatory sequence in intron 1 of the EGFR gene has been associated with its transcriptional activity with interethnic differences. Shorter CA repeats are associated with higher transcription activity and are observed more frequently in Caucasian than in Japanese patients [24]. In conclusion, previous studies [10,11,14] and our present study concur that the presence of EGFR mutations is a major determinant of gefitinib response. In future clinical trials, therefore, EGFR TK inhibitors should be considered in preference to chemotherapy as first-line treatment in lung adenocarcinomas harboring EGFR mutations.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.lungcan.2005.05.017.

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