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Multimodal treatment for resectable epithelial type malignant pleural mesothelioma

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Abstract

Background: Malignant pleural mesothelioma is a rare malignancy. The outcome remains poor despite complete surgical resection.

Patients and methods: Eleven patients with histologicaly proven epithelial type malignant pleural mesothelioma undergoing extrapleural pneumonectomy with systemic chemotherapy and/or radiotherapy before and after surgical resection were retrospectively reviewed.

Results: Ten out of 11 patients underwent complete surgical resection, of these 7 patients had stage I disease. Of these 7 patients, 5 are alive without any recurrence, a 2-year survival rate of 80% was observed in this group. There was no operative mortality or morbidity.

Conclusion: Extrapleural pneumonectomy with perioperative adjuvant treatment is safe and effective procedure for epithelial type malignant pleural mesothelioma.

Introduction

Malignant pleural mesothelioma (MPM) is a relatively rare entity among intrathoracic malignancies, as compared with lung cancer, although its prevalence has shown an increase in recent years [1]. Extrapleural pneumonectomy (EPP) is the surgical treatment of choice for MPM that do not extend in to the mediastinum or on to the chest wall, although its survival benefit is still not clear [2]. In a retrospective study of 189 Japanese cases [3], there were no significant differences in survival at 2-years between palliative surgery, such as decortication, and EPP (26% and 30%, respectively). EPP with adjuvant chemotherapy and/or radiotherapy has been reported to be effective against the MPM in its early stages [4,5]. Jaklitsch *et al.*, [6] advocated that EPP plus postoperative chemotherapy using paclitaxel and carboplatin with radiotherapy is effective for MPM if it's of epithelial histology, negative surgical margin, and if extrapleural lymph nodes are negative for metastasis. This study reports on resectable

Table 1: Clinical summary

Case	IMIG stage	BWH stage	Resection	Adjuvant therapy	Recurrence	Survival status/months
1 49 F	III(T3N2M0)	III	Complete	Pre CDDP/Hemithorax RTx	Pericardium	Died 22
2 63 M	III(T3N2M0)	III	Complete	Pre CDDP/Hemithorax RTx	Ipsilateral thorax	Died 30
3 61 M	III(T3N2M0)	III	Incomplete	Pre CDDP/Hemithorax RTx	Ipsilateral thorax	Died 1
4 59 F	III(T1N2M0)	III	Complete	Post CDDP/GEM/UFT	Lung	Dead 28
5 58 M	II(T2N0M0)	I	Complete	Pre CDDP/Hemithorax RTx	None	Alive 28
6 50 M	II(T2N0M0)	I	Complete	Pre CDDP/GEM/VNR	Ipsilateral thorax	Alive 35
7 55 M	II(T2N0M0)	I	Complete	Pre CDDP/GEM/VNR	Pericardium	Died 6
8 66 M	III(T3N0M0)	I	Complete	Pre CDDP/GEM/VNR	None	Alive 15
9 48 M	III(T3N0M0)	I	Complete	Post CDDP/GEM/UFT	None	Alive 32
10 57 M	I(T1N0M0)	I	Complete	Post CDDP/GEM/UFT	None	Alive 39
12 58 M	I(T1N0M0)	I	Complete	Pre CDDP/GEM/VNR	None	Alive 12

IMIG – International mesothelioma Interest group; BWH – Brigham and Women's Hospital; F – Female; M – male; Pre – Pre Operative; Post – Post operativ; CDDP – Cisplatin; RTx – Radiotherapy; GEM – gemcitabine; VNR – Vinorelbine; UFT – Uracil/tegafur

epithelial type MPM with perioperative treatment consisting of radiation and/or chemotherapy.

Patients and methods

Between 1995 and 2002, 10 patients with epithelial type MPM underwent EPP with postoperative or preoperative chemotherapy and/or radiotherapy. The clinical profiles of these patients are detailed in Table 1. The lesions were staged by computed tomographic (CT) scan using International Mesothelioma Interest Group (IMIG) classification. There were 6 stage III, 3 stage II and 1 stage I patient. A bone scan and Magnetic Resonance Imaging (MRI) of the brain were performed if metastasis was suspected. Using the Brigham and Womens Hospital (BWH) staging system of Sugarbaker *et al.* [4], after the surgical resection 6 patients were stage I, in terms of having completely resected primary tumors including chest wall invasion at the biopsy site.

A standard EPP was performed as described earlier [7]. Following a posterolateral incision, extrapleural space was entered from the 5th or 6th rib bed, and dissection was carried superiorly toward the apex, antero- and postero-laterally, and inferiorly toward the diaphragm. During the dissection, port site disease at the chest wall was resected *en block*. Following an antero-medial pericardiotomy, hilar vessels were resected using a mechanical stapler, followed by resection of the main bronchus. The diaphragm was divided from the peritoneum, and EPP was completed. The defects of pericardium and diaphragm were reconstructed with prosthetic patches. A complete mediastinal lymph node dissection was performed in all cases.

In a preoperative adjuvant setting, one course of concurrent chemoradiotherapy using cisplatin (CDDP) (80 mg/m^2, on days 1 and 29) with 40 Gy external beam radiotherapy to the hemithorax [5], was performed in 4 patients (Case 1, 2, 3 and 5) and 2 or 3 courses of chemotherapy using CDDP (40 mg/m^2 on days 1 and 8), gemcitabine (GEM) (800 mg/m^2, on days 1 and 8), and vinorelbine (VNR) (20 mg/m^2, on days 1 and 8) were given at intervals of 3 to 4 weeks in 4 patients (Case 6, 7, 8 and 11) (Table 1). Three patients received 2 courses of postoperative chemotherapy using CDDP (80 mg/m^2, on day 1 and 8), GEM (800 mg/m^2 on days 8 and 15) and UFT (tegafur/uracil) (400 mg/m^2 postoperative on days 1–15) with 3 to 4 weeks interval (Case 4, 9 and 10) (Table 1). One patient received 50 Gy postoperative radiation to the previous thoracic drainage site (Case 10).

Results

Postoperative course of the patients were uneventful, and no morbidity or mortality was experienced. Six patients experienced a relapse in the thorax. One patient underwent resection of the chest wall for recurrence at 12 months after EPP. Other 5 patients (Case 5, 6, 8, 9 and 11) are surviving without any disease. All the survivors had BWH stage I disease, which showed an 80%, 2-year survival. The survival in 4 patients with BWH stage II-III disease was 37% at 2-year. Postoperative chemotherapy was started 2 to 3 months after surgery, and grade 4 neutropenia was observed in all 3 cases, while grade 3 loss of appetite was observed in one. Of the 3 patients who underwent preoperative chemotherapy, a reduction in size of the tumors by 18 to 74% was seen following chemotherapy (Figure 1). Pathological examination of the resected specimens in all 3 cases showed extensive fibrosis with only a small focus of tumor cells (Figure 2). In Case 10, an exploratory thoracotomy was done for suspected recurrence, however, the intrathoracic lesion was found to be a herniated liver from the defect of the reconstructed diaphragm.

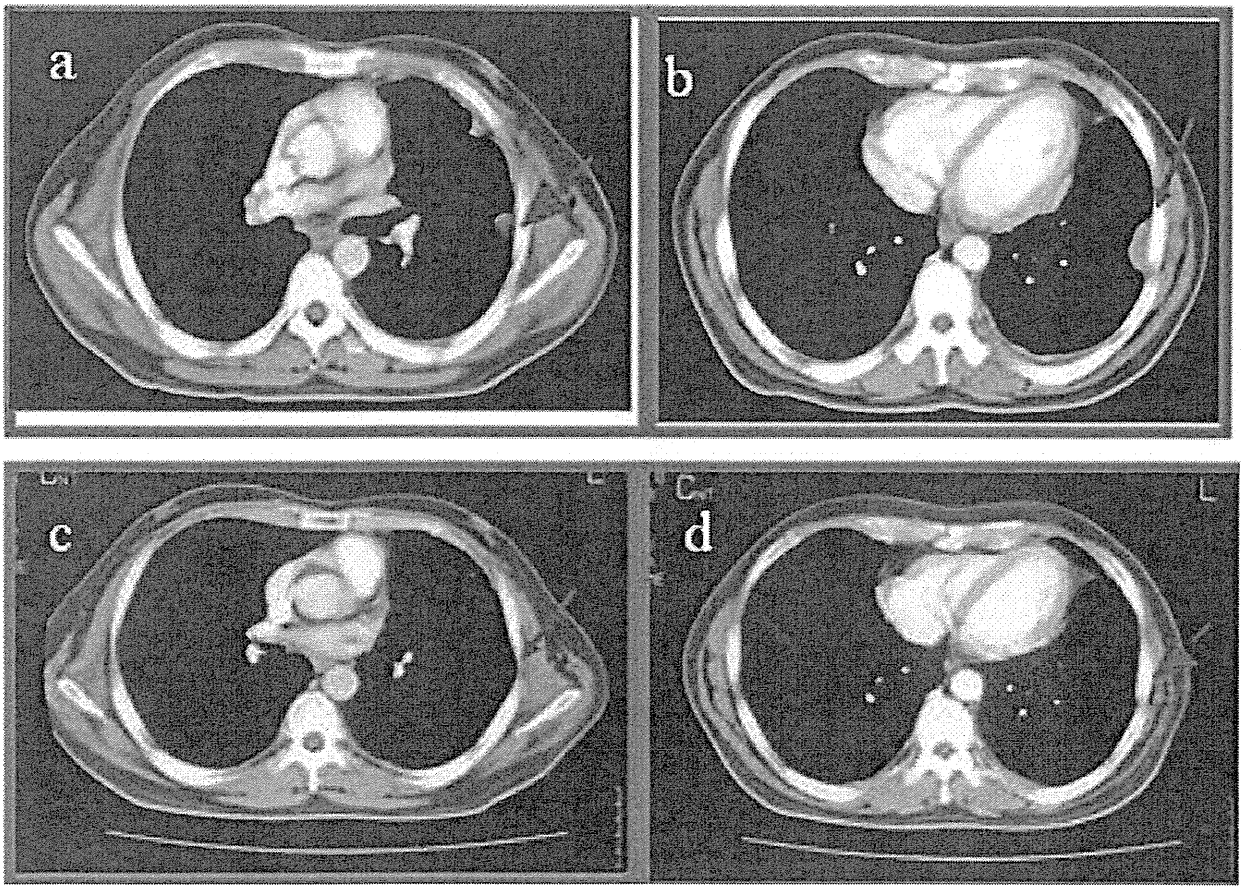

Figure 1
The effect of preoperative chemotherapy using CDDP/GEM/VNR in Case 5: Chest CT before (a, b) and after chemotherapy (c, d). The size of the primary tumors, measured two-dimensionally (arrows) decreased by 74% after chemotherapy.

Discussion

Early stage MPM, especially of the epithelial type, is a disease localized to the hemithorax. Therefore, EPP with or without perioperative adjuvant therapy should be effective, as is shown previously. Sugarbaker *et al.*, [4] reported that the treatment with EPP and adjuvant chemotherapy and hemithorax radiotherapy is effective for select patients with MPM. Nearly 50% of the cases who undergo complete resection of epithelial type MPM survive at 5 years. Rusch *et al.*, [5] showed favorable results with EPP followed by radiation. Survival rate at 5-years for patients with stage I/II IMIG classification was 40% [5].

The aim of the perioperative adjuvant therapy is to control tumor cells located at the front line and the lymphatic system and to sterilize the margin of EPP. However, a therapeutically active modality must be considered from the standpoint of patient benefit and safety. The mortality rates for EPP reported in literature are 3.8% by Sugarbaker *et al.*, [4] and 7.9% by Rusch *et al.*, [5]. In our series, all patients returned to active social life following their treatment, indicating that EPP with perioperative adjuvant therapy is well tolerated. Complete resection of capsulated MPM was achieved in 6 cases that had been designated as BWH stage I. Interestingly, as shown in table 1, BWH stage predicted the prognosis well however IMIG stage failed to do so. This indicated that local therapy for epithelial type MPM might be crucial for staging and prognosis as well.

Sugarbaker *et al.*, [4] started chemotherapy using carboplatin and paclitaxel within 4 weeks after EPP. In our series, chemotherapy was started 2 months after EPP in 4 patients who received CDDP/GEM/UFT. Of the 3 patients

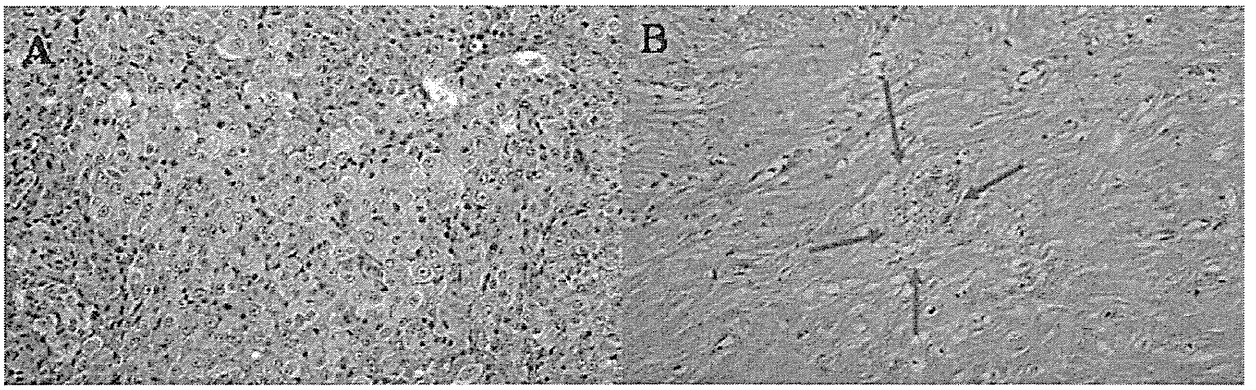

Figure 2
Photomicrograph of case number 5 showing the effect of preoperative chemotherapy. A). Histology of pretreatment biopsy and resected specimen in the above case (× 400, hematoxylin and eosin) B.) Only a small focus of epithelial type mesothelioma cells was found in the resected specimen.

receiving preoperative chemotherapy using CDDP/GEM/VNR, 2 patients received 2 courses and the other received 3 courses. EPP was performed within 5 weeks after cessation of chemotherapy. The clinical and pathological effects were remarkable. Among chemotherapeutic agents, GEM [8] and VNR [9] are reported to be active and the combination of them with CDDP was used in our study. Neutropenia was the main adverse effect of this regimen observed, which reversed with G-CSF. We therefore suggest that preoperative chemotherapy using such active agents followed by EPP is effective and safe procedure. However, this needs to be tested in a randomized controlled trial.

Competing interests
None delcared.

Authors' contributions
IY. Conceived of the study, participated in its design and coordination and drafted the manuscript.

MY. Carried out the literature search and helped in drafting the manuscript.

TO. Participated in the data retrieval and analysis also helped in literature search.

CU. Participated in the design of the study and helped in drafting the manuscript.

YI. Shape the idea for the study, coordinated the study and helped in editing the manuscript.

YM. Helped to shape the idea for the study, coordinated the study and edited the manuscript.

All authors read and approved the final manuscript.

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Clinical Patterns and Treatment Outcome of Elderly Patients in Clinical Stage IB/II Non-Small Cell Lung Cancer

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Background and Objectives: Surgery is a standard treatment in patients with clinical stage IB/II non-small cell lung cancer (NSCLC). We often have difficulty in treating of elderly patients due to their insufficient physiological function. To better manage such elderly patients, the clinical characteristics and prognosis of patients with these stages, who were 75 years of age or older, were reviewed.
Methods: From 1972 to 1999, 112 elderly patients with these stages were treated in our department. These patients comprised 88 men and 24 women. The histological types were 50 adenocarcinomas, 51 squamous cell carcinomas, 8 large cell carcinomas, and 3 adenosquamous carcinomas.
Results: Seventy-four patients (66%) underwent a surgical resection, including 60 surgery alone, 14 combined modality therapy. Radiotherapy, with or without chemotherapy, was given to 30 patients (27%), and chemotherapy alone to 5 (4.5%). In addition, 3 (2.7%) were given no therapy. The survivals of the surgery group at 2 and 5 years are 53% and 21% and those of the radiotherapy group are 35% and 3%, respectively. A multivariate analysis in radiotherapy group shows the predominant prognostic factor to be adenocarcinoma. The 2-year survival of the radiotherapy group in patients with adenocarcinoma is 58%, while that of patients with squamous cell carcinoma is 22%.
Conclusions: These above observations suggest that radiotherapy is an alternative strategy for patients who cannot undergo surgery, especially with adenocarcinoma.
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KEY WORDS: **non-small cell lung cancer; elderly; surgery; radiotherapy**

INTRODUCTION

Lung cancer is the leading cause of cancer deaths in Japan. The elderly population is growing rapidly, and thoracic surgeons and medical oncologists have an increasing chance to treat elderly patients with lung cancer. Surgical resection remains the primary modality for treatment of lung cancer [1], and recent studies have demonstrated that surgery for the elderly patients is still the most effective therapy for stage I/II non-small cell lung cancer (NSCLC) [2,3]. However, elderly patients tend to have lower cardiopulmonary functions and more co-morbidities, and therefore have a higher risk of surgery [4,5]. The careful selection of the candidates for operation is thus necessary for this group of patients. Recently, video-assisted thoracic surgery and limited resection is getting more popular and known as a useful technique for those patients of high risk in early stage NSCLC [6]. However, in clinical stage IB or II, a surgical resection tends to require more extended methods.

The 5-year survival of resected patients in clinical stage IA reaches 70%, however, that in stage IB/II is approximately 35–45% [1]. Although the long-term survival of the patients treated with surgical procedures

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has been reported to possibly not be influenced by the patients' age [2], it is often difficult for thoracic surgeons and medical oncologists in practice to determine which treatment modality should be performed for elderly patients with stage IB/II cancer, especially in patients 75 years of age or older. In the present study, we review the initial treatment patterns and outcomes of all the patients who are 75 years of age and older in clinical stage IB, IIA, IIB who were admitted to our department, in order to better manage such patients.

PATIENTS AND METHODS

Between January 1972 and December 1999, 2799 non-small lung cancer patients were admitted to the Department of Thoracic Oncology, National Kyushu Cancer Center. Among them, 390 patients (13.9%) were of 75 years of age or older, and about one-fourth of these patients had clinical stage IB and II disease (112 patients). These patients comprised of 88 men and 24 women. The histological types were 50 adenocarcinomas, 51 squamous cell carcinomas, 8 large cell carcinomas, and 3 adenosquamous carcinomas. The histological analysis of the tumor was based on the WHO classification for cell types [7]. The clinical stage of these patients was determined based on the TNM classification of the Union Internationale Contre Cancer (UICC) [8]. For TNM staging, almost all patients after 1980 underwent a computed tomography (CT) scan of the thorax and upper part of abdomen, and a bone scintigram. A brain CT or MRI was not mandatory unless neurologic symptoms were present. Before 1980, the majority of patients underwent chest roentgenography and conventional tomography and radionuclide scanning of the bone, brain, and liver. Statistical significance was evaluated using the χ^2 test, Fisher's extract test, or the Mann–Whitney test for various clinicopathologic factors. A survival analysis for each categorical variable on overall survival was estimated according to the Kaplan–Meier method. The terminal event was death due to any cause. The statistical significance of the differences between survival curves was evaluated by the log-rank test. The Cox proportional hazards model was applied to the univariate survival analysis. In the multivariate survival analysis, any variables with a *P*-value of less than 0.2 were further analyzed in a stepwise manner. Statistical difference was considered to be significant if the *P*-value was below 0.05.

RESULTS

Treatments

The details of the treatment of the elderly 112 patients are shown in Table I. A surgical resection is the first choice, and if it seems hard to undergo surgery because of low cardiopulmonary functions or poor performance status, we next choose radiotherapy either with or without chemotherapy. Seventy-four patients underwent surgery; 60 patients underwent surgery alone, 14 patients had combined modality therapy. Sixty-eight patients (91.9%) underwent standard lobectomy or more extended treatment, including three sleeve resection of main bronchus. Six patients (8.1%) had a limited resection. A complete resection was done for 72 patients (97.3%); 2 patients did not undergo mediastinal lymph nodes dissection. One patient underwent preoperative chemotherapy, and 6 were given postoperative chemotherapy. One patient had pre- and postoperative radiotherapy, 1 had postoperative radiotherapy alone, 4 had postoperative radiotherapy and chemotherapy, and 1 had preoperative radiotherapy and postoperative chemotherapy. Thirty-eight patients did not undergo surgery. Radiotherapy, with or without chemotherapy, was given for 30 patients, and chemotherapy alone for 5. Three were given no therapy. The reason why patients did not receive a surgical resection was as follows; a poor pulmonary function in 16 patients, a poor performance status in 5, heart disease in 3, avoidance of a pneumonectomy in 2, extended disease at exploratory surgery in 2, chronic renal failure in 1, and double lung cancer in 1. Eight patients refused undergoing surgery on their own volition. In radical radiotherapy, one patient received a total dose of 40 gray, 11 did 50 gray, 17 did 60 gray, and 1 did 80 Gy. In chemotherapy alone, 2 patients received the doublet combination of cisplatin and etoposide, 1 received the triplet regimen of cyclophosphamide, adriamycin, cisplatin (CAP), 1 received CAP plus mitomycin, 1 received adriamycin alone.

We divided these patients into two categories consisting of a surgery group who underwent surgical resection and radiotherapy group who received radiotherapy with or without chemotherapy. The patient characteristics of the two groups were summarized in Table II. There were

TABLE I. Details of the Treatment

	No.	Percent (%)
Surgery		
Total	74	66
Surgery alone	60	53.6
With chemotherapy	7	6.3
With radiotherapy	2	1.8
With chemotherapy and radiotherapy	5	4.5
Radiotherapy		
Total	30	26.8
Radiotherapy alone	20	17.9
With chemotherapy	10	8.9
Chemotherapy alone	5	4.5
No treatment	3	2.7

TABLE II. Comparison of Clinicopathological Characteristics Between the Surgery and Radiotherapy Groups

Category	S (n = 74) N	RT (n = 30) N	*P*
Age (75 ~ 79, 80 ~ 84, 85~)	58, 13, 3	18, 10, 2	0.147
Gender (Male, female)	55, 19	26, 4	0.169
Histologic type (Ad, Sq, La, Ad-sq)	36, 30, 6, 2	12, 18, 0, 0	0.863
Clinical stage (IB, IIA, IIB)	48, 2, 24	10, 1, 19	0.01
PS (0, 1, 2, 3)	35, 38, 1, 0	1, 23, 5, 1	<0.0001
FEV1/BSA (ml/m^2)	1291 ± 331	989 ± 339	<0.0001

PS, performance status; FEV1, forced expiratory volume in 1 sec; BSA, body surface area; Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Ad-sq, adenosquamous carcinoma; S, surgery group; RT, radiotherapy group; N, number; *P*, *P*-value.

TABLE III. Comparison of Clinicopathological Characteristics Between the Adenocarcinoma and Squamous Cell Carcinoma Patients in the Radiotherapy Groups

Category	Ad (n = 12) N	Sq (n = 18) N	*P*
Age (75 ~ 79, 80 ~ 84, 85~)	6, 4, 2	12, 6, 0	0.310
Gender (Male, female)	8, 4	18, 0	0.0085
Clinical stage (IB, IIA, IIB)	4, 1, 7	6, 0, 12	0.800
PS (0, 1, 2, 3)	0, 9, 3, 0	1, 14, 2, 1	0.612
Chemotherapy (+, −)	7, 5	3, 15	0.017
FEV1/BSA (ml/m^2)	1011 ± 355	975 ± 338	0.787

PS, performance status; FEV1, forced expiratory volume in 1 sec; BSA, body surface area; Ad, adenocarcinoma; Sq, squamous cell carcinoma; N, number; *P*, *P*-value.

no significant differences in the gender and histological type. In contrast, there were significant differences in the clinical stage, performance status, and pretreatment forced expiratory volume in 1 sec (FEV 1.0) per body surface area. Radiotherapy tended to be chosen more frequently in the patients with a poor risk, and with advanced disease. Surgery related deaths (death within 30 days after surgery) were found in 2 patients and the mortality rate was 2.7%. Major complications, which required artificial ventilation and the intensive care, were seen in 4 patients (5.4%) and minor complications were seen in 26 patients (35.1%).

Survival

The overall survivals of the two treatment groups are shown in Figure 1. The survivals of the surgery group at 2 and 5 years are 53% and 21% and those of the radiotherapy group are 35% and 3%, respectively. The median survivals times (MST) of both groups were 25 and 15 months. Table III shows a comparison of clinicopathological characteristics between the adenocarcinoma patients and the squamous cell carcinoma patients in the radiotherapy group. There was a significant difference in gender and the use of chemotherapy between the two categories. A univariate survival analysis indicated that adenocarcinoma was a prognostic factor in the radiotherapy group (Table IV); a multivariate analysis using a stepwise method also confirmed that adenocarcinoma was an independent prognostic factor among them Table V.

The survival curve of the radiation group is similar to that of the surgery group until 25 months in adenocarcinoma cases, but it began to decrease after that (Fig. 2A). In contrast, the same tendency was not seen in the squamous cell carcinoma cases (Fig. 2B).

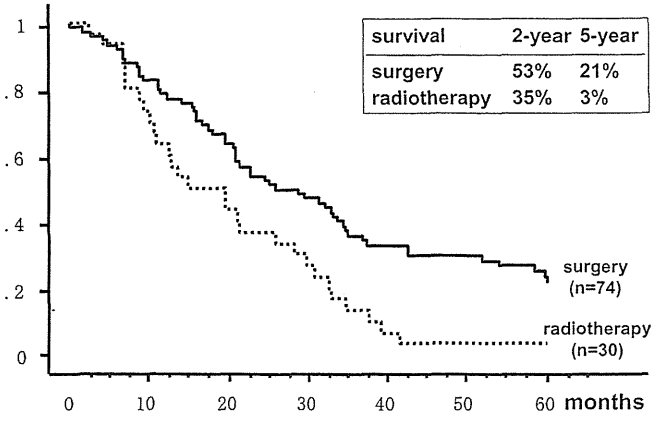

Fig. 1. The overall survival curves of surgery and radiotherapy groups.

DISCUSSION

The proportion of elderly individuals among lung cancer patients is increasing as a consequence of the aging population and the increased life expectancy in Japan. Recent studies demonstrated that surgery is the most effective therapy for early stage NSCLC for the elderly patients [2,9,10]. However, they have a tendency to have more underlying co-morbidities, compared with younger patients. We focused on the elderly population of clinical stage IB and II, and investigated the clinical patterns and treatment outcome, because more extended resection is sometimes required for these cases than those in clinical stage IA, and the treatment choice is often an extremely difficult choice for thoracic surgeons or oncologists.

TABLE IV. Univariate Analysis of Various Prognostic Factors Influencing Survival of Patients Treated With Radiotherapy

		Radiotherapy group			
Variable	Category	N	RR	95% CI	*P*
Age	75～79	18	1		
	80～84	10	0.79	0.36–1.74	0.559
	85～	2	0.71	0.16–3.10	0.645
Gender	Male	26	1		
	Female	4	0.36	0.12–1.12	0.078
Histologic type	Ad	12	1		
	Sq	18	3.1	1.32–7.29	0.010
Clinical stage	IB	10	1		
	II	20	1.21	0.55–2.64	0.634
PS	0/1	24	1		
	2/3	6	0.79	0.32–1.97	0.612
Chemotherapy	No	20	1		
	Yes	10	0.63	0.29–1.32	0.254

PS, performance status; Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Ad-sq, adenosquamous carcinoma; N, number; RR, relative risk; CI, confidence interval; *P*, *P*-value.

In our department, almost all patients undergo a stress electrocardiogram and respiratory function test, and the patients with heart disease or lower pulmonary function are checked. Patients who are predicted to have a FEV 1.0 per body surface which is less 600 ml/m^2 after surgery, are not indicated to undergo surgical treatment. Since the most frequent reason for no-surgery therapy was low pulmonary function, a significant difference was seen in the pulmonary functions and performance status between the surgery group and radiotherapy group (Table II). From the surgical point of view, more advanced disease is more likely to be treated without operation. In current studies, a pneumonectomy carries a higher mortality rate, especially on the right side. In our series, a pneumonectmy was done for only 1 elderly patient, and a standard lobectomy or bilobectomy were chosen for more than 90% of all patients in the surgery group. In these surgery patients, the co-morbidities were slight higher than in the younger patients, however, the mortality rate 2.7% was similar to that of other reported statistics [2]. These data demonstrate that standard

TABLE V. Multivariate Analysis of Various Prognostic Factors Influencing Survival of Patients Treated With Radiotherapy

		Radiotherapy group	
Variable	Category	RR (95% CI)	*P*
Histologic type	Ad	1	
	Sq	3.10 (1.32–7.29)	0.0096

Ad, adenocarcinoma; Sq, squamous cell carcinoma; RR, relative risk; CI, confidence interval; *P*, *P*-value.

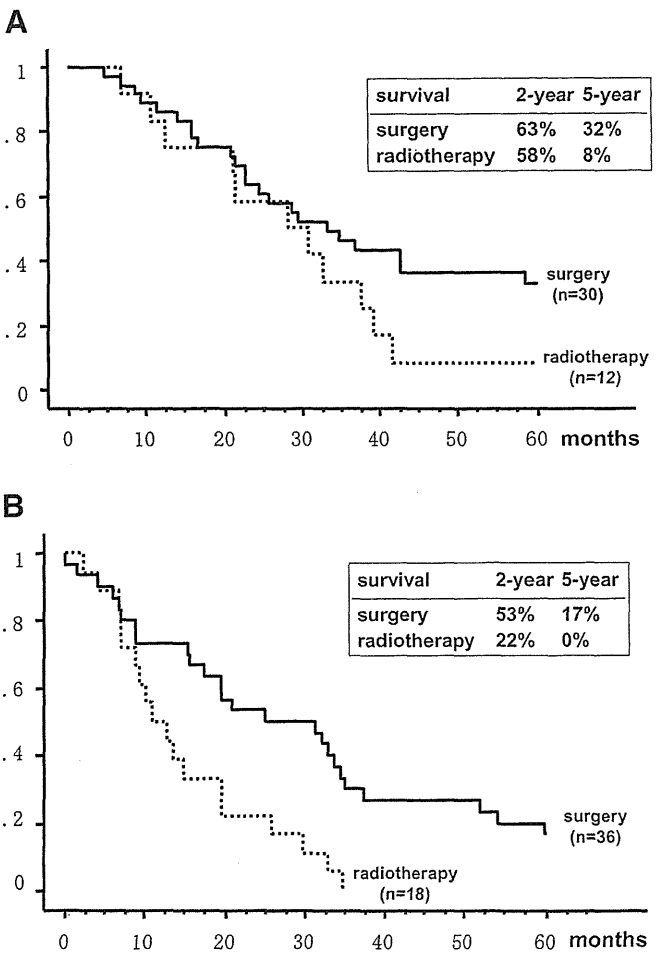

Fig. 2. **A**: Survival curves of the surgery and radiotherapy groups in the adenocarcinoma. **B**: Survival curves of the surgery and radiotherapy groups in the squamous cell carcinoma.

surgery, except for a pneumonectomy, is a safe treatment for elderly patients who are carefully selected.

Although there is no evidence from randomized trials to support the use of radical radiotherapy for stage I/II NSCLC, Many non-randomized trials and retrospective studies demonstrated that radiation treatment in stage I/II has a potential for curative treatment, and it is the common concept for most medical oncologists that patients who do not undergo surgical resection in this clinical stage should receive some type of radical treatment [2,11,12]. Radiation is capable of producing MST of 15–33 months and 5-year survivals of 0–42% for patients in stage I/II. Elderly patients can receive radical radiation treatment as safely as younger persons, and the survival results are the same [2,13]. We also use the radical radiotherapy for those who are considered to be inoperable because of insufficient physical function, or those who refuse surgical therapy. The survival of the patients who received radiotherapy in our department was not so good as those of published data. Regarding the causes, patients with stage IIB (63%) and those with low physical functions were the major population.

Many studies have demonstrated the prognostic factors of radiotherapy for early stages, including radiation dose [14], tumor size [15], nodal status [16]. There is only one study that shows the differences in survival based on the histological subtype, however, squamous cell carcinomas are known to have better results [17]. In our present study, patients with adenocarcinoma had a relatively better survival within 2 years of treatment; on the other hand, patients with squamous cell carcinoma had a worse survival. A multvariate survival analysis indicated that adenocarcinoma was the only prognostic factor in the radiotherapy group. The reason for this is unclear, however, one reason for it might be the difference of the biological future between the two histological types; for example, local proliferation of the squamous cell carcinoma is faster than adenocarcinoma. A report of the long-term survivors of NSCLC after radiotherapy shows that among progression free patients at the end of 2 years, 97% of all patients with squamous cell carcinoma survived without any evidence of disease for more than 5 years [18]. This phenomenon might possibly mean that a failure of sufficient disease control in squamous cell carcinoma results in a poor outcome.

CONCLUSIONS

Based on these data, the following conclusion was suggested. Surgery is still the predominant treatment for elderly patients with early stage lung cancer; however, radiotherapy is another preferable strategy for the patients who are unable to undergo surgery because of either a poor physical function or other reason, especially regarding adenocarcinoma patients.

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Original Article

Necessity of Preoperative Screening for Brain Metastasis in Non-small Cell Lung Cancer Patients without Lymph Node Metastasis

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Background: The exclusion of brain metastasis is important to determine the optimal treatment plan in patients with non-small cell lung cancer (NSCLC). However, a routine examination using magnetic resonance imaging (MRI) for the brain remains controversial in preoperative patients with resectable disease.

Methods: To assess the necessity of routine brain MRI for preoperative patients, a retrospective analysis for a consecutive series of 338 patients with NSCLC was performed. Among the 338 patients, 141 patients who were considered to have potentially resectable diseases through an examination of the chest plus an upper abdominal computed tomography scan and bone radioisotope scan with no neurological symptoms received MRI for examination of brain metastasis.

Results: The incidence of brain metastasis detected by MRI was 2.1% (three of 141) in all patients, 0% (zero of 80) in patients with N0 disease, 5.2% (one of 19) in N1, and 4.7% (two of 42) in N2 cases.

Conclusion: In patients with resectable NSCLC, a brain MRI is not considered to be useful due to the low incidence of asymptomatic brain metastasis. (Ann Thorac Cardiovasc Surg 2004; 10: 347–9)

Key words: non-small cell lung cancer, nodal metastasis, brain metastasis, magnetic resonance imaging

Introduction

The initial staging of non-small cell lung cancer (NSCLC) is essential for determining the appropriate treatment for patients.[1] However, in such patients without any manifestation of distant metastases in a physical examination including a detailed neurologic evaluation and complete blood chemistry study, it remains controversial as to whether or not a full examination should be routinely done. A number of authorities have recommended that an investigation of the most likely sites of metastatic disease such as the bone, brain, liver, and adrenal glands should be restricted to patients with symptoms or signs which suggest metastases.[2-5] Others have, however, suggested that imaging of the adrenal glands, head, bones, and liver, or several combinations thereof should be included in a routine investigation of all patients with lung cancer before a thoracotomy because of the possible existence of silent metastases.[6-12]

Regarding examinations for brain metastases, magnetic resonance imaging (MRI) is reported to have a greater sensitivity and specificity than computed tomography (CT) scans.[13,14] However, we hypothesize that the occurrence of brain metastasis is rare in patients who are considered to have resectable disease. In this retrospective study, we investigated the necessity of performing brain MRI for patients who are considered to have resectable disease based on chest CT, abdominal CT and bone scans as well as a physical examination.

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Patients and Methods

The medical records of all patients who underwent a clinical examination for the staging of NSCLC between April 1996 and December 1998 at National Kyushu Cancer Center, Fukuoka, Japan, were reviewed. The staging protocol during this period consisted of chest CT, radioisotope bone scanning, MRI or CT of the brain, and upper abdominal CT. Among a consecutive series of 338 patients with lung cancer, a total of 280 received brain MRI to evaluate their brain metastases, and the other 58 patients were evaluated by CT. Among the 280 patients, 139 patients were excluded from this study as unresectable cases because of an advanced intrathoracic stage such as T4 (a tumor with either malignant pleural effusion or malignant pericardial effusion, or one that invades either large vessels, the esophagus or vertebral body), cN3 disease, cM1 disease except for brain metastasis, and those considered to have some neurologic symptoms with brain metastasis.

The remaining 141 patients were all considered to have potentially resectable lung cancer, and were subjected to this analysis. The incidence of brain metastasis on MRI was reviewed. They consisted of 98 males and 43 females and their mean age was 63 years (range, 36 to 90 years). Seventy-six patients had T1-2N0, 2 had T3N0, 2 had T4N0, 16 had T1-2N1, 2 had T3N1, 1 had T4N1, 28 had T1-2N2, 12 had T3N2, 2 had T4N2, while 81 were adenocarcinomas (57.4%), 42 were squamous cell carcinomas (29.8%), 9 were small cell carcinomas (6.4%), and 4 were large cell carcinomas (2.8%). All patients had an ECOG performance status ranging from 0 to 2.

Results

The proportion of each TN subset of this cohort is summarized in Table 1. Regarding T3 diseases, seven patients had mediastinal pleural invasion, three chest wall invasion, one pericardium invasion, one direct invasion of another lobe and four invasion of the main bronchus measuring less than 2 cm distal to the carina. All T4 cases had separate nodule (s) in the same lobe of the primary tumor. As for N factor, N0 cases comprised the most common subset (56.7%), followed by N2 (31.0%) and N1 (13.3%).

Three (2.1%) of 141 patients had asymptomatic brain metastasis, one patient in each of the T2N1, T2N2 and T3N2 subsets had asymptomatic brain metastasis and the respective rates were 6.7% (1/15), 5.6% (1/18) and 8.3% (1/12). All three patients had adenocarcinomas. There was no patient whose asymptomatic brain metastasis was detected by MRI among the 76 patients with T1-2N0, four with T3N0-1 and five with T4N0-2.

Table 1. The proportion of evaluated patients and patients with silent brain metastasis as classified according to the presence of T and N factor

	T1	T2	T3	T4
N0	0/37 (0%)	0/39 (0%)	0/2 (0%)	0/2 (0%)
N1	0/1 (0%)	1/15 (6.7%)	0/2 (0%)	0/1 (0%)
N2	0/10 (0%)	1/18 (5.6%)	1/12 (8.3%)	0/2 (0%)

Discussion

The purpose of this study was to determine the value of routine screening for brain metastasis in patients with potentially resectable primary lung cancer. Asymptomatic brain metastasis detected by MRI was observed in three adenocarcinoma patients who had either T2N1, T2N2 or T3N2 disease. There were no patients with brain metastasis who had either a T1 or T4 status while all three patients with the brain metastasis had clinical nodal metastasis. These observations suggested that brain metastasis appeared to be more strongly associated with nodal metastasis than T factor, and with adenocarcinoma.

The present results showed that unless swollen lymph nodes were detected on chest CT, no brain metastasis was detected by MRI. In light of diagnosis for cerebral metastasis, Grant et al.[15] and Bilgin et al.[16] concluded that routine preoperative brain CT can eliminate unnecessary thoracotomies, and The Canadian Lung Oncology Group reported that full investigation including brain CT for all patients including asymptomatic cases may reduce the number of unnecessary thoracotomies.[17] The MRI screening of brain metastasis is more effective for distinguishing asymptomatic brain metastasis than CT.[18] Therefore MRI would eliminate unnecessary thoracotomies.

However, Tanaka et al. reported that routine brain MRI was not recommended due to its cost and its role in increasing the mental duress of the patients, since the examination period is the most stressful time for cancer patients.[19] Concerning the incidence of asymptomatic brain metastasis, Cole et al.[20] advocated that neither MRI nor an enhanced CT scan is indicated for preoperative staging unless some clinical findings are observed. Hochstenbag et al.[21] also reported that the incidence of

brain metastasis was only 3% in NSCLC patients with clinical stage I and II disease before brain MRI screening. In a consensus report of International Association for the Study of Lung Cancer[22] and American Thoracic Society and European Respiratory Society,[23] brain MRI is therefore not considered to be essential for the initial staging of NSCLC.

In conclusion, routine brain MRI is therefore considered to be unnecessary in patients who are considered to have potentially resectable diseases, no distant lesions, and no neurological symptoms.

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Activating Mutations in the Tyrosine Kinase Domain of the Epidermal Growth Factor Receptor Are Associated with Improved Survival in Gefitinib-Treated Chemorefractory Lung Adenocarcinomas

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Abstract **Purpose:** Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) confer a strong sensitivity to gefitinib, a selective tyrosine kinase inhibitor of EGFR.
Experimental Design: We examined *EGFR* mutations at exons 18, 19, and 21 in tumor tissue from 68 gefitinib-treated, chemorefractory, advanced non–small cell lung cancer patients from the United States, Europe, and Asia and in a highly gefitinib-sensitive non–small cell lung cancer cell line and correlated their presence with response and survival. In addition, in a subgroup of 28 patients for whom the remaining tumor tissue was available, we examined the relationship among *EGFR* mutations, CA repeats in intron 1 of *EGFR*, *EGFR* and *caveolin-1* mRNA levels, and increased *EGFR* gene copy numbers.
Results: Seventeen patients had *EGFR* mutations, all of which were in lung adenocarcinomas. Radiographic response was observed in 16 of 17 (94.1%) patients harboring *EGFR* mutations, in contrast with 6 of 51 (12.6%) with wild-type *EGFR* ($P < 0.0001$). Probability of response increased significantly in never smokers, patients receiving a greater number of prior chemotherapy regimens, Asians, and younger patients. Median survival was not reached for patients with *EGFR* mutations and was 9.9 months for those with wild-type *EGFR* ($P = 0.001$). *EGFR* mutations tended to be associated with increased numbers of CA repeats and increased *EGFR* gene copy numbers but not with *EGFR* and *caveolin-1* mRNA overexpression (P = not significant).
Conclusions: The presence of *EGFR* mutations is a major determinant of gefitinib response, and targeting EGFR should be considered in preference to chemotherapy as first-line treatment in lung adenocarcinomas that have demonstrable *EGFR* mutations.

Platinum-based chemotherapy as first-line treatment in advanced non–small cell lung cancer (NSCLC) yields limited survival benefit. A retrospective analysis of advanced NSCLC patients showed that response rates decreased with each successive chemotherapy regimen: first line, 21%; second line, 16%; third line, 2%; fourth line, 0% (1). Aberrant epidermal growth factor receptor (EGFR) signaling limits sensitivity to anticancer agents, and ligand-independent tyrosine kinase activation of EGFR, often caused by EGFR mutations in the extracellular domain, has been observed in various tumor types, including glioblastoma multiforme (2). Pharmacologic inhibitors of EGFR, such as gefitinib (Iressa), disrupt EGFR activity by binding the ATP pocket within the catalytic domain containing a critical ATP-binding site, Lys^{745} (K745). Gefitinib and related tyrosine kinase inhibitors occasionally yield dramatic and durable "Lazarus responses" (3), yet response rates are variable, with higher rates in patients with adenocarcinoma, female gender, Asian origin, and never-smoker status (4, 5).

The value of EGFR inhibitors as an NSCLC treatment approach has been limited by the lack of reliable methods for predicting which patients are likely to respond. The logical supposition that tumors overexpressing EGFR would respond best to EGFR inhibitors has not been borne out either in preclinical models(6, 7) or in clinical trials (8, 9). However, recent discoveries of *EGFR* mutations in the tyrosine kinase

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domain have shed light on the relationship between EGFR and sensitivity to both gefitinib and the related kinase inhibitor erlotinib. Accumulated data from three studies (10–12) show that 25 of 31 (81%) tumors from NSCLC patients with partial response or marked clinical improvement contained mutations in the *EGFR* tyrosine kinase domain. In contrast, none of 29 specimens from patients refractory to EGFR inhibitors had such mutations ($P < 0.0001$). The mutations included small in-frame deletions (746-750) adjacent to K745 (ELREA amino acids) and missense mutations, mainly L858R adjacent to the DFG motif in the COOH-terminal lobe in the activation loop of the kinase (10–12). These *EGFR* mutations are bona fide somatic mutations in NSCLC and have not been identified in other primary tumor types (10, 13, 14), with the exception of colorectal tumors. One of 293 tumors contained a G719S point mutation (15) that had previously been reported in NSCLC (11), and recently, 4 of 33 tumors harbored point mutations in exons 19 and 20 (16). *In vitro* studies of lung cancer cell lines with endogenous *EGFR* mutations displayed elevated activation of downstream antiapoptotic targets like AKT and signal transducer and activation of transcription (STAT5 and STAT3), conferring enhanced gefitinib sensitivity and increased cisplatin resistance (17).

The transcription activity of the *EGFR* gene is closely related to the enhancer region in intron 1 that is located near a polymorphic CA single sequence repeat containing 14 to 21 CA dinucleotides. Decreased numbers (<19) of CA dinucleotides in this CA sequence correlate with increased *EGFR* transcription (18, 19), and in breast cancer, this CA sequence is a frequent target for *EGFR* gene alterations (20). Moreover, interethnic studies have found that Japanese breast cancer patients carry increased numbers (>19) of CA dinucleotides than Caucasian patients (20). It has been shown that the number of repeats itself affects the mutation rate of nucleotide repeats (21).

A variety of cell surface receptors, including EGFR, as well as intracellular signaling molecules, are concentrated in specialized plasma membrane domains known as caveolae (22). *Caveolin-1* mRNA expression is elevated in multidrug-resistant cultured cancer cells (23), and up-regulation of caveolin-1 and caveolae organelles has been observed in drug-resistant human and ovarian cancer cell lines (24). In addition, high *caveolin-1* mRNA expression has been observed in potentially chemoresistant NSCLC cell lines established from metastatic NSCLCs (25). We therefore hypothesized that tumors harboring EGFR mutations might be associated with higher levels of *caveolin-1* mRNA.

In the present study, we examined *EGFR* mutations in tumor tissue from gefitinib-treated, chemorefractory, advanced NSCLC patients from the United States, Europe, and Asia and in a highly gefitinib-sensitive NSCLC cell line (26) and correlated their presence with response and survival. In addition, in a subgroup of patients for whom remaining tumor tissue was available, we examined the relationship among *EGFR* mutations, number of CA repeats, *EGFR* and *caveolin-1* mRNA levels, and increased *EGFR* gene copy numbers.

Materials and Methods

Patients. Patients with pretreated NSCLC received gefitinib, based on the attending oncologist's decision at the time of chemotherapy failure, at a daily dose of 250 mg given until disease progression. Patients were selected for the present study based on the availability of tumor tissue, without scoring tumor response at the time of selection. Acquisition of tissue specimens and examination of clinical records was approved by the ethics committees of participating institutions. A total of 68 patients were included: 32 Asians (19 Japanese and 13 Chinese) and 36 Caucasians (23 Spanish, nine German, three North American, and one English patient resident in Hong Kong). Assessment of *EGFR* mutations was done for all 68 patients. After this initial analysis, sufficient genomic DNA remained to perform additional related analyses in a subgroup of 28 patients.

Patients were divided into smokers and nonsmokers (having smoked <100 cigarettes in their lifetimes; ref. 27). Tumor response was defined according to the Response Evaluation Criteria in Solid Tumors (28). Survival was calculated from the start of gefitinib treatment. Follow-up was calculated from the start of gefitinib treatment; median follow-up was 11.4 months (range, 1.7-40.3 months).

Epidermal growth factor receptor sequencing. Pure tumor genomic DNA was derived from paraffin-embedded tissue obtained by laser capture microdissection (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 for PCR amplification in nested reactions for exons 18, 19, and 21 of EGFR (Genbank accession no. X00558) were as follows: exon 18 (first PCR, forward 5′-CAAATGAGCTGGCAAGTGCCGTGTC-3′ and reverse 5′-GAGTTTCCCA-AACACTCAGTGAAAC-3′; nested PCR, forward 5′-CAAGTGCCGTGTCC-TGGCACCCAAGC-3′ and reverse 5′-CCAAACACTCAGTGAAACAAAG-AG-3′); exon 19 (first PCR, forward 5′-GCAATATCAGCCTTAGGT GCGGCTC-3′ and reverse 5′-CATAGAAAGTGAACATTTAGGATGTG-3′; nested PCR, forward 5′-GTGCATCGCTGGTAACATCC-3′ and reverse 5′-TGTGGAGATGAGCAGGGTCT-3′); exon 21 (first PCR, forward 5′-CTAA-CGTTCGCCAGCCATAAGTCC-3′ and reverse 5′-GCTGCGAGCT-CACCCAGAATGTCTGG-3′; nested PCR, forward 5′-GCTCAGAGCCTGG-CATGAA-3′ and reverse 5′-CATCCTCCCCTGCATGTGT-3′). Sequencing was done using forward and reverse nested primers with the ABI Prism 3100 DNA Analyzer (Applied Biosystems, Foster City, CA). Electropherograms were analyzed for the presence of mutations using Seqscape v2.1.1 software in combination with Factura to mark heterozygous positions. The human NSCLC cell line (PC9) derived from an adenocarcinoma (Kyushu Cancer Center, Fukuoka, Japan) was also examined using the same methods.

CA repeats in intron 1. In the subgroup of 28 patients, genomic DNA from peripheral blood or adjacent normal lung tissue was used to determine the number of CA repeats in intron 1. PCR amplification was done with 50 ng of genomic DNA; the primer sequences specific for this microsatellite marker were as follows: forward 5′-FAMGGGCTCACAG-CAAACTTCTC-3′ and reverse 5′-AAGCCAGACTCGCTCATGTT-3′. One microliter of each PCR product was mixed with 0.5 μL of size standard (GenScan-350 Rox Standard, Applied Biosystems) and denatured in 18 μL of formamide at 95°C for 5 minutes. Separation was done with a four-color laser-induced fluorescence capillary electrophoresis system (ABI Prism 3100 DNA Analyzer, Applied Biosystems). The collected data was evaluated with the GeneScan Analysis Software (Applera, Norwalk, CT). DNA from the tumor cell line Hep-2 was used as a control for PCR amplified microsatellite fragment length.

Quantitative PCR. In the subgroup of 28 patients, total RNA was derived from paraffin-embedded tissue obtained by laser capture microdissection. After standard tissue sample deparaffinization using xylene and alcohols, samples were lysed in a Tris-chloride, EDTA, SDS, and proteinase K containing buffer. RNA was then extracted with phenol-chloroform-isoamyl alcohol followed by precipitation with isopropanol in the presence of glycogen and sodium acetate. RNA was resuspended in RNA storage solution (Ambion, Inc., Austin, TX) and treated with DNase I to avoid DNA contamination. cDNA was synthesized using Moloney murine leukemia virus retrotranscriptase enzyme. Template cDNA was added to Taqman Universal Master Mix (Applied Biosystems) in a 12.5-μL reaction with specific primers and

probe for each gene. The primer and probe sets were designed using Primer Express 2.0 Software (Applied Biosystems). Quantification of gene expression was done using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Primers and probe for *EGFR* and *caveolin-1* mRNA expression analysis were designed according to the Ref Seq NM_005228 and NM_001753, respectively (http://www.ncbi.nlm.nih.gov/LocusLink). The primers and labeled fluorescent reporter dye probe were as follows: β-actin, forward 5′-TGAGCGCGGCTACAGCTT-3′, reverse 5′-TCCTTAATGTCACGCACGATTT-3′, probe 5′-FAMACCACCACGGCCGAGCGG-3′TAMRA; EGFR, forward 5′-GGAAATTACCTATGTGCAGAGGAATT-3′, reverse 5′-TAACCAGCCACCCCCTGGAT-3′, MGB probe 5′-FAMTGATCTTTCCTTCTTAAAGAC-3′; Caveolin-1, forward 5′-CGACCCTAAACACCTCAACGA-3′, reverse 5′-GGTTCTGCAATCACATCTTCAAAG-3′, MGB probe 5′-FAMCGTGGTCAAGATTG-3′. Relative gene expression quantification was calculated according to the comparative C_t method using β-actin as an endogenous control and commercial RNA controls (Stratagene, La Jolla, CA) as calibrators. Final results were determined as follows: $2^{-(\Delta Ct\ sample\ -\ \Delta Ct\ calibrator)}$, where ΔC_t values of the calibrator and sample are determined by subtracting the C_t value of the target gene from the value of the *β-actin* gene. In all experiments, only triplicates with a SD of the $C_t < 0.20$ were accepted. In addition, for each sample analyzed, a retrotranscriptase minus control was run in the same plate to assure lack of genomic DNA contamination.

To distinguish between high and low gene expression levels, median levels obtained were used as cutoffs: 3.28 for *EGFR* and 0.52 for *caveolin-1* mRNA expression.

Fluorescence in situ hybridization assay. For each patient in the subgroup of 28 patients, two sections of 3- to 5-μm paraffin-embedded tumor tissue were placed over silenized treated slides. Another section was stained with H&E and confirmed to contain tumor tissue components. The silenized slides were left overnight at 60°C; deparaffinized in two changes of xylene for 10 minutes; rehydrated in 100% ethanol, 90% ethanol, and 70% ethanol for 1 minute each; and left in deionized water for 5 minutes. After tissue hydration, sections were placed in citrate buffer and heated in a microwave twice for 5 minutes at 800 W each. Slices were then digested by proteinase K treatment for 15 minutes at 37°C, fixed with formalin solution (pH 7.5), and washed in 2× SSC buffer. The hybridization was done using Vysis probes (LSI EGFR/CEP 7 Dual Color, Downers Grove, IL) following the manufacturer's instructions. Briefly, 5 μL of the probe solution were added to each slide and covered by a coverslip. Slides and probes were denatured for 3 minutes at 85°C in a slide warmer plaque (Hybrite, Vysis) and left at 37°C overnight. The coverslips were removed and the slides washed in 2× SSC/0.3%NP40 solution for 2 minutes at 72°C followed by an additional wash in 2× SSC/0.3%NP40 solution for 10 seconds at room temperature. Finally, slides were counterstained using a 4′-6′-diamidine-2-phenylindole-containing medium that specifically binds to DNA. For each patient, 100 nuclei from the selected tumor region were analyzed in a fluorescence microscope. The ratio of the average number of EGFR spots/nucleus by the average number of CEP 7 (centromeric chromosome 7) spots/nucleus was used for the scoring criteria. EGFR status in tumors was scored as follows: (a) single copy, up to four specific signals of both EGFR and CEP 7 probes with a ratio equal to 1; (b) polysomy, more than four specific signals of both probes per nucleus and a ratio <2; (c) amplification, more than four specific signals of EGFR probe per nucleus compared with CEP 7 with a ratio >2. Tumors scored as polysomy and/or amplification were labeled as having increased EGFR copy numbers.

Statistical methods. The primary objective of this study was to compare clinical characteristics, response rates, and survival in gefitinib-treated patients with and without mutations in the EGFR tyrosine kinase domain. In the subgroup of 28 patients, further analyses were done to examine the correlation among EGFR mutations, the number of CA repeats in intron 1 of EGFR in normal tissue, *EGFR* and *caveolin-1* mRNA expression levels in tumor tissue, and *EGFR* gene copy numbers.

The nonparametric Mann-Whitney *U* test and one-way ANOVA test were used to analyze differences in EGFR mutation status, number of CA repeats in intron 1 of EGFR, EGFR and caveolin-1 mRNA expression, and *EGFR* gene copy numbers. Normality of the distribution of continuous variables was assessed with the Kolmogorov-Smirnov test. The χ^2 and Fisher's exact tests were used to compare differences in response according to EGFR mutation status, number of CA repeats in intron 1, EGFR and caveolin-1 mRNA expression, and gene copy numbers. Univariate Cox regression models were used to measure hazard ratios. To identify relevant variables of influence, a multivariable logistic regression model was used, and the fit of the models was evaluated with the Hosmer-Lemeshow likelihood ratio test. The Wald test was used to test the statistical significance of each variable in the model. Survival curves were drawn with the Kaplan-Meier product limit method and *P* values assessed with the Tarone-Ware test. All reported *P* values are two sided; $P < 0.05$ was considered statistically significant. SPSS software version 11.5 (SPSS, Inc., Chicago, IL) was used for all analyses.

Results

Table 1 shows characteristics for all patients according to *EGFR* mutation status. Seventeen of the 68 patients harbored *EGFR* mutations in the tyrosine kinase domain. Mutations were not observed in DNA from peripheral blood or adjacent normal lung tissue, indicating that all mutations were somatic. All mutations were identified in adenocarcinomas (Table 1); 10 were heterozygous and six were homozygous (Table 2). Eleven tumors had in-frame nucleotide deletions in exon 19, adjacent to K745; five were delE746-A750, which was also observed in

Table 1. Characteristics of all patients according to *EGFR* mutation status

	EGFR mutation status		*P*
	Mutation	Wild-type	
No. patients	17	51	0.8
Age (range)	60 (34-84)	59 (39-86)	
Sex (%)			
Male	6 (35.3)	39 (76.5)	0.003
Female	11 (64.7)	12 (23.5)	
Histology (%)			
Adenocarcinoma	17 (100)	30 (58.8)	0.007
Large cell carcinoma	—	5 (9.8)	
Squamous cell carcinoma	—	11 (21.6)	
Other	—	5 (9.8)	
Smoking history (%)			
Smokers	3 (17.6)	43 (84.3)	0.0001
Nonsmokers	14 (82.4)	8 (15.7)	
No. prior regimens (range)	1 (0-3)	2 (0-6)	0.04
Response to gefitinib (%)			
Complete and partial response	16 (94.1)	6 (11.8)	<0.0001
Stable disease	1 (5.9)	8 (15.7)	
Progressive disease	—	34 (66.7)	
Not evaluable	—	3 (5.8)	
Duration of gefitinib treatment			
Months (range)	9.4 (1.1-23.1)	4.2 (0.2-41.9)	0.07

Table 2. Clinical characteristics and *EGFR* mutation status in 22 responders to gefitinib

Country of origin	Age	sex	Smoking status	Pathol	Prior regimens	Response	Overall survival (mo)	Survival status	*EGFR* mutation 1 AA sequence	*EGFR* mutation 2 AA sequence	Mutational status
Spain	60	F	Yes	ADC	1	PR	6.7	D	wt		wt
Spain	52	F	Yes	ADC	1	PR	8.3	D	wt		wt
Germany	65	M	Yes	ADC	1	PR	22.1	D	wt		wt
Japan	53	F	No	LCC	3	PR	18.4	D	wt		wt
Japan	76	M	Yes	ADC	3	PR	10.8	A	wt		wt
Japan	68	F	No	ADC	2	PR	24.3	D	wt		wt
Spain	71	F	No	ADC	1	PR	8.9	A	delE746.A750		Hetero
Spain	63	M	Yes	ADC	3	PR	17.8	A	delE746.T751InsA		Hetero
Germany	66	F	No	ADC	2	PR	13.7	D	delE746.S752InsV		Hetero
China	67	M	No	ADC	0	PR	22.0	A	delL747.P753InsS	L861Q	Hetero
China	34	F	No	ADC	2	PR	6.1	A	L858R		Homo
China	61	F	No	ADC	2	PR	14.3	A	L858R		Hetero
China	49	M	Yes	ADC	1	PR	25.4	A	L858R		Homo
China	37	M	No	ADC	0	PR	15.9	A	delE746.S752InsV		Hetero
Japan	71	F	No	ADC	1	PR	14.2	A	del719G(G)C to GC		Hetero
Japan	66	F	No	ADC	0	PR	9.5	A	delE746.T751		Homo
Japan	54	F	No	ADC	2	PR	18.7	D	delE746.A750		Homo
Japan	60	F	No	ADC	3	PR	15.3	A	L718P		Hetero
Japan	50	M	No	ADC	0	PR	18.4	A	delL747.T751InsF		Hetero
Japan	52	M	Yes	ADC	1	PR	8.9	A	delE746.A750		Homo
Japan	42	F	No	ADC	2	CR	18.9	A	delE746.A750		Homo
USA	84	F	No	ADC	1	PR	11.7	D	delE746.A750		Hetero

Abbreviations: Pathol, pathologic diagnosis; ADC, adenocarcinoma; LCC, large cell carcinoma; PR, partial response; CR, complete response; A, alive; D, dead; AA, amino acid; Homo, homozygogous; Hetero, heterozygous; wt, wild type.

the PC9 cell line; one was delE746-T751; four contained an amino acid insertion (one delE746-T751insF, one delE746-T751insA, and two delE746-752insV); and one tumor contained both an amino acid insertion (delL747-P753insS) and a missense mutation L861Q in exon 21. Four tumors contained an L858R mutation in exon 21. One tumor had an L718P mutation and another had a nucleotide deletion (guanine) in codon 719, both in exon 18. This second mutation was heterozygous. The G deletion affects the reading frame 5′ downstream of this position. The protein is nonfunctional, and the new sequence has a stop codon in codon 747 (TAA instead of TTA).

Twenty-two patients (32%) achieved a partial response to gefitinib. Table 2 shows the clinical characteristics of all responders. Sixteen of the 17 patients (94.1%) carrying EGFR mutations attained a partial radiographic response in contrast with 6 (12.6%) of the 51 patients with wild-type EGFR ($P < 0.0001$) Patients with *EGFR* mutations had 17.1 times greater probability of response ($P = 0.02$). Probability of response was also increased in nonsmokers, patients receiving a greater number of prior chemotherapy regimens, Asians, and younger patients (Table 3).

In general, patients harboring *EGFR* mutations obtained dramatic responses. For example, a Japanese female with adenocarcinoma underwent three pulmonary resections between 1999 and 2002; two of the three resected tumors contained an EGFR mutation (delE746-T751). The patient developed multiple bilateral lung metastases and did not respond to several chemotherapy regimens. After 2 months of gefitinib treatment, almost complete response was attained and the patient remains in remission at the time of submitting this article (Fig. 1). A second patient, a Spanish

Table 3. Adjusted odds ratio for the joint effect on response of different covariates

	Odds ratio (95% confidence interval)	*P*
Odds ratio adjusted by covariates		
EGFR mutations	17.1 (5.1-58.8)	0.002
EGFR mutations by sex (female)	8.7 (2.2-34.6)	0.0001
EGFR mutations by smoking status (nonsmoker)	37.4 (3.1-426)	0.005
EGFR mutations by no. prior chemotherapy regimens	73.1 (7.6-462)	0.005
EGFR mutations by ethnicity	61.7 (5.9-639)	0.001
EGFR mutations by age	105.0 (11.4-981)	0.00001
Crude odds ratio		
Sex (female)	1.4 (0.6-3.4)	0.4
Smoking status (smoker)	0.6 (0.4-0.8)	0.001
No. prior chemotherapy regimens	0.7 (0.5-0.9)	0.003
Ethnicity (Asian)	4.0 (1.7-9.2)	0.001
Age	0.9 (0.98-0.99)	0.008

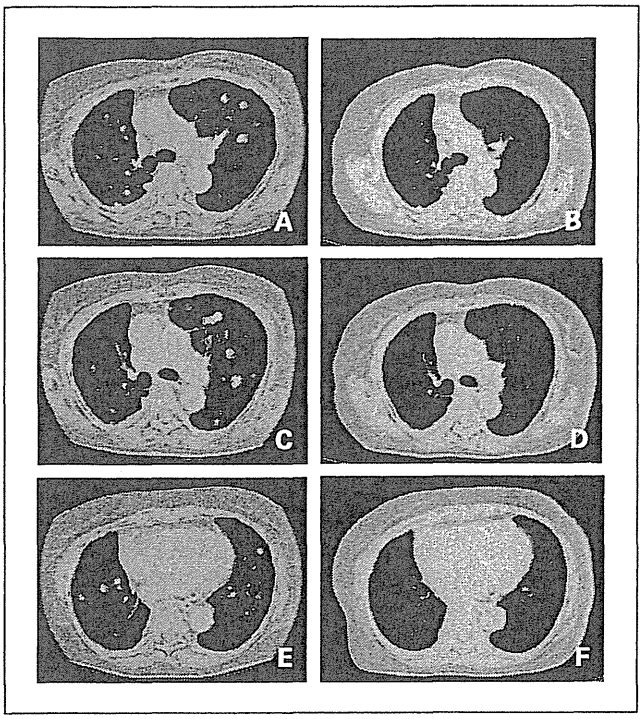


Fig. 1. Example of response to gefitinib in a representative patient with recurring NSCLC after three lines of chemotherapy. Computed tomography slices before gefitinib treatment (*A*, *C*, and *E*) and after 8 weeks of gefitinib treatment (*B*, *D*, and *F*).

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,

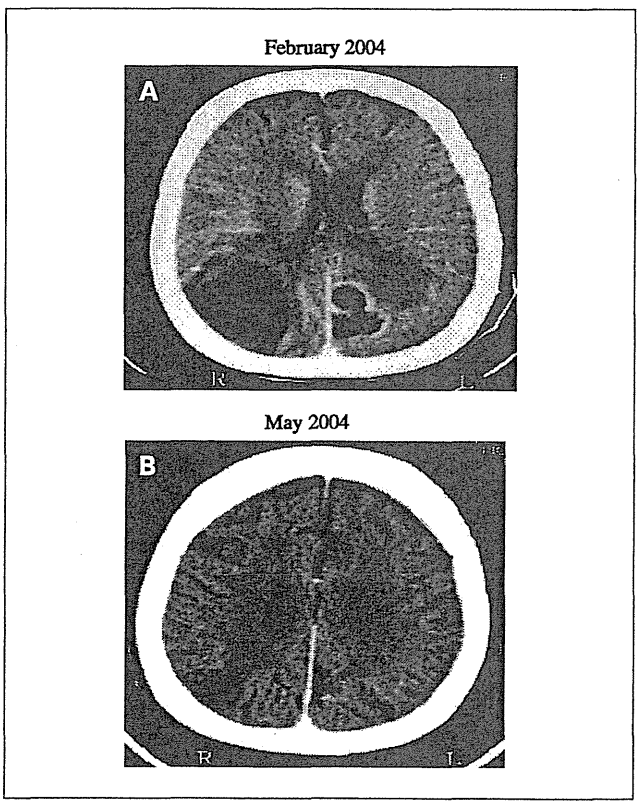


Fig. 2. Example of response to gefitinib in a lung adenocarcinoma patient with brain metastases. Computed tomography before gefitinib treatment (*A*) and after 8 weeks of gefitinib treatment (*B*). *A*, enlarged ventricles were observed in the pretreatment computed tomography. *B*, after treatment, with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

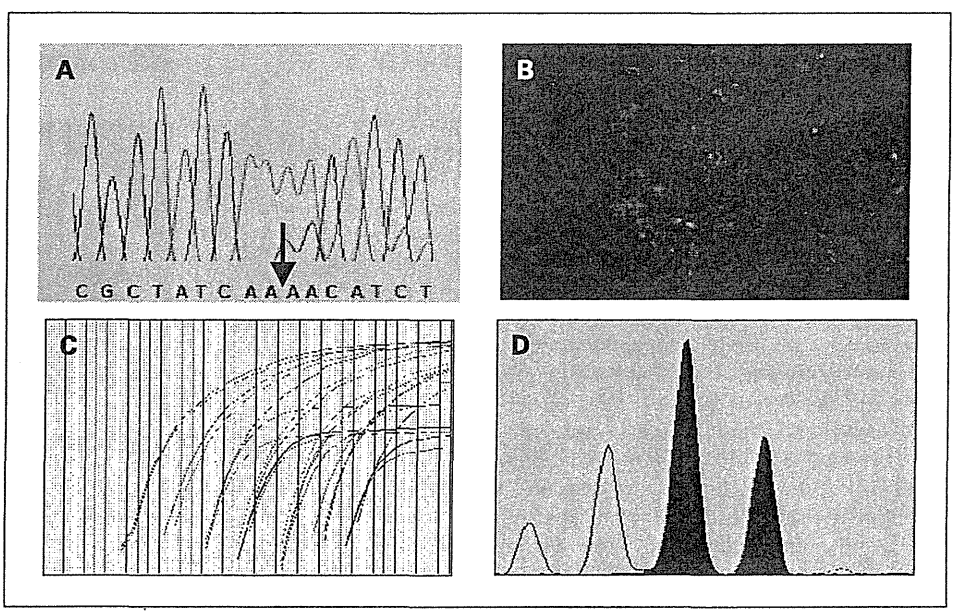
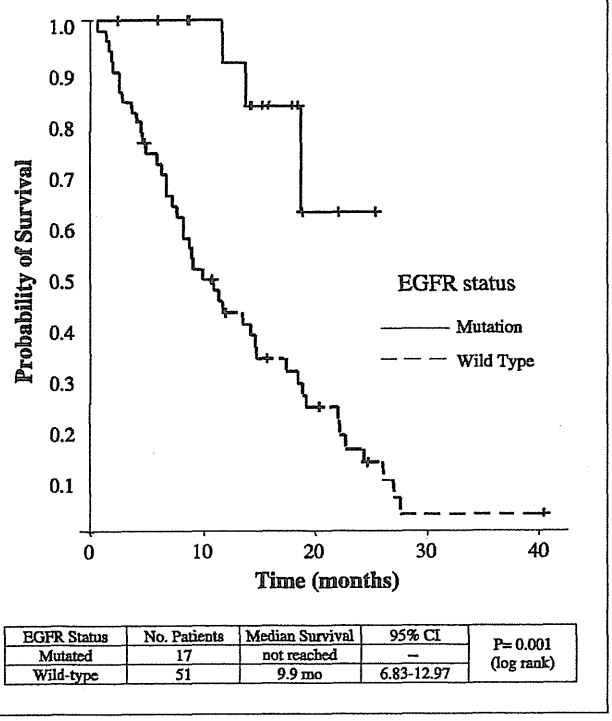


Fig. 3. Gefitinib responder showing, clockwise from top left: (*A*) an *EGFR* mutation (del E746-A750); (*B*) a high level of gene amplification (spots); (*C*) high *EGFR* and *caveolin-1* mRNA levels (superimposed one on the other); (*D*) and increased numbers of CA repeats. *C*, cDNAs for the gene 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.