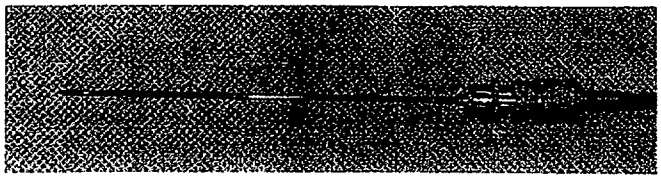

FIG. 1. A specially designed single-needle electrode, 150 mm in length and 1.6 mm in diameter, was inserted 20 mm into the normal lung.

MATERIALS AND METHODS

Measurements of Thermal Response

Animal studies were performed with the approval of the Institutional Committee for Ethical Research Animal Care. Adult beagles (10-15 kg) were given artificial respiration under general anesthesia with 30 mg/kg of phentobarbital sodium intravenously and placed in the left lateral recumbent position. Using an aseptic technique, thoracotomy was performed through the 5th intercostal space. A specially designed single-needle electrode (MD-16CBT-10/150, Azwell, Osaka, Japan, Fig. 1) that is 150 mm in length and 1.6 mm in diameter was inserted 20 mm into the normal lung. Then, tissue coagulation was performed using a microwave generator (Microtaze HSD-20W, Azwell, Fig. 2) that emitted 2450 MHz microwaves of 12 cm wavelength at a power output of 20, 40, and 60 W for 4 min. Temperature change was continuously monitored for 4 min using a K-type electric thermometer at 5 mm and 10 mm from the electrode with a sensor inserted 10 mm into the normal lung. The data of temperature were plotted for every 15 s. Measurements of temperature change were performed three times in each condition. Three beagles were used for this study.

Measurements of Coagulation Area

Microwave electrodes were inserted into normal lung tissue of beagles using the same procedure as mentioned above. Microwave coagulation was performed three times under each condition at power outputs of 20, 40, and 60 W for 1, 2, 3, and 4 min. Three beagles were used for this study. Shortly after microwave coagulation, the beagles were euthanized with an intravenous phentobarbital sodium overdose and pneumonectomy was performed. The resected canine lungs were inflated with bubbling air and 10% buffered formalin from the bronchial stump using an enema syringe pump and preserved in 10% buffered formalin for tissue fixation.

Under the same conditions, microwave coagulation was performed for normal human fresh lung tissue after resection of central type lung carcinoma, inflated with bubbling air using an enema syringe pump from the bronchial stump. Coagulation was performed once under each condition using two fresh lung lobes after resections. Informed consent was obtained in all cases. Tissue fixation was performed in the same manner as in the animal experiment.

The fixed lung tissue was transected perpendicular to the direction of the inserted electrode. The longest dimension of the maximum coagulation area of fixed lung tissue was measured.

Histological Examinations after Microwave Coagulation

Microwave coagulations were performed in three beagles at a power output of 40 W for 3 min. One beagle was euthanized with an intravenous phentobarbital sodium overdose immediately, and the other two beagles were followed up to assess histological change of the coagulated tissue. The normal activity and condition of each beagle was monitored daily. These beagles were euthanized at 3 and 6 months after the procedure. Histological changes of normal lung tissue immediately, 3 and 6 months after microwave coagulation were investigated by H-E staining and Elastica von Gieson staining.

Statistical Analysis

Data were expressed as means ± standard deviations (SD), and statistical analyses were done using Student's t test with computer software (Microsoft Excel, version 2002). A P value of less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

Measurements of Thermal Response

The data of thermal response of normal canine lung tissue to microwave coagulation is shown in Fig. 3. At 5 mm from the electrode (Fig. 3A), the temperature rose rapidly over 80°C (15 s for 60 W: 83.1 ± 13.0°C; 30 s for 40 W: 83.9 ± 3.4°C), and thereafter the temperature reached a plateau around 90-100°C at both 40 and 60 W. There was no significant difference between the two groups for each time point. At 20 W, the temperature rose gradually to only 65°C and reached a plateau. At 10 mm from the electrode (Fig. 3B), the temperature rose gradually to 70°C (45 s for 60 W: 70.6 ± 11.6°C; 90 s for 40 W: 70.9 ± 13.0°C), and thereafter the temperature reached a plateau around 80°C at 40 and 60 W. There was no significant difference between the two groups for each time point. At 20 W, the temperature rose to only 50°C after 90 s and reached a plateau. It appeared that 20 W was not enough for coagulation. The same thermal re-

FIG. 2. Microwave coagulation was performed using a microwave generator that emitted 2450 MHz microwaves of 12 cm wavelength at a power output of 20, 40, and 60 W.

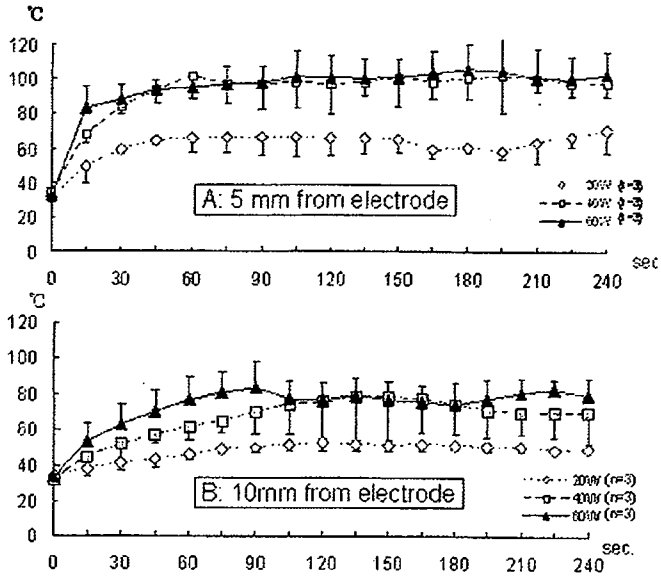

FIG. 3. (A) At 5 mm from the electrode, the temperature rose rapidly to 85°C (for 15 s at 60 W and 30 s at 40 W). The temperature reached a plateau around 90-100°C at 40 and 60 W. There was no significant difference between 40 and 60 W for each time point. (B) At 10 mm from the electrode, the temperature rose gradually to 70°C (45 s at 60 W and 90 s at 40 W). The temperature reached a plateau around 80°C at 40 and 60 W. There was no significant difference between 40 and 60 W for each time point.

sponse was obtained in the two groups of 40 W and 60 W at same distances from the electrode.

Measurements of Coagulation Area

The data of the diameter of the maximum coagulation area in the animal model is shown in Fig. 4. The maximum coagulation area increased with increased power and coagulation time. The diameter of the maximum coagulation area was 18.3 ± 10.4 mm at 40 W for 4 min and 21.7 ± 2.9 mm at 60 W for 4 min. There was no significant difference in the coagulation area at each time period when using 40 W and 60 W.

The diameter of the maximum coagulation area in normal human fresh lung tissue after resection of central-type lung cancer is shown in Fig. 5. The maximum coagulation area increased with increased power and coagulation time until 3 min. After 3 min, the diameter of the maximum coagulation area was 25 mm at 40 W and 26 mm at 60 W. At 40 and 60 W for 4 min, the diameter of the maximum coagulation area shrank to 15 mm. There was no difference between 40 and 60 W for each period of coagulation.

Histological Examinations after Microwave Coagulation

All beagles tolerated the procedure well. During 6 months of follow up to assess histological change of the coagulated tissue, the normal activity and condition of each beagle was monitored daily. There was no death due to serious complications such as hemoptysis or pneumothorax during the period.

The histological changes after microwave coagulation are shown in Fig. 6. Histological findings shortly after microwave coagulation showed degeneration and thickening of collagen fiber and exfoliation and ulceration of bronchial epithelium surrounding the electrode. No surrounding bronchioli or veins were destroyed. No blood clots or debris were observed in surrounding veins (Fig. 6A). After 3 months, histological findings showed stromal edema and loose collagen fiber, immature neoangiogenesis, progression of bronchial epithelial hyperplasia, infiltration of inflammatory cells at the boundary zone (lymphocyte > plasma cell > neutrophil) between the central coagulation area and normal tissue (Fig. 6B). After 6 months, coagulated tissue became scar tissue that showed disappearance of stromal edema, tight collagen fiber, mature capillaries, disappearance of inflammatory cells, and completion of epithelial hyperplasia (Fig. 6C).

DISCUSSION

With the increasing use of high-resolution CT scans, the rate detection of small nodules in the peripheral lung, such as early-stage lung cancer, small metastases, or AAH has increased. Kaneko *et al.* demonstrated that the detection rate of peripheral lung carcinoma by mass screening using CT scan was 0.45% (15 of 3457 examinations), 73% of which were detected by low-dose spiral CT but were not visible on standard chest radiography [5]. Noguchi *et al.* investigated 236 surgically resected small-size peripheral adenocarcinomas measuring 2.0 cm or less in greatest dimension and demonstrated that type A (localized bronchioloalveolar carcinoma: LBAC) and type B (LBAC with foci of structural collapse of alveoli) that showed ground glass opacity (GGO) on CT scanning images demonstrated

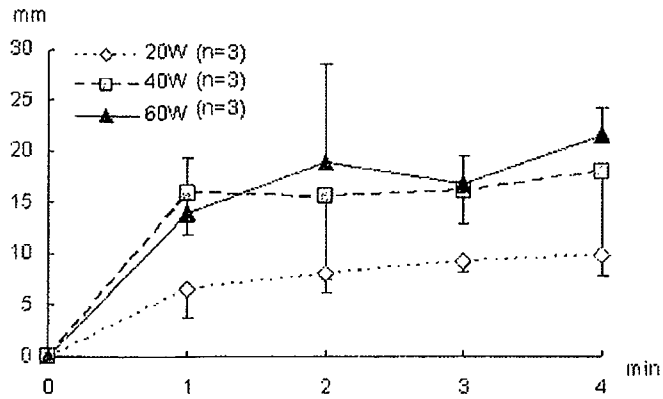

FIG. 4. The diameter of the maximum coagulation area was 18 mm at 40 W for 4 min and 22 mm at 60 W for 4 min. There was no significant difference between 40 W and 60 W for each coagulation time.

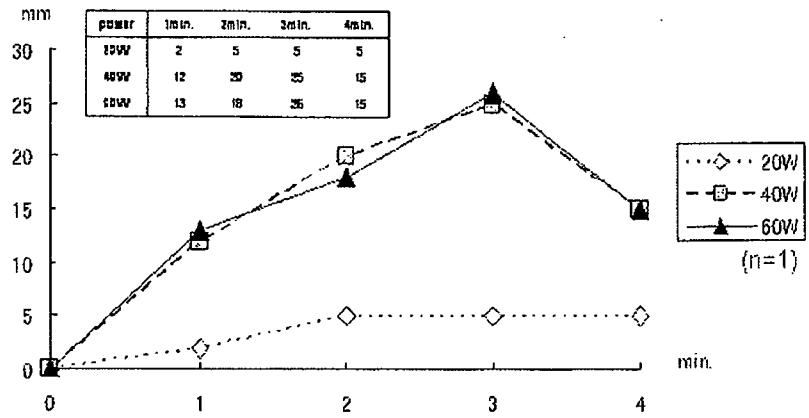

FIG. 5. The diameter of the maximum coagulation area was 25 mm at 40 W and 60 W for 3 min. There was no difference between 40 and 60 W for each time. The diameter of the maximum coagulation area shrank to 15 mm at 40 and 60 W for 4 min.

no lymph node metastasis and had the best 5-year survival rate (100%) [6]. Meanwhile, according to a pathological study on lymph node metastasis of primary lung carcinoma after surgery, the rate of metastasis (N1 and N2) was 0% with a tumor 1.0 cm or less, 17% with a tumor 1.1 to 2.0 cm, and 37% with a tumor 2.1 to 3.0 cm in diameter [7]. As a result, it seems to be possible to control 83% of lung carcinomas smaller than 2.0 cm in size by local treatment. Also, the rate of local lymph node metastasis of metastatic lung tumor is known to be low.

We usually perform surgery for such patients as a possible cure, but this may lead to considerable lung damage with significant loss of function. Although VATS has widened the indication of surgery recently, some patients are unable to undergo even VATS due to poor cardiopulmonary function, severe adhesion, or advanced age, etc. Radical radiotherapy or chemotherapy or both may be offered with curative intent to such patients, but the prospect of cure is substantially worse than with surgical options, while it may also lead to considerable lung damage with significant loss of function caused by radiation fibrosis, systemic toxicity due to the anticancer agent. Therefore, new modalities for local treatment that effectively destroy tumor but are less invasive and less damaging to normal lung tissue are required. Recently, several investigators tried to use radiofrequency ablation (RFA) [8–10] or photodynamic therapy (PDT) for peripheral lung tumors [11, 12].

We are interested in microwave coagulation therapy (MCT), which has been successfully used to perform coagulation of hepatic tumors. In 1978, hepatic surgery with MCT was introduced by Tabuse [1], and recently the effectiveness of percutaneous microwave coagulation therapy (PMCT) under ultrasonography or CT scan guidance for small hepatocellular carcinoma was demonstrated [3, 4]. We considered this modality to be applicable for patients with lung tumors who are poor surgical candidates, as well as patients with hepatic tumors, and evaluated the efficacy and safety of MCT for lung tissue experimentally.

The microwave generator emits a higher frequency wavelength than electrocautery and generates dielectric heat energy due to friction of water molecules when irradiating living tissue. MCT applies this mechanism to achieve tumor necrosis. Because the dielectric heat energy cannot be generated in the presence of air, selective tumor damage may be achieved, with limited damage to the surrounding normal air-filled lung tissue. We considered it essential to know how MCT affects normal lung tissue before performing PMCT clinically for peripheral lung malignancies. An experimental study was deemed necessary to evaluate the thermal response, coagulation extent, and histological changes in the air-filled normal lung.

With regard to thermal response, the temperatures of normal canine lung tissue rose with increased microwave power and coagulation time. The temperatures in normal lung tissue rose to 90-100°C at 5 mm from the electrode after 60 s and 70-80°C at 10 mm after 90 s, thereafter reaching a plateau. A power of 20 W was not sufficient to coagulate lung tissue. These data suggested that the same thermal response could be obtained at 40 and 60 W. The coagulation area in normal canine lung tissue increased to 18 mm and 22 mm at 40 W and 60 W for 4 min, respectively. Therefore, it may be possible to coagulate a diameter of approximately 20 mm. In solid tumors, there is a possibility to achieve more extensive coagulation. In human resected normal lung with central-type lung carcinoma, the coagulation area increased to 25 mm at 40 and 60 W for 3 min and shrank to 15 mm for 4 min. This phenomenon may be explained by shrinking of lung tissue due to rapid elevation of the temperature in the tissue, because there is no radiator effect in the resected lung due to the lack of blood supply. From the current study, we concluded the optimal condition in clinical PMCT to be 40-60 W for 3–4 min of coagulation.

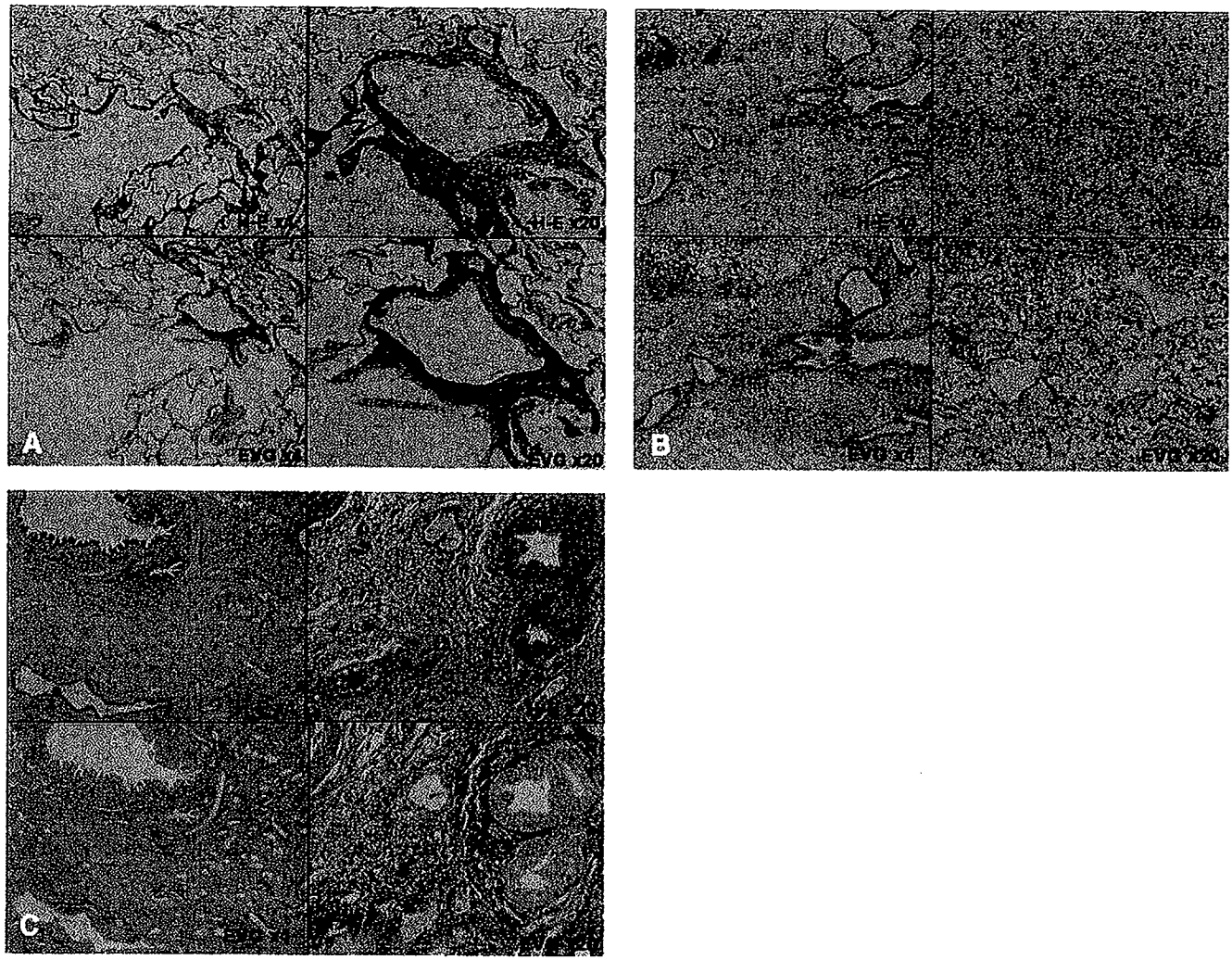

FIG. 6. (A) Histological findings shortly after microwave coagulation showed degeneration and thickening of collagen fiber and exfoliation and ulceration of bronchial epithelium surrounding the electrode. Surrounding bronchial and veins were not destroyed. (B) After 3 months, histological findings showed stromal edema and loose collagen fiber, immature neoangiogenesis, progression of bronchiolus epithelial hyperplasia, infiltration of inflammatory cells at the boundary zone between the central coagulation area and normal tissue. (C) After 6 months, coagulated tissue became scar tissue that showed disappearance of stromal edema, tight collagen fiber, mature capillaries, disappearance of inflammatory cells and completion of epithelial hyperplasia. (Color version of figure is available online.)

Histological analysis following MCT for normal canine lung tissue demonstrated exfoliation and ulcer formation of the epithelium in the bronchioli and degeneration and thickening of collagen fiber in the parenchyma by heat coagulation shortly after MCT. The coagulated lesions were gradually repaired by progression of epithelial hyperplasia and infiltration of inflammatory cells, showing stromal edema and granulation tissue after 3 months and finally becoming scar tissue after 6 months. We concluded MCT to be a safe modality for lung tissue because no destruction of bronchioles or veins was seen in the specimens during 6 months.

The present studies of MCT for peripheral lung tissue demonstrated that this new modality had no serious adverse effects and could be performed safely. However, the incidence of pneumothorax by CT-guided RFA was demonstrated to be 38.5% (3/8) in a rabbit model [8] and 33.3% (1/3) and 53.8% (7/13) in clinical cases [9, 10], which seems to be relatively high. Therefore, the development of a fine electrode with a cooling system will be necessary to prevent complications such as pneumothorax and heat sensation for clinical use.

From our experimental studies, the advantages of PMCT are the fact that this modality is minimally invasive, may be performed by local anesthesia, and is applicable for patients with poor cardiopulmonary function. In addition, the microwave generator is a very simple device, maintenance free, easy to handle, and portable, and the procedure is easy compared with RFA and PDT. The possibility of pneumothorax, heat

sensation or pain, or both during treatment and the limited coagulation area are considered the disadvantages for clinical use at present. Nevertheless, our results demonstrated the possibility of MCT for patients with small peripheral lung tumors with the intent of curative treatment. Although MCT is considered to be a useful modality as minimally invasive therapy for small peripheral lung tumors, further comparative research is necessary with other modalities such as RFA and PDT for peripheral lung tumors.

REFERENCES

1. Tabuse, K. A new operative procedure of hepatic surgery using a microwave tissue coagulation. *Arch. Jpn. Chir.* **48:** 160, 1979.
2. Hamazoe, R., Hirooka, Y., Ohtani, S., *et al.* Intraoperative microwave tissue coagulation as treatment for patients with nonresectable hepatocellular carcinoma. *Cancer* **75:** 794, 1995.
3. Seki, T., Wakabayashi, M., Nakagawa, T., *et al.* Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. *Cancer* **74:** 817, 1994.
4. Ohmoto, K., Miyake, I., Tsuduki, M., *et al.* Percutaneous microwave coagulation therapy for unresectable hepatocellular carcinoma. *Hepatogastroenterology* **46:** 2894, 1999.
5. Kaneko, M., Eguchi, K., Ohmatsu, H., *et al.* Peripheral lung cancer: screening and detection with low-dose spiral CT versus radiography. *Radiology* **201:** 798, 1996.
6. Noguchi, M., Morikawa, A., Kawasaki, M., *et al.* Small adenocarcinoma of the lung. Histologic characteristics and prognosis. *Cancer* **75:** 2844, 1995.
7. Ishida, T., Yano, T., Maeda, K., *et al.* Strategy for lymphadenopathy in lung cancer three cm or less in diameter. *Ann. Thorac. Surg.* **50:** 708, 1990.
8. Goldberg, S. N., Gazelle, G. S., Compton, C. C., *et al.* Radiofrequency tissue ablation in the rabbit lung: efficacy and complications. *Acad. Radiol.* **2:** 776, 1995.
9. Dupuy, D. E., Zagoria, R. J., Akerley, W., *et al.* Percutaneous radiofrequency ablation of malignancies in the lung. *AJR Am. J. Roentgenol.* **174:** 57, 2000.
10. Herrera, L. J., Fernando, H. C., Perry, Y., *et al.* Radiofrequency ablation of pulmonary malignant tumors in nonsurgical candidates. *J. Thorac. Cardiovasc. Surg.* **125:** 929, 2003.
11. Fielding, D. I., Buonaccorsi, G. A., MacRobert, A. J., *et al.* Fine-needle interstitial photodynamic therapy of the lung parenchyma. *Chest* **155:** 502, 1999.
12. Okunaka, T., Kato, H., Tsutsui, H., *et al.* Photodynamic therapy for peripheral lung cancer. *Lung Cancer* **43:** 77, 2004.

VOLUME 23 · NUMBER 11 · APRIL 10 2005

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Mutations of the Epidermal Growth Factor Receptor Gene Predict Prolonged Survival After Gefitinib Treatment in Patients With Non–Small-Cell Lung Cancer With Postoperative Recurrence

Tetsuya Mitsudomi, Takayuki Kosaka, Hideki Endoh, Yoshitsugu Horio, Toyoaki Hida, Shoichi Mori, Shunzo Hatooka, Masayuki Shinoda, Takashi Takahashi, and Yasushi Yatabe

From the Departments of Thoracic Surgery, Thoracic Oncology, and Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital; and Division of Molecular Oncology, Aichi Cancer Center Research Institute, Aichi Cancer Center, Nagoya, Japan.

Submitted September 10, 2004; accepted January 11, 2005.

Supported in part by Grant-in-Aid (16591424) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Terms in blue are defined in the glossary, found at the end of this issue and online at www.jco.org.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to Tetsuya Mitsudomi, MD, Department of Thoracic Surgery, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan; e-mail: mitsudom@aichi-cc.jp.

0732-183X/05/2311-2513/$20.00

DOI: 10.1200/JCO.2005.00.992

ABSTRACT

Purpose
To evaluate the relationship between mutations of the epidermal growth factor receptor (*EGFR*) gene and the effectiveness of gefitinib treatment in patients with recurrent lung cancer after pulmonary resection.

Patients and Methods
We sequenced exons 18-21 of the *EGFR* gene using total RNA extracted from 59 patients with lung cancer who were treated with gefitinib for recurrent lung cancer. Gefitinib effectiveness was evaluated by both imaging studies and change in serum carcinoembryonic antigen (CEA) levels.

Results
EGFR mutations were found in 33 patients (56%). Of these mutations, 17 were deletions around codons 746-750 and 15 were point mutations (12 at codon 858, three at other codons), and one was an insertion. *EGFR* mutations were significantly more prevalent in females, adenocarcinoma, and never-smokers. Gefitinib treatment resulted in tumor shrinkage and/or CEA decrease to less than half of the baseline level in 26 patients, tumor growth and/or CEA elevation in 24 patients, and gefitinib effect was not assessable in nine patients. Female, never-smoking patients with adenocarcinoma tended to respond better to gefitinib treatment. Gefitinib was effective in 24 of 29 patients with *EGFR* mutations, compared with two of 21 patients without mutations ($P < .0001$). Of note, del746-750 might be superior to L858R mutations for prediction of gefitinib response. Patients with *EGFR* mutations survived for a longer period than those without the mutations after initiation of gefitinib treatment ($P = .0053$).

Conclusion
EGFR mutations were a good predictor of clinical benefit of gefitinib in this setting.

J Clin Oncol 23:2513-2520. © 2005 by American Society of Clinical Oncology

INTRODUCTION

Lung cancer has long been the leading cause of cancer death in North America. In 1998, it became the leading cause of cancer death in Japan, and now claims more than 55,000 lives annually.[1] Lung cancer is divided into two morphologic types: small-cell lung cancer and non–small-cell lung cancer (NSCLC). NSCLCs are further subdivided into adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. Adenocarcinoma is the predominant histologic subtype, and is increasing among patients with lung cancer who are candidates for surgical treatment in Japan. In our institution, adenocarcinoma accounted for 76% of 407 patients who were operated on from 2001 through 2003. Adenocarcinomas are characterized by a high degree of morphologic heterogeneity. Analyses of various cancer-associated genes, including *K-ras*,[2] *p53*,[3,4] cyclin D1,[5] $p27^{Kip1}$,[6] and cyclooxygenase-2,[7]

suggests a different molecular pathway for carcinogenesis in lung adenocarcinomas at least partly accounts for this heterogeneity. In addition, the NSCLC frequently overexpresses receptors of the ErbB family, including the epidermal growth factor receptor (EGFR) encoded by ErbB1 (HER-1).[8,9]

EGFR is a 170 kd receptor tyrosine kinases (TK) that dimerizes and phosphorylates several tyrosine residues upon binding of several specific ligands including epidermal growth factor and transforming growth factor alpha.[8] These phosphorylated tyrosines serve as the binding sites for several signal transducers that initiate multiple signaling pathways resulting in cell proliferation, migration and metastasis, evasion from apoptosis, or angiogenesis, all of which are associated with cancer phenotypes.[8] Downstream pathways include ras-raf-MEK-ERK, phosphatidylinositol-3 kinase-Akt, and PAK-JNKK-JNK.[8]

Gefitinib is an orally bioavailable small molecule that specifically inhibits EGFR tyrosine phosphorylation.[10] Clinical trials revealed that there is significant variability in response to gefitinib. Good clinical responses have been observed most frequently in women, in nonsmokers, in patients with adenocarcinomas, and in Japanese patients.[11,12] However, it was not possible to predict gefitinib sensitivity by levels of EGFR overexpression as determined by immunohistochemistry[13] or immunoblotting.[14] The factors that determine gefitinib sensitivity have long been an enigma. Recently, it has been reported that activating mutations of *EGFR* are present in a subset of pulmonary adenocarcinomas and that tumors with *EGFR* mutations are highly sensitive to gefitinib[15-17] or erlotinib, another EGFR TK inhibitor. Furthermore, the incidence of *EGFR* mutations is significantly higher in female, never-smoking, Japanese patients with adenocarcinoma.[15] These features coincide with those of good responders to gefitinib.

In this study, we studied patients who had recurrent disease after pulmonary resection for NSCLC and who were subsequently treated with gefitinib. We searched for mutations of the *EGFR* gene in tumor specimens taken at the time of surgery and we correlated *EGFR* mutations with gefitinib effectiveness, including tumor response and patient survival.

PATIENTS AND METHODS

Patients

Seventy-five patients were treated with gefitinib for their recurrent diseases after they had undergone surgery between 1999 and 2003. We studied 59 patients whose tumors were available for RNA extraction, which was a sole determinant of inclusion into the present study. There were 32 men and 27 women with ages ranging from 48 to 79 years. Fifty patients had adenocarcinomas, five had squamous cell carcinomas, three had large-cell carcinomas, and one had adenosquamous carcinoma. Eight patients had stage IA disease; seven stage IB; three stage IIA; five stage IIB; 24 stage IIIA; eight stage IIIB; and three stage IV at the time of surgery. Lobectomy had been performed in 57, and pneumonectomy and partial resection in one patient each. Four patients received postoperative adjuvant chemotherapy (two with oral uracil/tegafur and two with gemcitabine monotherapy). Forty patients had had chemotherapy before gefitinib treatment (23 patients, platinum doublet; 16 patients, monotherapy with vinorelbine or gemcitabine, one patient, oral uracil/tegafur). Gefitinib treatment with a daily dose of 250 mg was initiated between July 2002 and May 2004, with the median interval between operation and gefitinib treatment being 778 days (range, 107 to 1,931 days). Fifty patients had distant metastatic tumors, eight patients had pleural dissemination and malignant effusion, and one patient had hilar lymph-node metastasis at initiation of gefitinib treatment.

Molecular Analysis of Lung Cancer Specimens

After we obtained appropriate approval from the institution and written informed consent for comprehensive use of molecular and pathologic analysis from the patients, tumor samples were collected during surgery, rapidly frozen in liquid nitrogen and stored at −80°C. A surgical pathologist (Y.Y.) grossly dissected the frozen tumor specimens to enrich the tumor cell population as much as possible. Total RNA was isolated using the RNeasy kit (Qiagen, Valencia, CA).

The first four exons (exons 18-21) of the seven exons (exons 18-24) that code for TK domain of the *EGFR* gene (which includes all the mutations reported so far[15-17]) was amplified with primers F1 (5′-AGCTTGTGGAGCCTCTTACACC-3′) and R1 (5′-TAAAATTGATTCCAATGCCATCC-3′) in a one-step reverse transcription polymerase chain reaction (RT-PCR) using the QIAGEN OneStep RT-PCR Kit (Qiagen). The cDNA sequence of the *EGFR* gene was obtained from GenBank (accession number NM 005228). The RT-PCR conditions were: one cycle of 50°C for 30 minutes, 95°C for 15 minutes, 40 cycles of 94°C for 50 seconds, 62°C for 50 seconds, and 72°C for 60 seconds, followed by one cycle of 72°C for 10 minutes.

RT-PCR products were diluted and cycle-sequenced using the Big Dye Terminator v3.1/1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Sequencing products were electrophoresed on an ABI PRISM 3100 (Applied Biosystems). Both the forward and reverse sequences obtained were analyzed by BLAST (basic local alignment search tool) and chromatograms by manual review. High-quality sequence variations found in both directions were scored as candidate mutations.

Definition of Effectiveness of Gefitinib

Because this study was a retrospective analysis of the daily clinical practice of oncology, the evaluation of tumor response could not be performed strictly according to predefined criteria, such as Response Evaluation Criteria in Solid Tumors (RECIST).[18] RECIST are not necessarily applicable or complete in such a context and the evaluation may instead be based on a subjective medical judgment that results from clinical and laboratory data.[18] Therefore, gefitinib treatment was judged as effective when the tumors showed at least a 30% decrease in tumor diameter in imaging studies. However, because of the nature of the study, confirmation of tumor response no less than 4 weeks apart, as in RECIST,[18] was not necessarily required.

As patients with recurrent lung cancer often do not have measurable disease, we also included change in serum carcinoembryonic antigen (CEA) level (cut off, 5 ng/mL) as an evaluation

criterion to avoid underestimating gefitinib effectiveness. CEA has been reported as a useful clinical therapeutic marker.[19] When the elevated CEA level decreased to a level less than half of the baseline level, gefitinib treatment was judged as effective. On the other hand, gefitinib treatment was judged as ineffective when the tumors showed any growth or a new lesion appeared in the imaging studies, or when the serum CEA level increased. Any patient who did not fit either of these criteria was classified as not assessable. All these evaluations were done before the *EGFR* gene analysis, without knowledge of mutational status of the *EGFR* gene.

Statistical Analysis

For comparisons of proportions, the χ^2 test or Fisher's exact test was used. The Kaplan-Meier method was used to estimate the probability of survival as a function of time, and survival differences were analyzed by the log-rank test. The two-sided significance level was set at $P < .05$. To identify which independent factors had a joint significant influence on gefitinib effectiveness, the logistic regression modeling technique was used, and for multivariate analysis of the overall survival, the Cox proportional hazards modeling technique was applied. All analyses were performed using StatView version 5 (SAS institute Inc, Cary, NC) software on a Macintosh computer.

RESULTS

EGFR *Mutations*

Mutations of the *EGFR* gene were detected in 33 (56%) of 59 patients. Seventeen were deletions, 15 were point mutations, and one was an insertion. Details of these mutations are shown in Figure 1. As previously reported,[15-17] *EGFR* mutations were significantly associated with adenocarcinoma histology, female sex, and never-smoking status (Table 1). However, the mutations were not associated with the age or stage of the patients. Furthermore, median time from the original surgery to

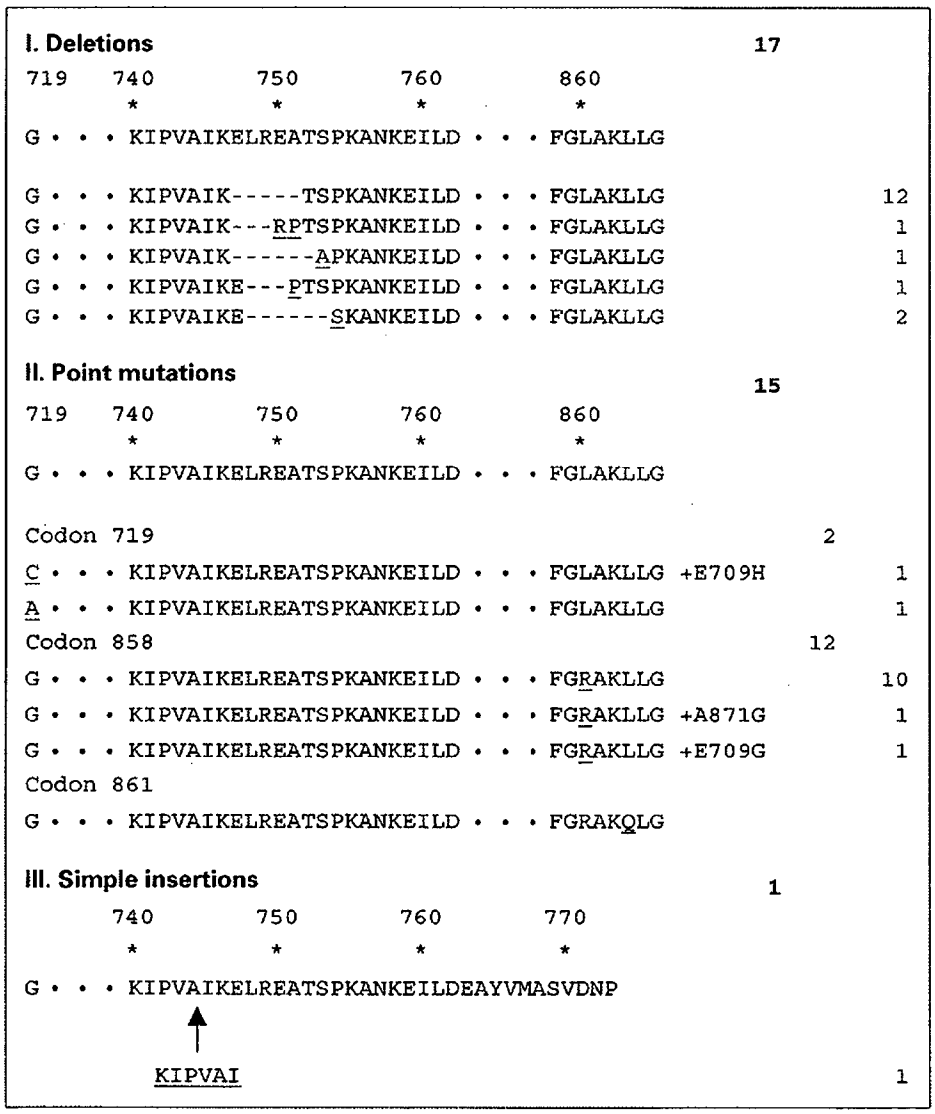

Fig 1. Analysis of 33 epidermal growth factor receptor (EGFR) mutations in tyrosine kinase domain of the *EGFR* gene found in unselected patients with lung cancer.

Table 1. Incidence of EGFR Mutations and Clinical and Pathologic Features

Variable	EGFR Mutation		EGFR Wild-Type	*P*
	No. of Patients	%		
All cases	33	56	26	
Sex				
Male	14	44	18	.0402
Female	19	70	8	
Age, years				
≤ 64	22	55	18	.8342
> 64	11	58	8	
Histologic type				
Adenocarcinoma	32	64	18	.0033
Nonadenocarcinoma	1	11	8	
Squamous cell carcinoma	0	0	5	
Large-cell carcinoma	0	0	3	
Adenosquamous carcinoma	1	100	0	
Smoking status				
Never smoker	20	71	8	.0227
Former or current smoker	13	42	18	
Stage				
I-II	12	50	12	.4472
III-IV	21	60	14	

Abbreviation: EGFR, epidermal growth factor receptor.

recurrence was almost identical in patients with *EGFR* mutations (362 days) and in those without *EGFR* mutations (363 days; $P = .8265$).

Clinical Improvement After Gefitinib Treatment

Forty-one of 59 patients had measurable disease at recurrence with imaging studies. Of these, 20 showed appreciable tumor shrinkage after gefitinib treatment, whereas 17 tumors increased in size, and there was no change in tumor size in four patients. All of these 20 tumors (pulmonary metastases in 11, pleural disseminated nodules in two, hepatic metastases in two, mediastinal lymph node swelling in two, brain metastases in two, and chest wall tumor in one) showed at least a 30% decrease in diameter. Figure 2 shows representative imaging studies. A computed tomography scan of the chest in patient L703 (73-year-old woman, adenocarcinoma) showed masses in the right-lower lobe and marked improvement 8 weeks after gefitinib initiation. A computed tomography scan of the liver in patient L1492 (52-year-old woman, adenocarcinoma) showed masses in the right lobe of the liver and dramatic improvement 10 days after gefitinib initiation. A large chest-wall mass in the left back of patient L1362 (62-year-old man, adenosquamous carcinoma) before gefitinib treatment almost disappeared 13 weeks after gefitinib initiation. A left-lung tumor in patient L1171 (70-year-old woman, adenocarcinoma) was smaller 6 weeks after gefitinib initiation.

CEA was above the upper normal limit (5 ng/mL) at baseline in 32 patients. Serum CEA level decreased to < 10%, < 50%, and to > 50% of the baseline level in three, 12, and five patients, respectively, whereas CEA level increased in 12 patients. When we combined the results of imaging studies with CEA and judged according to our criteria, gefitinib treatment was effective in 26 (52%), not effective in 24 (48%), and not assessable in nine patients (Table 2). There was a good correlation between these two examinations. The imaging studies and change in CEA levels did not conflict in any patients. In 17 patients with measurable diseases and whose baseline CEA level was elevated, the CEA level decreased in all 11 patients showing tumor shrinkage and increased in all five patients showing tumor growth, except for one patient whose tumors showed no change in size ($P < .001$, Fisher's exact test), supporting the validity of our criteria.

We searched for a relation between gefitinib effectiveness and various clinical and pathologic features (Table 2). Never-smokers and patients with adenocarcinoma had a significantly higher incidence of gefitinib effect. However, we could not detect significant difference in gefitinib sensitivity by sex or presence of prior chemotherapy, probably because of the small sample size, although there was a trend that female and chemotherapy-naïve patients were more responsive.

Relationship Between Clinical Response to Gefitinib Treatment and EGFR *Mutations*

The incidence of *EGFR* mutations in terms of response to gefitinib treatment as judged by imaging studies and CEA levels is shown in Table 3. Of 20 patients who showed tumor shrinkage, 19 (95%) had mutations of the *EGFR* gene. On the other hand, two (12%) of 17 patients whose tumors grew after gefitinib treatment harbored *EGFR* mutations ($P < .001$, Fisher's exact test). In Figure 2, patient L703, L1492, and L1362 had *EGFR* mutations (delE746-A750, L858R, and E746-S752insA, respectively). Of three, 12, and five patients whose CEA level decreased to less than 10%, less than 50%, and to more than 50% of the baseline level after gefitinib treatment, three (100%), 10 (83%), and four (80%) had *EGFR* mutations, respectively. On the other hand, of 12 patients whose CEA level increased, three (25%) had *EGFR* mutations ($P = .004$, Fisher's exact test).

When we used our criteria combining the results of imaging studies with CEA, gefitinib was effective in 24 (83%) of 29 patients with *EGFR* mutations, whereas it was effective only in two (10%) of 21 patients without *EGFR* mutations ($P < .0001$; Table 2). There were three patients with *EGFR* mutations (two with L858R and one with G719A) whose CEA level increased after gefitinib treatment but did not have measurable diseases. There were also two patients with *EGFR* mutations, one with L858R+E709H and one with I744-K745 ins KIPVAI whose tumor progressed.

Logistic regression analysis (Table 4) showed that *EGFR* mutation was the only significant factor contributing to gefitinib sensitivity.

On the other hand, patient L1171, who showed a decrease in size of multiple pulmonary metastatic nodules

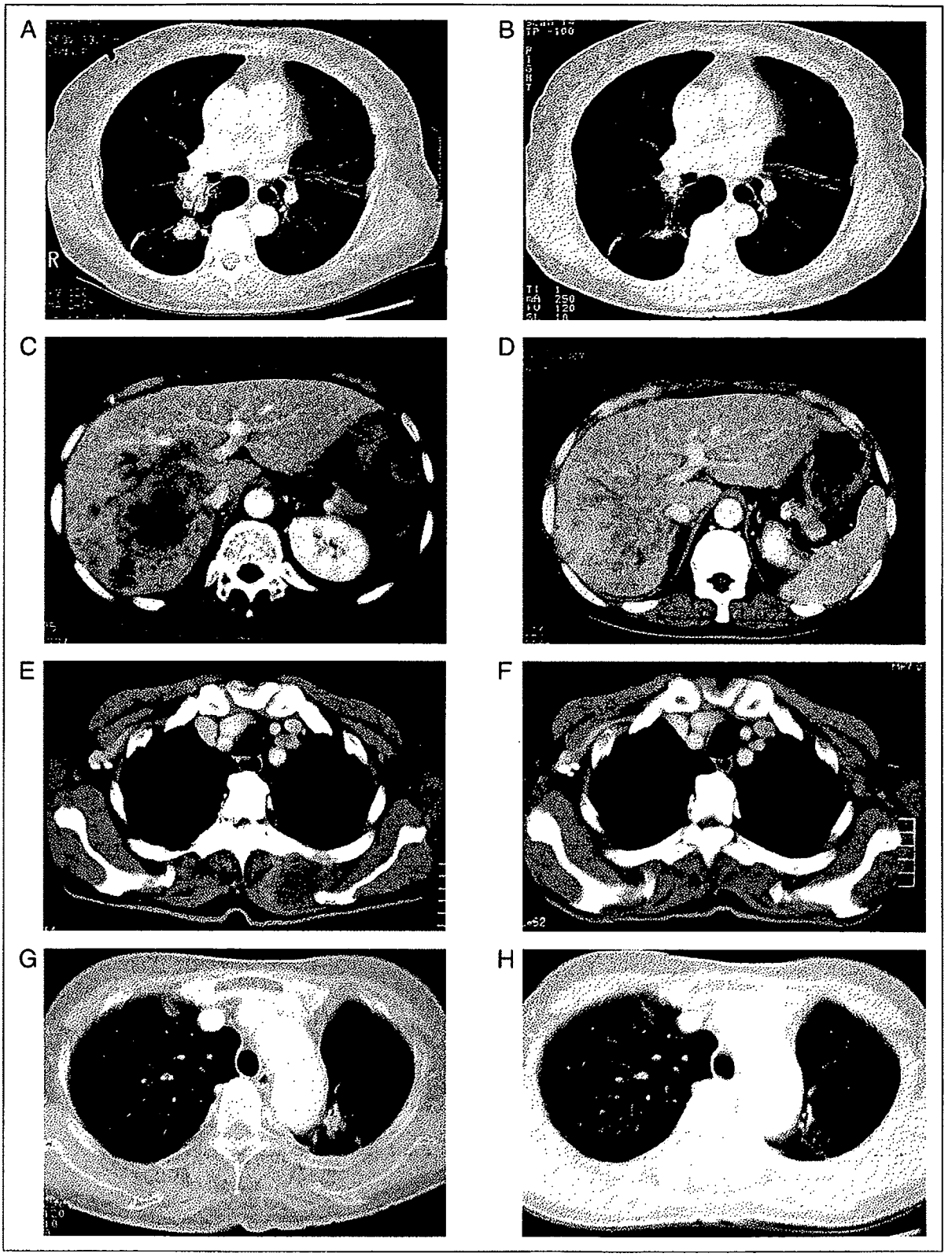

Fig 2. Examples of the response to gefitinib in representative four patients with recurrent non–small-cell lung cancer. Computed tomography (CT) scans before gefitinib treatment (A, C, E, G) and after the gefitinib was initiated (B, D, F, H) are shown. CT scans of patient L703 (A, B), patient L1492 (C, D), patient L1362 (E, F), and patient L1171 (G, H).

Table 2. Relation Between Gefitinib Effectiveness and Various Clinical and Pathologic Features

Variable	Effective: No. of Patients	Effective: %*	Not Effective	Not Assessable	P†
All patients	26	52	24	9	
Sex					
Male	11	41	16	5	.0842
Female	15	65	8	4	
Smoking status					
Never-smoker	17	68	8	3	.0235
Former or current smoker	9	36	16	6	
Histologic type					
Adenocarcinoma	25	58	18	7	.0313
Nonadenocarcinoma	1	14	6	2	
Prior chemotherapy					
Present	17	47	19	4	.2782
Absent	9	64	5	5	
EGFR mutation					
Mutation	24	83	5	4	< .0001
Deletion	16	100	0	1	.0108‡
Insertion	0	0	1	0	
Point mutation	8	67	4	3	
Wild-type	2	10	19	5	

Abbreviation: EGFR, epidermal growth factor receptor.
*Percentages were calculated excluding patients who were not assessable.
†*P* values were calculated excluding patients who were not assessable.
‡*P* value for Fisher's exact test comparing deletion mutants with the other mutants.

(Figs 2G and H) and a decrease in CEA level from 16.8 to 4.3 ng/mL, did not have *EGFR* mutations. In this patient, we extended our search for mutations to exons 22 and 23 of the *EGFR* gene, and still found none. Another patient without *EGFR* mutation in whom gefitinib was effective was a 59-year-old man who showed a decrease in serum CEA level from 10.6 to 1.5 ng/mL after 2 weeks of gefitinib treatment; this low level of CEA was maintained at least for 7 months.

When we further analyzed gefitinib response by classes of *EGFR* mutation, we found that there was a difference of response between patients with deletion mutations and those with the other types of mutations. Gefitinib was effective in all 16 patients with deletions, and effective in eight of 13 with other types of mutation ($P = .0108$).

Effect of EGFR *Mutation on Patient Survival After Gefitinib Treatment*

Patients with *EGFR* mutations survived for a significantly longer time calculated from the day of gefitinib initiation than those without *EGFR* mutations ($P = .0053$, log-rank test; Fig 3). Likewise, 26 gefitinib responders survived for a longer time than 24 nonresponders ($P = .0320$, log-rank test; not shown). Multivariate analysis revealed that *EGFR* mutation was the only factor that significantly and independently affected overall survival (Table 5). *EGFR* mutation class did not affect overall survival (not shown).

DISCUSSION

Recurrence after complete resection of NSCLC often presents as a form of distant metastases.[20] In clinical practice, chemotherapy is given to these patients except for a small number in whom re-resection of the tumor is indicated. Many studies have shown that chemotherapy prolongs survival and improves quality of life in unresectable stage IV tumors.[21] However, patients with unresectable tumors and patients with recurrent diseases may not be the same. There have been no large-scale randomized clinical trials addressing whether chemotherapy improves survival of patients with recurrence. Yoshino et al[22] found that chemotherapy for recurrence only tended to prolong survival in 118 of 468 consecutive patients who had recurrence after pulmonary resections. After introduction of gefitinib to clinical practice in 2002 in Japan, some patients with recurrent disease showed dramatic responses to gefitinib treatment, but many others did not respond. It has been unclear which patients respond to gefitinib and also whether gefitinib treatment prolongs survival in these patients.

Recent studies have showed striking correlation between gefitinib sensitivity and *EGFR* mutations both in vitro and in clinical studies.[15-17] Because this study was a retrospective analysis of response to gefitinib prescribed as routine care, judgment of gefitinib effectiveness tended to be less strict than that in a prospective clinical trial. Yet, changes in serum CEA level never conflicted with imaging studies. We were able to confirm a relation between *EGFR*

Table 3. Response to Gefitinib Treatment in 59 Patients With Recurrent Disease

CEA Level	Imaging Results: Shrinkage	No Change	Not Measurable	Growth	Total
Decreased					
<10% of the baseline	**3 (3)**				3 (3)
<50% of the baseline	**6 (5)**	**1 (1)**	**5 (4)**		12 (10)
>50% of the baseline	**2 (2)**		*3 (2)*		5 (4)
Not assessable	**9 (9)**	*3 (1)*	*3 (1)*	12 (2)	27 (13)
Elevated			7 (3)	5 (0)	12 (3)
Total	20 (19)	4 (2)	18 (10)	17 (2)	59 (33)

NOTE. Numbers in bold indicate that gefitinib treatment resulted in clinical improvement in these patients; numbers with underlines indicate the treatment resulted in progression of the disease; numbers in parentheses show number of patients with EGFR mutations in each category; and italicized numbers indicate that gefitinib treatment could not be assessed.
Abbreviations: EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen.

Table 4. Logistic Regression Analysis of Various Factors That Predict EGFR Effectiveness

Variable	Odds Ratio	95% CI	P
Sex			
Male/female	1.139	0.130 to 9.953	.9063
Smoking status			
Never/former/current	1.496	0.165 to 13.535	.7202
Histologic type			
Adenocarcinoma/ nonadenocarcinoma	1.727	0.091 to 33.33	.7159
Prior chemotherapy			
Yes/no	0.427	0.060 to 3.027	.3948
EGFR mutation			
Mutant/wild-type	40.000	6.024 to 2750	< .0001

Abbreviation: EGFR, epidermal growth factor receptor.

mutations and gefitinib sensitivity in a slightly different clinical setting. We correlated *EGFR* mutations found in specimens taken at the time of surgery with response to gefitinib, often after several courses of cytotoxic chemotherapy for recurrent disease. Multivariate analysis revealed that *EGFR* mutation was the only independent predictor for gefitinib response among several allegedly contributing factors. As in previous studies, *EGFR* mutation was not a perfect predictor of gefitinib effectiveness.[15-17] Two patients without *EGFR* mutations showed response to gefitinib. It is not clear at this time whether *EGFR* mutations are present in other parts of the gene or whether mechanisms other than *EGFR* mutations govern sensitivity in these patients.

We found a significant difference in gefitinib sensitivity according to classes of *EGFR* mutations. All 16 patients with deletion mutants responded to gefitinib, compared with eight of 12 patients with other mutations (P = .0108). It is not clear whether this difference is based on differences in biologic activity of these mutant proteins.

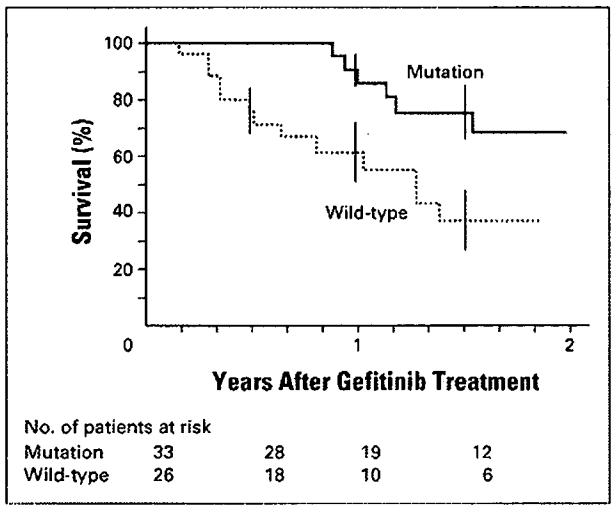

Fig 3. Effect of epidermal growth factor receptor mutations on survival, calculated from the day of initiating gefitinib treatment in patients who had recurrent disease after surgery (P = .0053, log-rank test).

Table 5. Cox Proportional Hazards Model for Survival Analysis

Variable	Hazard Ratio	95% CI	P
Sex			
Female/male	0.359	0.068 to 1.900	.2280
Smoking status			
Never/former/current	0.511	0.092 to 2.854	.4445
Histologic type			
Adenocarcinoma/ nonadenocarcinoma	0.335	0.095 to 1.184	.0894
Prior chemotherapy			
Yes/no	0.653	0.222 to 1.923	.4397
Stage			
I-II/III-IV	0.848	0.322 to 2.232	.7380
Age, years			
> 64/≤ 64	0.964	0.342 to 2.717	.9457
EGFR mutation			
Mutant/wild-type	0.342	0.117 to 0.998	.0496

Abbreviation: EGFR, epidermal growth factor receptor.

Gefitinib sensitivity was essentially the same in COS cells transfected with L858R and in cells transfected with del L747-P753insS.[16] A more recent study showed that the tyrosine residue at codon 845 is highly phosphorylated in L858R mutants, but not in deletion mutants after epidermal growth factor binding.[23] This might explain the difference in gefitinib response between tumors with L858R and those with deletions.

Although our criteria for tumor response are soft, these are merely a surrogate marker for the effect on survival. We were able to show, for the first time, that *EGFR* mutation was the only significant and independent predictor for a prolonged survival after gefitinib treatment. In a previous study, we showed that *EGFR* mutation itself is not a predictor for better postoperative survival in 236 unselected patients with adenocarcinoma,[24] and in the present study, median disease-free interval was almost identical in patients with or without *EGFR* mutations. A recent placebo-controlled clinical trial showed that treatment with erlotinib, another oral EGFR TK inhibitor, significantly prolongs survival after first and second chemotherapy for NSCLC,[25] although *EGFR* mutation frequency is reported to be around 10% in Western countries.[15-17] This result is interpreted to mean that a subset of patients without mutations have also benefited from erlotinib therapy. The present study suggests that if patients were selected by presence of *EGFR* mutations, it would be possible to concentrate patients with benefits from gefitinib treatment, avoiding unnecessary adverse reactions such as fatal interstitial lung disease, which is relatively common in Japanese patients.[26] Furthermore, our results provide a basis for postoperative adjuvant gefitinib treatment in NSCLC patients with *EGFR* mutations, as adjuvant treatment is considered the earliest treatment of metastatic disease. These possibilities should be tested in future clinical trials.

It is common for patients to show progressive disease soon after presenting an initial striking response to

gefitinib. However, we could not detect any evidence that differences in classes of *EGFR* mutations are associated with duration of response (data not shown).

In conclusion, tumors with *EGFR* mutations showed good, but not perfect, correlation with clinical response in patients with postoperative recurrence of NSCLC. Furthermore, patients with *EGFR* mutations survived for a significantly longer period than those without *EGFR* mutations. Future clinical trials using gefitinib should examine *EGFR* mutations for effective selection of patients who are most likely to benefit from this molecular-targeted drug.

Acknowledgment

We thank Kaori Hayashi-Hirano for excellent technical assistance in molecular analysis of tumors, and Ryuzo Ohno, President of Aichi Cancer Center, for special encouragement and support.

Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Honoraria: Tetsuya Mitsudomi, AstraZeneca Japan, Bristol-Myers Squibb Japan, TAIHO Pharmaceutical. For a detailed description of this category, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and Disclosures of Potential Conflicts of Interest found in Information for Contributors in the front of each issue.

REFERENCES

1. Kuroishi T, Hirose K, Tajima K: Cancer mortality in Japan, in Tominaga S, Oshima A (eds): Gann Monograph Cancer Research. Tokyo, Japan Scientific Societies Press, 1999, pp 1-38
2. Mitsudomi T, Viallet J, Linnoila RI, et al: Mutations of ras genes distinguish a subset of non-small cell lung cancer cell lines from small cell lung cancer cell lines. Oncogene 6:1353-1362, 1991
3. Mitsudomi T, Oyama T, Nishida K, et al: p53 nuclear immunostaining and gene mutations in non-small-cell lung cancer and their effects on patient survival. Ann Oncol 6:S9-13, 1995
4. Nishio M, Koshikawa T, Kuroishi T, et al: Prognostic significance of abnormal p53 accumulation in primary, resected non-small-cell lung cancers. J Clin Oncol 14:497-502, 1996
5. Nishio M, Koshikawa T, Yatabe Y, et al: Prognostic significance of cyclin D1 and Rb expression in combination with p53 abnormalities in primary, resected non-small cell lung cancer. Clin Cancer Res 3:1051-1058, 1997
6. Yatabe Y, Masuda A, Koshikawa T, et al: p27kip1 in human lung cancers: Differential changes in small cell and non-small cell carcinomas. Cancer Res 58:1042-1047, 1998
7. Hida T, Yatabe Y, Achiwa H, et al: Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res 58:3761-3764, 1998
8. Arteaga CL: Overview of epidermal growth factor receptor biology and its role as a therapeutic target in human neoplasia. Semin Oncol 29:3-9, 2002
9. Bunn PA, Jr., Franklin W: Epidermal growth factor receptor expression, signal pathway, and inhibitors in non-small cell lung cancer. Semin Oncol 29:38-44, 2002
10. Ciardiello F, Tortora G: A novel approach in the treatment of cancer: Targeting the epidermal growth factor receptor. Clin Cancer Res 7:2958-2970, 2001
11. Kris MG, Natale RB, Herbst RS, et al: Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: A randomized trial. JAMA 290:2149-2158, 2003
12. Fukuoka M, Yano S, Giaccone G, et al: Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol 21:2237-2246, 2003
13. Cappuzzo F, Gregorc V, Rossi E, et al: Gefitinib in pretreated non-small-cell lung cancer (NSCLC): Analysis of efficacy and correlation with HER2 and epidermal growth factor receptor expression in locally advanced or metastatic NSCLC. J Clin Oncol 21:2658-2663, 2003
14. Suzuki T, Nakagawa T, Endo H, et al: The sensitivity of lung cancer cell lines to the EGFR-selective tyrosine kinase inhibitor ZD1839 ('Iressa') is not related to the expression of EGFR or HER-2 or to K-ras gene status. Lung Cancer 42:35-41, 2003
15. Paez JG, Janne PA, Lee JC, et al: EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 304:1497-1500, 2004
16. Lynch TJ, Bell DW, Sordella R, et al: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129-2139, 2004
17. Pao W, Miller V, Zakowski M, et al: EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 101:13306-13311, 2004
18. Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors. European Organisation for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000
19. Salgia R, Harpole D, Herndon JE, 2nd, et al: Role of serum tumor markers CA 125 and CEA in non-small cell lung cancer. Anticancer Res 21:1241-1246, 2001
20. Mitsudomi T, Nishioka K, Maruyama R, et al: Kinetic analysis of recurrence and survival after potentially curative resection of nonsmall cell lung cancer. J Surg Oncol 63:159-165, 1996
21. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomized trials. Non-Small Cell Lung Cancer Collaborative Group. BMJ 311:899-909, 1995
22. Yoshino I, Yohena T, Kitajima M, et al: Survival of non-small cell lung cancer patients with postoperative recurrence at distant organs. Ann Thorac Cardiovasc Surg 7:204-209, 2001
23. Sordella R, Bell DW, Haber DA, et al: Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 305:1163-1167, 2004
24. Kosaka T, Yatabe Y, Endoh H, et al: Mutations of the epidermal growth factor receptor gene in lung cancer: Biological and clinical implications. Cancer Res 64:8919-8923, 2004
25. Shepherd FA, Pereira J, Ciuleanu TE, et al: A randomized placebo-controlled trial of erlotinib in patients with advanced non-small cell lung cancer following failure of 1st line or 2nd line chemotherapy. Proc Am Soc Clin Oncol 22:622s, 2004 (abstr 7022)
26. Inoue A, Saijo Y, Maemondo M, et al: Severe acute interstitial pneumonia and gefitinib. Lancet 361:137-139, 2003

Annals of Oncology Advance Access published January 14, 2005

Original article

Annals of Oncology
doi:10.1093/annonc/mdi081

Phase I–II study of amrubicin and cisplatin in previously untreated patients with extensive-stage small-cell lung cancer

Y. Ohe[1]*, S. Negoro[2], K. Matsui[3], K. Nakagawa[4], T. Sugiura[5], Y. Takada[6], Y. Nishiwaki[7], S. Yokota[8], M. Kawahara[9], N. Saijo[7], M. Fukuoka[4] & Y. Ariyoshi[10]
On behalf of the Amrubicin SCLC Study Group

[1]Department of Internal Medicine, National Cancer Center Hospital, Tokyo; [2]Department of Clinical Oncology, Osaka City General Hospital, Osaka; [3]Department of Thoracic Malignancy, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino, Osaka; [4]Department of Medical Oncology, Kinki University School of Medicine, Osakasayama, Osaka; [5]Department of Internal Medicine, Aichi Cancer Center Hospital, Nagoya, Aichi, [6]Department of Radiology, Hyogo Medical Center for Adults, Akashi, Hyogo; [7]Division of Thoracic Oncology, National Cancer Center Hospital East, Kashiwa, Chiba; [8]Department of Internal Medicine, National Hospital Organization Toneyama National Hospital, Toyonaka, Osaka; [9]Department of Internal Medicine, National Hospital Organization Kinki-Chuo Chest Medical Center, Sakai, Osaka; [10]Aichi Prefectural Hospital, Okazaki, Aichi, Japan

Received 2 September 2004; revised 14 October 2004; accepted 22 October 2004

Background: Amrubicin, a totally synthetic 9-amino-anthracycline, demonstrated excellent single-agent activity for extensive-stage small-cell lung cancer (ED-SCLC). The aims of this trial were to determine the maximum-tolerated doses (MTD) of combination therapy with amrubicin and cisplatin, and to assess the efficacy and safety at their recommended doses (RD).

Patients and methods: Eligibility criteria were patients having histologically or cytologically proven measurable ED-SCLC, no previous systemic therapy, an Eastern Cooperative Oncology Group performance status of 0–2 and adequate organ function. Amrubicin was administered on days 1–3 and cisplatin on day 1, every 3 weeks.

Results: Four patients were enrolled at dose level 1 (amrubicin 40 mg/m^2/day and cisplatin 60 mg/m^2) and three patients at level 2 (amrubicin 45 mg/m^2/day and cisplatin 60 mg/m^2). Consequently, the MTD and RD were determined to be at level 2 and level 1, respectively. The response rate at the RD was 87.8% (36/41). The median survival time (MST) was 13.6 months and the 1-year survival rate was 56.1%. Grade 3/4 neutropenia and leukopenia occurred in 95.1% and 65.9% of patients, respectively.

Conclusions: The combination of amrubicin and cisplatin has demonstrated an impressive response rate and MST in patients with previously untreated ED-SCLC.

Key words: anthracycline, cisplatin, phase I–II, small-cell lung cancer

Introduction

Small-cell lung cancer (SCLC) is one of the most chemosensitive solid tumors, and the outcome of SCLC patients is slowly but surely improving. Combination chemotherapy consisting of cisplatin plus etoposide and concurrent twice-daily thoracic radiotherapy has yielded a 26% 5-year survival rate in limited-stage (LD) patients [1]. Despite the high response rate to combination chemotherapy, however, local and distant failure is very common, especially in extensive-stage (ED) patients. Moreover, resistance to chemotherapeutic agents develops easily after failure of initial treatment. Thus, long-term survivors are still very rare among patients with ED-SCLC. To improve the outcome of SCLC patients, several strategies, such as high-dose chemotherapy, dose-intensive chemotherapy, alternating chemotherapy and introduction of new drugs, have been investigated [2–6]. However, only the introduction of new agents has improved the outcome of SCLC patients. Combination chemotherapy with etoposide plus cisplatin or etoposide plus cisplatin alternating cyclophosphamide, doxorubicin and vincristine had been mainly used for SCLC in North America. Recently, a Japanese trial [Japan Clinical Oncology Group (JCOG) 9511] demonstrated the superiority of the combination of irinotecan and cisplatin for ED-SCLC patients over the combination of etoposide and cisplatin [6]. The development of more active chemotherapy, and especially the introduction of effective new drugs, is therefore essential to improve the survival of SCLC patients.

Amrubicin (SM-5887) is a totally synthetic anthracycline and a potent topoisomerase II inhibitor [7–14]. It has antitumor activity, and is more potent than doxorubicin against various mouse experimental tumors and human tumor

*Correspondence to: Dr Y. Ohe, Department of Internal Medicine, National Cancer Center Hospital 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Tel: +81-3-3542-2511; Fax: +81-3-3542-7006; E-mail: yohe@ncc.go.jp

xenografts. Amrubicin and its 13-hydroxy metabolite, amrubicinol, inhibit purified human DNA topoisomerase II [11]. Amrubicinol is 10–100 times more cytotoxic than amrubicin [9]. The potent therapeutic activity of amrubicin is caused by the selective distribution of its highly active metabolite, amrubicinol, in tumors [9]. In an experimental animal model, amrubicin did not exhibit any chronic cardiotoxicity potential, and no deleterious effects on doxorubicin-induced cardiotoxicity in dogs was observed [14]. In a phase II study of amrubicin using a schedule of 45 mg/m^2 on days 1–3 every 3 weeks, in 33 previously untreated ED-SCLC patients, an overall response rate of 76% and a complete response (CR) rate of 9% were reported [15]. Moreover, median survival time (MST) was 11.7 months in the single-agent phase II study of amrubicin. Amrubicin is one of the most active new agents for SCLC. Thus, we conducted a phase I/II study of amrubicin plus cisplatin for untreated ED-SCLC, because cisplatin is considered as one of the most important drugs in the treatment of SCLC. The aims of this trial were to determine the maximum-tolerated doses (MTD) of combination therapy of amrubicin with cisplatin, to assess the efficacy and safety for ED-SCLC at their recommended doses (RD), and to examine the pharmacokinetics of the drug combination.

Patients and methods

Patient selection

Patients with histologically and/or cytologically documented SCLC were eligible for this study. Each patient was required to meet the following criteria: extensive-stage disease [16]; no prior therapy for primary lesion; measurable lesion; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2; expected survival time >2 months; age 20–74 years; adequate hematological function [white blood cell (WBC) count 4000–12 000/mm^3, neutrophils ≥2000/mm^3, platelets ≥100 000/mm^3, hemoglobin ≥10 g/dl]; adequate hepatic function [total bilirubin within 1.5× the upper limit of normal; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within 2.5× the upper limit of normal]; adequate renal function (creatinine within the upper limit of normal); partial pressure of arterial oxygen 60 torr; no abnormality requiring treatment on electrocardiogram; left ventricle ejection fraction >60%; written informed consent. Patients with symptomatic brain metastasis, pleural effusion that required drainage, non-steroidal anti-inflammatory drug or glucocorticoid use for >50 days, pericarditis carcinomatous, active infection, varicella, superior vena cava syndrome, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), gastric and/or duodenal ulcer, severe heart disease, severe renal disease, active concomitant malignancy, symptomatic pneumonitis and/or pulmonary fibrosis and pregnant/nursing women were excluded. This study was approved by the Institutional Review Board at each hospital.

Patient evaluation

Pretreatment evaluation consisted of complete blood cell counts, differential, routine chemistry measurements, progastrin-releasing peptide (ProGRP), neuron-specific enolase, electrocardiogram, echocardiography, chest radiograph, chest and abdominal computed tomography (CT) scan, whole-brain magnetic resonance imaging (MRI) or CT scan, and isotope bone scan. Complete blood cell counts, differential and routine chemistry measurements were performed at least once a week during the chemotherapy.

Treatment schedule

At level 1, chemotherapy consisted of cisplatin 60 mg/m^2 on day 1 and amrubicin 40 mg/m^2 on days 1–3. Amrubicin was administered as an intravenous injection over 5 min and cisplatin was administered as a drip infusion over 60–120 min with adequate hydration. At level 2 the dose of amrubicin was increased to 45 mg/m^2 on days 1–3. Level 3 was planned with cisplatin 80 mg/m^2 on day 1 and amrubicin 45 mg/m^2 on days 1–3. The chemotherapy was repeated every 3 weeks for four to six courses. Intrapatient dose escalation was not allowed. Administration of granulocyte colony-stimulating factor (G-CSF) was permitted prophylactically for patients expected to experience grade 3 neutropenia during the first course. Prophylactic administration of G-CSF was only permitted at second or later courses.

The administrations of both cisplatin and amrubicin were postponed if patients met the following criteria: WBC <3000/mm^3; neutrophils <1500/mm^3; platelets <100 000/mm^3; AST and ALT >5× the upper limit of normal; total bilirubin >1.5× the upper limit of normal; creatinine >1.3× the upper limit of normal; ECOG PS 3 or 4; active infection; grade 2 or worse non-hematological toxicity, except for alopecia, anorexia, nausea, vomiting or fatigue.

The administrations of both cisplatin and amrubicin were withdrawn if patients met the following criteria: tumor regression <15% after first course or <30% after second course; WBC <3000/mm^3; neutrophils <1500/mm^3; platelets <100 000/mm^3; no recovery from grade 3 or 4 non-hematological toxicity at 6 weeks after the start of previous chemotherapy; abnormality of electrocardiogram requiring treatment for more than 6 weeks; left ventricle ejection fraction <48%; treatment delay of >4 weeks.

The dose of amrubicin was decreased 5 mg/m^2/day if patients met the following criteria: grade 4 leukopenia or neutropenia for ≥4 days; grade 3 neutropenia with fever; platelets <20 000/mm^3 during the previous course. The dose of cisplatin was decreased to 75% if creatinine increased to >1.5× the upper limit of normal during the previous course.

The dose-limiting toxicity (DLT) was defined as follows: grade 4 leukopenia or neutropenia for ≥4 days; grade 3 febrile neutropenia; platelets <20 000/mm^3; grade 3 or worse non-hematological toxicity except for nausea, vomiting, anorexia, fatigue, hyponatremia and infection. Initially, three patients were treated at each dose level. If DLT was not observed in any of the three patients, dose escalation was carried out. If DLT was observed in one of three patients, an additional three patients were entered at the same dose level. If DLT was observed in three or more of six patients, or two or three of the initial three patients, we considered that dose to be the MTD. If DLT was observed in one or two of six patients, dose escalation was also carried out. Dose escalation was determined based only on the data from the first course of chemotherapy.

Response and toxicity evaluation

Response was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) and tumor markers were excluded from the criteria [17]. CR was defined as the complete disappearance of all clinically detectable tumors for at least 4 weeks and no new lesions. Partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameters of target lesion, taking as reference the baseline sum longest diameter, the required non-progression in non-target lesions and no new lesions for at least 4 weeks. Stable disease (SD) included: regression of target lesions insufficient to meet the criteria for PR, a <20% increase in the sum of the longest diameter of target lesion, taking as reference the smallest sum longest diameters recorded since the treatment started, the required non-progression in non-target lesions and no new lesions for at least 6 weeks. Progressive disease (PD) indicated a >20% increase in the sum of the longest diameters of target lesion, taking as reference the smallest sum longest diameter recorded since the treatment started

and/or unequivocal progression of existing non-target lesions and/or appearance of new lesions. The evaluation of objective tumor response for all patients was performed by an external review committee.

Toxicity grading criteria of the National Cancer Institute Common Toxicity Criteria (version 2.0) was used for evaluation of toxicity.

Statistical analysis

This study was designed to reject response rates of 70% (P0) at a significance level of 0.05 (one-tailed) with a statistical power of 80% to assess the activity of the regimen as a 85% response rate (P1) at the recommended dose. The upper limit of rejection was 29 responses (CR + PR) among 37 evaluable patients. Overall survival was defined as the interval between the first administration of the drugs in this study and death or the last follow-up visit. Median overall survival was estimated using the Kaplan–Meier method [18].

Table 1. Characteristics of treated patients

	Phase I	Phase II	Total
Number of patients	7	37	44
Gender			
Male	5	31	36
Female	2	6	8
Age (years)			
Median	65	64	64.5
Range	54–73	50–74	50–74
ECOG PS			
0	0	5	5
1	7	32	39
2	0	0	0
Stage			
IIIB	0	2	2
IV	7	35	42
Prior therapy			
Yes	0	1	1
No	7	36	43
Serum ALP			
Normal	7	29	36
Elevated	0	7	7
Serum LDH			
Normal	3	14	17
Elevated	4	23	27
Na			
Normal	6	35	41
Decreased	1	2	3
Number of metastases			
0	0	2	2
1	4	27	31
2	3	6	9
3	0	1	1
4 or more	0	1	1

In one patient, serum ALP level could not be measured.
ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed in patients entering the phase I section of this study. One milliliter of the blood was taken from the patients before administration of amrubicin, and at 0 min, 15 min, 1, 2, 3, 4, 8 and 24 h after administration on days 1 and 3 in the first course of chemotherapy. Concentrations of amrubicin and its active metabolite, amrubicinol, in plasma and red blood cells were measured as reported elsewhere [9].

Results

Patient characteristics

Between April 2001 and December 2002, 45 patients with ED-SCLC were enrolled and 44 were treated in this study (Table 1). One patient did not receive the protocol treatment because atrial fibrillation was observed just before administration on day 1 of the first course. All treated patients were assessed for response, survival and toxicity. The median age of the treated patients was 64.5 years (range 50–74). There were 36 males and eight females. Five patients had an ECOG PS 0 and 39 patients had PS 1. Only one patient received surgery for brain metastasis as a prior therapy.

MTD and DLT in the phase I study

Four patients were enrolled at dose level 1 (amrubicin 40 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1) and three patients at level 2 (amrubicin 45 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1). Toxicities in the phase I study are listed in Table 2. No DLT were observed during the first course of level 1. At level 2, grade 4 neutropenia for ≥4 days and febrile neutropenia occurred in one patient, and febrile neutropenia and grade 3 constipation occurred in another patient. Consequently, the MTD and RD were determined to be level 2 and level 1, respectively.

Pharmacokinetics of amrubicin and its active metabolite, amrubicinol

Pharmacokinetic parameters of amrubicin in plasma were almost identical on days 1 and 3 at the two dose levels (Table 3). No clear dose relationship in the area under the concentration–time curve (AUC) of amrubicin in the plasma was observed. The AUC of amrubicinol in red blood cells tended to increase on day 3 at both doses (Table 4). No clear dose relationship in the AUC of amrubicinol in red blood cells was observed. Combination with cisplatin did not alter the pharmacokinetics of amrubicin and amrubicinol (data not shown).

Treatment received in patients treated at the RD

Forty-one patients were treated at the RD: amrubicin 40 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1. Of 41 patients, 32 (78%) patients received more than three

Table 2. Toxicities during the first course in the phase I study

	Level 1 ($n=4$)					Level 2 ($n=3$)				
Amrubicin	40 mg/m^2 days 1–3					45 mg/m^2 days 1–3				
Cisplatin	60 mg/m^2 day 1					60 mg/m^2 day 1				
	Grade (NCI CTC)					Grade (NCI CTC)				
	0	1	2	3	4	0	1	2	3	4
Leukopenia	0	1	1	2	0	0	0	1	1	1
Neutropenia	0	0	0	2	2	0	0	0	0	3
Febrile neutropenia	4	–	–	0	0	1	–	–	2	0
Hemoglobin	1	1	2	0	0	2	1	0	0	0
Thrombocytopenia	1	2	0	1	0	0	2	0	1	0
Stomatitis	3	0	1	0	0	3	0	0	0	0
Nausea	1	1	2	0	–	1	1	0	1	–
Constipation	3	0	1	0	0	1	0	1	1	0
Hyponatremia	2	1	0	0	1	1	2	0	0	0
Hypocalcemia	3	0	1	0	0	3	0	0	0	0

Dose limiting toxicity at level 2: febrile neutropenia, two patients; grade 4 neutropenia ≥4 days, one patient; grade 3 constipation, one patient.
NCI CTC, National Cancer Institute Common Toxicity Criteria.

Table 3. Pharmacokinetics of amrubicin in plasma

Dose	n	Day	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	V_d (l)	CL (l/h)	$AUC_{0-24\,h}$ (ng h/ml)
40 mg/m^2	4	1	0.11 ± 0.04	2.29 ± 0.31	46.6 ± 11.0	13.6 ± 1.8	2995 ± 434
	4	3	0.08 ± 0.01	2.89 ± 0.34	50.0 ± 10.6	11.6 ± 1.9	3511 ± 514
45 mg/m^2	3	1	0.13 ± 0.05	2.39 ± 0.34	56.3 ± 10.6	14.9 ± 1.8	3052 ± 402
	3	3	0.09 ± 0.03	2.27 ± 0.18	51.9 ± 3.7	14.2 ± 2.3	3217 ± 479

$T_{1/2\alpha}$, half-life at distribution phase; $T_{1/2\beta}$, half-life at elimination phase; V_d, volume of distribution; CL, clearance; AUC, area under the concentration–time curve.

courses of chemotherapy, and 10 (31%) of these 32 patients needed dose reduction of amrubicin at the fourth course (Table 5). Of 41 patients, 22 (54%) patients completed four courses of chemotherapy without dose modification. The main cause of dose reduction was myelosuppression, especially leukopenia and neutropenia.

Objective tumor response and overall survival

The objective tumor responses are given in Table 6. Four CRs and 32 PRs occurred, for an objective response rate of 87.8% [95% confidence interval (CI) 73.8% to 95.9%] in 41 patients treated at the RD. The objective response rate for all 44 patients was 88.6% (95% CI 75.4% to 96.2%). The overall survival times of the 41 patients treated at the RD are shown in Figure 1. The MST of the 41 patients was 13.6 months (95% CI 11.1–16.6), with a median follow-up time for eight censored patients of 16.4 months (95% CI 14.2–18.8). The 1- and 2-year survival rates were 56.1% and 17.6%, respectively. The MST of all 44 patients was 13.8 months (95% CI 11.1–16.6). The 1- and 2-year survival rates of all 44 patients were 56.8% and 21.4%, respectively.

Table 4. Pharmacokinetics of amrubicinol in red blood cells

Dose	n	Day	$T_{1/2}$ (h)	$AUC_{0-24\,h}$ (ng·h/ml)
40 mg/m^2	4	1	21.0 ± 3.1	1412 ± 314
	4	3	20.7 ± 4.8	2159 ± 622
45 mg/m^2	3	1	19.6 ± 6.1	1098 ± 277
	3	3	18.1 ± 5.7	2027 ± 332

$T_{1/2}$, elimination half-life; AUC, area under the concentration–time curve.

Table 5. Treatment received in patients treated at the recommended dose

Cycle	n	Amrubicin (mg/m^2)			Cisplatin (mg/m^2)	
		40	35	30	60	45
1	41	41			41	
2	36	30	6		36	
3	33	26	5	2	33	
4	32	22	8	2	32	
5	18	9	5	4	18	
6	13	6	3	4	12	1

Table 6. Response rates

	n	CR	PR	SD	PD	NE	Response rate (%) (95% CI)
All	44	4	35	3	0	2	88.6 (75.4–96.2)
Treated at RD	41	4	32	3	0	2	87.8 (73.8–95.9)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; 95% CI, 95% confidence interval; RD, recommended dose.

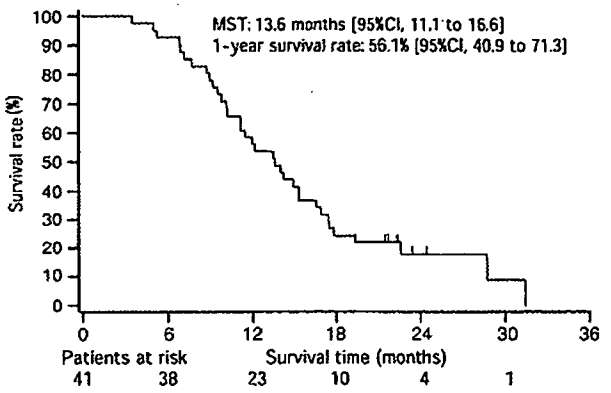

Figure 1. Overall survival of patients with extensive-stage small-cell lung cancer who were treated with amrubicin and cisplatin at the recommended dose. MST, median survival time; 95% CI, 95% confidence interval.

Toxicity in patients treated at the RD

The worst grades of hematological and non-hematological toxicities experienced by each patient are listed in Table 7. Hematological toxicity, especially leukopenia and neutropenia, was common and relatively severe. Grade 3 or worse leukopenia and neutropenia occurred in 65.9% and 95.1% of patients, respectively. Febrile neutropenia was observed in two patients at level 2. Grade 3 or worse anemia and thrombocytopenia occurred in 53.7% and 24.4% of patients, respectively. Four patients received platelet transfusions. Common non-hematological toxicities were gastrointestinal toxicity, such as anorexia, nausea, vomiting, constipation, diarrhea and stomatitis. Gastric ulcers developed in three patients. Hepatic and renal toxicity were not common in this study. Grade 3 or worse hyponatremia and hypokalemia occurred in 22% and 9.8% of patients, respectively. One patient developed myocardial infarction; however, cardiac toxicity was not common. No treatment-related deaths were observed.

Discussion

Doxorubicin and epirubicin are classified as active agents for SCLC, for which single-agent activity is a >20% response rate [19]. Doxorubicin has been used as a constituent of combination therapy for SCLC in the CAV (cyclophospamide, doxorubicin and vincristine) and CAP (cyclophosphamide, doxorubicin and cisplatin) regimens. Epirubicin has shown

Table 7. Toxicity in patients treated at the recommended dose ($n=41$)

	Grade (NCI CTC)					Grade 3/4 (%)
	0	1	2	3	4	
Leukopenia	1	0	13	20	7	65.9
Neutropenia	0	1	1	7	32	95.1
Febrile neutropenia	41	–	–	0	0	0.0
Hemoglobin	1	8	10	17	5	53.7
Thrombocytopenia	9	14	8	10	0	24.4
Stomatitis	22	13	5	1	0	2.4
Anorexia	1	14	13	13	0	31.7
Nausea	3	15	14	9	0	22.0
Vomiting	20	8	11	2	0	4.9
Constipation	24	1	13	3	0	7.3
Diarrhea	26	12	1	2	0	4.9
Gastric ulcer	38	0	1	2	0	4.9
Bilirubin	24	12	4	1	0	2.4
Hyponatremia	18	14	–	7	2	22.0
Hypokalemia	31	6	–	4	0	9.8
Hyperkalemia	33	3	4	1	0	2.4
Hypocalcemia	31	5	4	0	1	2.4

NCI CTC, National Cancer Institute Common Toxicity Criteria.

50% and 48% response rates in two clinical studies in 41 and 80 previously untreated patients, respectively, with ED-SCLC [20, 21]. However, currently, combination modalities containing doxorubicin or epirubicin are not being used in the therapy of SCLC, in preference to combination therapy with cisplatin and etoposide. Since amrubicin has shown excellent single-agent activity [15], it can be expected to be superior to other anthracyclines in the treatment of SCLC. Additionally, the present results of combination therapy with cisplatin support the view that amrubicin may be a promising agent that overcomes the therapeutic plateau of SCLC.

Amrubicin is one of the most promising new agents for the treatment of SCLC. In a previous phase II study of amrubicin 45 mg/m^2 on days 1–3 every 3 weeks as a monotherapy for chemonaive ED-SCLC, a 76% overall response rate and 11.7 month MST were observed [15]. The overall response rate and MST were comparable to those achieved with standard combination chemotherapy, such as etoposide plus cisplatin [5, 6]. Moreover, only a few patients treated in the phase II study received salvage chemotherapy consisting of cisplatin and etoposide [15]. The major toxicity of amrubicin as a monotherapy was hematological toxicity: grade 4 leukopenia and neutropenia were seen in 12.1% and 39.4% of patients, respectively, and thrombocytopenia and anemia of grade 3 or worse in 21.2%. Hepatic, renal and cardiac toxicities with amrubicin were not common. Cisplatin is a key drug for the treatment of SCLC and its hematological toxicity, such as leukopenia and neutropenia, is not severe. Thus, we conducted a phase I–II study of amrubicin and cisplatin treatment for chemonaive ED-SCLC to determine the MTD of this combination therapy, to

assess the efficacy and safety of the drugs delivered at their RD in chemonaive ED-SCLC, and to examine pharmacokinetics.

The topoisomerase I inhibitor, irinotecan, is also very effective for SCLC [6]. Combinations of topoisomerase I and topoisomerase II inhibitors, such as irinotecan plus etoposide, have been reported as active combination chemotherapy for SCLC [22]. Thus, combination of irinotecan and amrubicin is another candidate for new combination chemotherapy for SCLC. A phase I study of irinotecan and amrubicin for chemonaive non-SCLC was performed in National Cancer Center Hospital (unpublished data). However, the MTD was less than irinotecan 60 mg/m^2 on days 1 and 8 and amrubicin 35 mg/m^2 on days 2–4, due to relatively severe myelotoxicity. We considered that amrubicin <35 mg/m^2 on days 2–4 with irinotecan 60 mg/m^2 on days 1 and 8 was insufficient to treat SCLC.

In this study, we determined the RD to be amrubicin 40 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1 every 3 weeks, and 41 patients were treated at the RD. Main toxicities of this combination chemotherapy were myelosuppression, especially leukopenia and neutropenia, and gastrointestinal toxicities including anorexia, nausea, vomiting, constipation, diarrhea, stomatitis and gastric ulcer. Of 41 patients, 32 (78%) patients received four or more courses of chemotherapy, and 22 (54%) patients completed four courses of chemotherapy without dose modification. One patient developed myocardial infarction; however, other cardiac toxicity, including decrease in left ventricle ejection fraction, was not observed in up to six courses of chemotherapy. The total dose of amrubicin was 720 mg/m^2. Grade 3 or 4 hyponatremia occurred in nine (22%) patients; however, most of the patients were asymptomatic. No unexpected toxicities and no treatment-related deaths were observed in this study. Toxicities observed in this study were manageable.

Four CRs and 32 PRs occurred, for an objective response rate of 87.8% (95% CI 73.8% to 95.9%) in 41 patients treated at the RD. In most patients, ProGRP levels changed in parallel with tumor responses. The MST of the 41 patients was 13.6 months, and the 1-year survival rate was 56.1%. These results were better than recently reported results for irinotecan and cisplatin in chemonaive ED-SCLC: an objective response rate of 84% and MST of 12.8 months [6]. The combination of amrubicin and cisplatin has demonstrated an impressive response rate and MST in patients with previously untreated ED-SCLC. A possible reason for the better results is overselection of patients, because we used unusual exclusion criteria such as non-steroidal anti-inflammatory drug or adrenal cortical steroid use for >50 days, and gastric and/or duodenal ulcer. However, in a phase II study, this kind of bias is not uncommon.

Combination chemotherapy with etoposide plus cisplatin or etoposide plus cisplatin, alternating with cyclophosphamide, doxorubicin and vincristine, had been considered as standard chemotherapy for SCLC in North America and Japan. A Japanese phase III trial (JCOG 9511) demonstrated that treatment with four cycles of irinotecan plus cisplatin every 4 weeks yielded a highly significant improvement in survival in ED-SCLC patients over standard etoposide plus cisplatin, with less myelosuppression [6]. Based on the results of the JCOG 9511 trial, irinotecan plus cisplatin is considered to be the reference chemotherapy arm for ED-SCLC in future trials in Japan [23]. The JCOG are preparing a phase III clinical trial of amrubicin and cisplatin for previously untreated ED-SCLC to compare combination therapy of irinotecan with cisplatin.

Acknowledgements

This study was supported by Sumitomo Pharmaceuticals Co., Ltd., Osaka, Japan. Previously presented in part at the Annual Meetings of the American Society of Clinical Oncology, Chicago, IL, 31 May to 3 June 2003 and New Orleans, LA, 5–8 June 2004.

References

1. Turrisi A, Kim K, Blum R et al. Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. N Engl J Med 1999; 340: 265–271.
2. Armitage JO. Bone marrow transplantation. N Engl J Med 1994; 330: 827–838.
3. Furuse K, Fukuoka M, Nishiwaki Y et al. Phase III study of intensive weekly chemotherapy with recombinant human granulocyte colony-stimulating factor versus standard chemotherapy in extensive-disease small-cell lung cancer. J Clin Oncol 1998; 16: 2126–2132.
4. Murray N, Livingston RB, Shepherd FA et al. Randomized study of CODE versus alternating CAV/EP for extensive-stage small-cell lung cancer: an Intergroup Study of the National Cancer Institute of Canada Clinical Trials Group and the Southwest Oncology Group. J Clin Oncol 1999; 17: 2300–2308.
5. Fukuoka M, Furuse K, Saijo N et al. Randomized trial of cyclophosphamide, doxorubicin, and vincristine versus cisplatin and etoposide versus alternation of these regimens in small-cell lung cancer. J Natl Cancer Inst 1991; 83: 855–861.
6. Noda K, Nishiwaki Y, Kawahara M et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 2002; 346: 85–91.
7. Ohe Y, Nakagawa K, Fujiwara Y et al. In vitro evaluation of the new anticancer agents KT6149, MX-2, SM5887, menogaril, and liblomycin using cisplatin- or adriamycin-resistant human cancer cell lines. Cancer Res 1949; 49: 4098–4102.
8. Noguchi T, Ichii S, Morisada S et al. In vivo efficacy and tumor-selective metabolism of amrubicin to its active metabolite. Jpn J Cancer Res 1998; 89: 1055–1060.
9. Noguchi T, Ichii S, Morisada S et al. Tumor-selective distribution of an active metabolite of the 9-aminoanthracycline amrubicin. Jpn J Cancer Res 1998; 89: 1061–1066.
10. Yamaoka T, Hanada M, Ichii S et al. Cytotoxicity of amrubicin, a novel 9-aminoanthracycline, and its active metabolite amrubicinol on human tumor cells. Jpn J Cancer Res 1998; 89: 1067–1073.
11. Hanada M, Mizuno S, Fukushima A et al. A new antitumor agent amrubicin induces cell growth inhibition by stabilizing topoisomerase II-DNA complex. Jpn J Cancer Res 1998; 89: 1229–1238.
12. Yamaoka T, Hanada M, Ichii S et al. Uptake and intracellular distribution of amrubicin, a novel 9-amino-anthracycline, and its active metabolite amrubicinol in P388 murine leukemia cells. Jpn J Cancer Res 1999; 90: 685–690.

13. Obara N, Imagawa S, Nakano Y et al. Hematological aspects of a novel 9-aminoanthracycline, amrubicin. Cancer Sci 2003; 94: 1104–1106.
14. Noda T, Watanabe T, Kohda A et al. Chronic effect of a novel synthetic anthracycline derivative (SM-5887) on normal heart and doxorubicin-induced cardiomyopathy in beagle dogs. Invest New Drugs 1998; 16: 121–128.
15. Yana T, Negoro S, Takada Y et al. Phase II study of amrubicin (SM-5887), a 9-amino-anthracycline, in previously untreated patients with extensive stage small-cell lung cancer (ES-SCLC): a West Japan Lung Cancer Group trial. Proc Am Soc Clin Oncol 1998; 17: 450a.
16. Stahel RA, Ginsberg R, Havemann K et al. Staging and prognostic factors in small cell lung cancer: a consensus report. Lung Cancer 1989; 5: 119–126.
17. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 200; 92: 205–216.
18. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53: 457–481.
19. Grant SC, Gralla RJ, Kris MG et al. Single-agent chemotherapy trials in small-cell lung cancer, 1970 to 1990: The case for studies in previously treated patients. J Clin Oncol 1992; 10: 484–494.
20. Blackstein M, Eisenhauer EA, Weirzbicki R et al. Epirubicin in extensive small-cell lung cancer: A phase II study in previously untreated patients: A National Cancer Institute of Canada Clinical Trials Group study. J Clin Oncol 1990; 8: 385–389.
21. Eckhardt S, Kolaric K, Vukas D et al. Phase II study of 4'-epi-doxorubicin in patients with untreated, extensive small cell lung cancer. Med Oncol Tumor Pharmacother 1990; 7: 19–23.
22. Masuda N, Matsui K, Negoro S et al. Combination of irinotecan and etoposide for treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 1998; 16: 3329–3334.
23. Ohe Y, Saijo N. Results of recent Japanese clinical trials in lung cancer. Clin Lung Cancer 2002; 3: 243–248.