Table 3. Twice-daily versus once-daily radiotherapy for LD-SCLC

Author	Chemotherapy	Radiotherapy	*n*	MST (Months)	5-Year Survival	*P* Value
Turrisi[10]	CDDP + ETOP × 4	1.5 Gy × 2/Day, 45 Gy, 1st cycle, continuous	211	23	26%	
	CDDP + ETOP × 4	1.8 Gy/Day, 45 Gy, 1st–2nd cycles, continuous	206	19	16%	$P = 0.04$
Bonner[72] Schild[73]	CDDP + ETOP × 6	1.5 Gy × 2/Day, 48 Gy, 4th–5th cycles, split	130	20.6	22%	
	CDDP + ETOP × 6	1.8 Gy/Day, 50.4 Gy, 4th–5th cycles, continuous	132	20.6	21%	$P = 0.68$

CDDP, cisplatin; ETOP, etoposide

from anticancer drugs by the blood-brain barrier. Several phase III trials have demonstrated that prophylactic cranial irradiation (PCI) reduces the incidence of brain metastasis in patients with SCLC, but no phase III trials have demonstrated a survival benefit of PCI for patients with SCLC.[74–76] A meta-analysis, using individual data on 987 patients with SCLC in complete remission (CR) who took part in seven trials that compared PCI with no PCI demonstrated a survival benefit of PCI.[77] The relative risk of death in the PCI group as compared with the no-PCI group was 0.84 (95% CI, 0.73 to 0.97; $P = 0.01$), which corresponds to a 5.4% increase in the rate of survival at 3 years (15.3% in the no-PCI group vs 20.7 % in the PCI group). This absolute improvement in 3-year survival (5.4%) was the same as that shown in a meta-analysis comparing chemotherapy with chemoradiotherapy for SCLC.[70,77] Thus, PCI for SCLC, in patients who have achieved a complete response (CR), has a power to improve survival similar to that of thoracic radiotherapy for LD-SCLC.

The state-of-the-art treatment for LD-SCLC is considered to be four cycles of combination chemotherapy with cisplatin plus etoposide, combined with early concurrent twice-daily thoracic irradiation (45 Gy). If patients achieve a CR, PCI should be administered. A 5-year survival rate of approximately 25% is expected with the state-of-the-art treatment for LD-SCLC.

Future directions

The JCOG conducted a randomized multicenter phase III study of irinotecan plus cisplatin versus etoposide plus cisplatin for previously untreated patients with ED-SCLC (JCOG 9511).[8] One hundred and fifty-four patients were randomized, 77 into each arm. The median survival time was 12.8 months in the irinotecan-plus-cisplatin arm and 9.4 months in the etoposide-plus-cisplatin arm. The irinotecan-plus-cisplatin arm showed significantly better survival compared with standard treatment with etoposide plus cisplatin ($P = 0.002$; unadjusted one-sided log-rank test). Treatment with four cycles of irinotecan plus cisplatin every 4 weeks in ED-SCLC patients yielded a highly significant improvement in survival, with less myelosuppression, over the standard etoposide plus cisplatin.[8] Thus, the incorporation of

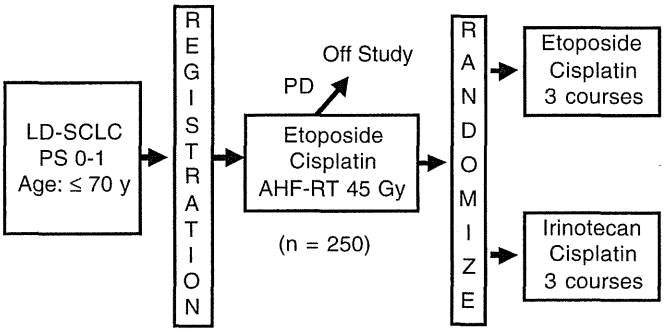

Fig. 1. Ongoing randomized phase III trial in patients with limited-stage small cell lung cancer (LD-SCLC) by Japan Clinical Oncology Group (JCOG; JCOG 0202MF). *PS*, performance status; *PD*, progressive disease; *AHF-RT*, accelerated hyperfractionated radiotherapy

irinotecan into the treatment for LD-SCLC is considered to be one of the most important strategies for improving the survival of LD-SCLC patients. Concurrent twice-daily thoracic radiotherapy with combination chemotherapy consisting of irinotecan and cisplatin may be the most powerful treatment for LD-SCLC patients if it is possible to use the full dose of irinotecan with acceptable toxicity. Previously, the JCOG conducted a dose-finding study of irinotecan and cisplatin plus concurrent radiotherapy for unresectable stage III NSCLC (JCOG 9405).[32] The dose intensity of irinotecan in the study was low, because of the need to omit irinotecan administration on days 8 and/or 15 as a result of leukopenia or diarrhea, and the radiotherapy completion rate was also low. This was a very small study, however, and chemotherapy with full-dose irinotecan and cisplatin plus concurrent radiotherapy was deemed unacceptable based on the results of the JCOG 9405 study. Full-dose chemotherapy consisting of etoposide and cisplatin can be used in combination with concurrent radiotherapy. However, when irinotecan is used as a single agent with concurrent radiotherapy, the dose of irinotecan must be reduced from 100 mg/m^2 to 60 mg/m^2 in a weekly schedule.[78] This dose reduction of irinotecan likely reduces the efficacy of irinotecan in the treatment of LD-SCLC patients. The JCOG is conducting a phase III study (JCOG 0202-MF) of concurrent twice-daily thoracic radiotherapy with four

cycles of etoposide and cisplatin as the standard arm versus, concurrent twice-daily thoracic radiotherapy with etoposide and cisplatin, followed by three cycles of chemotherapy with the standard dose of irinotecan and cisplatin (Fig. 1).

Conclusion

Chemoradiotherapy is considered to be the standard treatment for both unresectable locally advanced NSCLC and LD-SCLC. Cisplatin-based chemotherapy with concurrent thoracic radiotherapy yields a 5-year survival rate of approximately 15% for patients with unresectable locally advanced NSCLC. Cisplatin plus etoposide with concurrent twice-daily thoracic radiotherapy yields a 5-year survival rate of approximately 25% for patients with LD-SCLC. Several new strategies are currently underway to investigate improvements in survival for these patients. The incorporation of target-based drugs, such as gefitinib, is considered to be the most promising strategy for unresectable locally advanced NSCLC. The incorporation of irinotecan is also a promising strategy to improve the survival of patients with LD-SCLC. The JCOG is presently conducting clinical trials to develop a new strategy for the treatment of both unresectable locally advanced NSCLC and LD-SCLC.

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CLINICAL INVESTIGATION **Lung**

A PHASE II STUDY OF HYPERFRACTIONATED ACCELERATED RADIOTHERAPY (HART) AFTER INDUCTION CISPLATIN (CDDP) AND VINORELBINE (VNR) FOR STAGE III NON–SMALL-CELL LUNG CANCER (NSCLC)

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Purpose: The purpose was to assess the feasibility and efficacy of hyperfractionated accelerated radiotherapy (HART) after induction chemotherapy for Stage III non–small-cell lung cancer.

Methods and Materials: Treatment consisted of 2 cycles of cisplatin 80 mg/m^2 on Day 1 and vinorelbine 25 mg/m^2 on Days 1 and 8 every 3 weeks followed by HART, 3 times a day (1.5, 1.8, 1.5 Gy, 4-h interval) for a total dose of 57.6 Gy.

Results: Thirty patients were eligible. Their median age was 64 years (range, 46–73 years), 24 were male, 6 were female, 8 had performance status (PS) 0, 22 had PS 1, 9 had Stage IIIA, and 21 had Stage IIIB. All but 1 patient completed the treatment. Common grade ≥3 toxicities during the treatment included neutropenia, 25; infection, 5; esophagitis, 5; and radiation pneumonitis, 3. The overall response rate was 83%. The median survival was 24 months (95% confidence interval [CI], 13–34 months), and the 2-year overall survival was 50% (95% CI, 32–68%). The median progression-free survival was 10 months (95% CI, 8–20 months).

Conclusion: Hyperfractionated accelerated radiotherapy after induction of cisplatin and vinorelbine was feasible and promising. Future investigation employing dose-intensified radiotherapy in combination with chemotherapy is needed. © 2005 Elsevier Inc.

Non–small-cell lung cancer, Hyperfractionated accelerated radiation therapy, Chemoradiotherapy.

INTRODUCTION

Lung cancer is the leading cause of cancer-related death for men and the second for women in Japan. During 2001, approximately 55,000 patients died of lung and bronchus cancer (1). Surgery is the standard of care for patients with Stage I–II non–small-cell lung cancer (NSCLC), but a combination of chemotherapy and thoracic radiotherapy with or without surgery is indicated for the majority of patients with Stage III disease. Cisplatin (CDDP) based chemotherapy with conventional radiotherapy improved survival compared to conventional radiotherapy alone (2–6) and was the standard of care in the 1990s. Recently, concurrent chemoradiotherapy has been revealed to be superior to sequential chemoradiotherapy (7, 8), but it is difficult to give full-dose chemotherapy using newer cytotoxic agents concurrently with radiotherapy, and the optimal combination has not yet been clarified. In the meantime, continuous hyperfractionated accelerated radiotherapy (CHART) with 3 daily fractions to intensify the local effect of radiotherapy has been found to be superior to conventional radiotherapy (9). The survival benefit of CHART was encouraging, but the protocol including treatments on weekends and 6-h intervals between fractions had some difficulties in practicality. Mehta *et al.* introduced hyperfractionated accelerated radiotherapy (HART) (modified CHART) with 3 daily fractions and 4-h interfraction intervals with weekend breaks and also showed promising results similar to those using sequential chemoradiotherapy (10). After these results, we started a Phase II trial to evaluate the feasibility and efficacy of induction chemotherapy with HART for patients with Stage III NSCLC.

METHODS AND MATERIALS

Eligibility criteria

Eligibility criteria included previously untreated patients with pathologically proven NSCLC with clinical tumor-node-metastasis system Stage III, and pathologic N2 was also required for Stage

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IIIA; age, 20 to 74 years; performance status (PS) (based on Eastern Cooperative Oncology Group [ECOG] scale) 0 to 1; measurable disease; adequate hematologic (WBC count ≥4,000/mm^3, platelet count ≥100,000/mm^3, and hemoglobin ≥9.5 g/dL), hepatic (AST and ALT level ≤2 times the upper limit of normal and total bilirubin level ≤ the upper limit of normal), and renal (creatinine ≤1.2 mg/dL and creatinine clearance ≥60 mL/min) functions; PaO_2 ≥70 torr; no pleural and pericardial effusion; radiation field encompassed one-half or less of the ipsilateral lung; and no serious comorbidity. All patients signed written informed consent in accordance with our institutional review board.

Pretreatment evaluation included history and physical examination; serum chemistries (lactate dehydrogenase, alkaline phosphatase, AST, ALT, bilirubin, albumin, creatinine, and calcium); chest radiograph; CT scan of the chest; ultrasound of the abdomen; MRI or CT scan of the brain; and bone scintigraphy.

Treatment details

The treatment consisted of 2 cycles of CDDP 80 mg/m^2 on Day 1 and vinorelbine (VNR) 25 mg/m^2 on Days 1 and 8 every 3 weeks followed by HART; 3 times a day with minimal interval of 4 hours for a total dose of 57.6 Gy in 36 fractions over 2.5 weeks.

Radiation therapy was started after the patient recovered from the toxicity of chemotherapy and was delivered with megavoltage equipment. Lung heterogeneity corrections were not used. The first and third fraction of each day consisted of anterior-posterior opposed fields that encompassed the primary tumor, the metastatic lymph nodes, and the regional lymph nodes with a 1.5 to 2-cm margin. The fraction size was 1.5 Gy. Regional nodes excluding the contralateral hilar and supraclavicular nodes were included in these fractions. However, lower mediastinal nodes were included only if the primary tumor was located in the lower lobe of the lung. The second fraction of each day consisted of bilateral oblique fields that encompassed the primary tumor and the metastatic lymph nodes with a 1.5 to 2-cm margin; the fraction size was 1.8 Gy. Attempts were made to design the field of the second fraction to minimize the irradiated volume of the esophagus without compromising the margin around the tumor or spinal cord.

Toxicity assessment

Patients were observed weekly during treatment to monitor toxicity. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). Late toxicity was graded according to the Radiation Therapy Oncology Group (RTOG)/European Organization for Research and Treatment of Cancer late radiation morbidity scoring scheme. Late toxicity was defined as that occurring more than 90 days after treatment initiation.

Follow-up evaluation

The following evaluations were performed until disease progression every 2 months for the first year, every 3 months for the second year, and every 6 months thereafter: physical examination, toxicity assessment, and chest radiograph. CT scan of the chest was performed at 1, 3, 6, 9, 12, 18, and 24 months after the treatment and when indicated thereafter. Restaging at 6 months after the treatment was also performed with ultrasound of the abdomen, MRI or CT scan of the brain, and bone scintigraphy.

Response assessment

Complete response (CR) was defined as complete disappearance of all measurable and assessable lesions for ≥4 weeks, partial response (PR) was defined as a decrease of 50% or more from baseline in the sum of products of perpendicular diameters of all measurable lesions for ≥4 weeks, and progressive disease (PD) was defined as an increase of 25% or more from baseline in the sum of products of perpendicular diameters of all measurable lesions or the appearance of any new lesion. Stable disease was defined as the remainder of evaluable patients without CR, PR, or PD.

Pattern of failure

Patterns of failure were defined as first site of failure. Local/regional failure included the primary tumor and regional lymph nodes. Distant failure included any site beyond the primary tumor and regional lymph nodes.

Statistics

A Simon's two-stage optimal design was used for this study with the assumption that a protocol compliance rate of less than 60% would not be feasible, and protocol compliance rate of 80% or greater with α error of 0.10 and β error of 0.10 would warrant further investigation of this regimen. In the first stage, 11 assessable patients were entered. If fewer than 7 patients completed the treatment, accrual would be stopped with the conclusion that the regimen was not feasible for further investigation. If 7 or more patients completed the treatment, an additional 27 patients would be accrued in the second study. According to this design, this study would be determined to be feasible and be proceeded to a multicenter Phase II study if 27 patients completed the treatment. The actuarial median survival time and 2-year survival were estimated by the Kaplan–Meier method (11).

RESULTS

Patient population

Between July 1999 and March 2001, 30 patients were enrolled in the study. The accrual was stopped, because 29 of 30 patients completed the treatment, and conclusions could be drawn at that time. The patients' median age was 64 years (range, 46–73 years), 24 were male, and 6 were female. The patient and tumor characteristics are summarized in Table 1.

Treatment compliance and toxicity

All patients completed 2 cycles of induction chemotherapy. Six of 30 patients required dose modification, and 13 patients had treatment delay. The median time to start of HART from start of chemotherapy was 49 days (range, 41–62 days). Twenty-nine of 30 patients completed HART, and the median overall treatment time of HART was 17 days (range, 16–22 days). In total, 29 of 30 patients (97%; 95% confidence interval [CI], 83–100%) completed this combined treatment.

The toxicity profile of the treatment is shown in Tables 2 and 3. Common Grade 3 or greater acute toxicities were neutropenia, 25 (83%); infection, 5 (17%); esophagitis, 5 (17%); and radiation pneumonitis, 3 (19%). There were 2 cases of treatment-related death due to radiation pneumonitis. As of the date of this analysis, 2 cases with Grade

Table 1. Patient and tumor characteristics

Number of patients	30
Age	
Median	64
Range	46–73
Gender	
Male	24
Female	6
Performance status	
0	8
1	22
Weight loss	
<5%	25
≥5%	5
Tumor and lymph nodes	
T1N2	3
T1N3	1
T2N2	5
T2N3	5
T3N2	1
T4N0	1
T4N1	4
T4N2	9
T4N3	1
Stage	
IIIA	9
IIIB	21
Histology	
Squamous	13
Nonsquamous	17

3 s.c. tissue fibrosis and 1 case with spontaneous rib fracture were observed as late toxicities.

Response and survival

Of 30 patients, 2 achieved CR, and 23 achieved PR with a response rate of 83% (95% CI, 65–94%). Five patients remained in a stable disease state, and there were no PD patients. With a median follow-up period of 40 months for surviving patients, the median survival and the 2-year and 3-year survivals (Fig. 1) were 24 months (95% CI, 13–34 months), 50% (95% CI, 32–68%), and 32% (95% CI, 15–49%), respectively. The median progression-free survival and the 1-year progression-free survival (Fig. 2) were 10 months (95% CI, 8–20 months) and 47% (95% CI, 29–65%), respectively.

Pattern of failure

At the time of this analysis, 22 of 30 patients (73%) showed tumor progression, 2 patients (7%) had died as a result of treatment, and 6 patients (20%) were alive without disease progression. The patterns of first failure were as follows: local/regional only, 13 (43%); local/regional and distant, 4 (13%); distant only, 5 (17%).

DISCUSSION

In the 1970s, treatment of locally advanced NSCLC was by conventional radiotherapy alone. In the 1980s, sequential chemotherapy and conventional radiotherapy were revealed to be superior to conventional radiotherapy alone. In the 1990s, optimal sequences of chemoradiotherapy and radiation fractionation were investigated. The West Japan Lung Cancer Group compared sequential vs. concurrent radiotherapy with induction CDDP, vindesine, and mitomycin (7). In an RTOG 9410 trial, induction CDDP and vinblastine plus sequential standard radiotherapy, CDDP and vinblastine plus concurrent standard radiotherapy, and CDDP and etoposide plus concurrent twice-daily hyperfractionated radiotherapy were compared (8). Both trials showed similar results; concurrent chemoradiotherapy was superior to the sequential approach and achieved 5-year survivals for concurrent and sequential approach of approximately 20% and 10%, respectively. However, twice-daily hyperfractionated radiotherapy, which seemed to be promising in a preceding RTOG 9015 trial (12), failed to show a survival advantage over standard once-daily radiotherapy, and concurrent chemotherapy and once-daily radiotherapy is the standard of care today. Recently, a Czech randomized Phase II trial (13) suggested a similar advantage of the concurrent approach using CDDP and VNR, a newer cytotoxic agent. However, there remains some argument that newer cytotoxic agents cannot be delivered as full-dose chemotherapy with concurrent radiotherapy, and the survival advantage of newer cytotoxic agents over old ones has not yet been demonstrated in Stage III NSCLC patients. The optimal schedule and fractionation of thoracic radiotherapy in combination with chemotherapy also remains to be determined.

Another promising regimen was altered fractionation of radiotherapy such as CHART or HART, 3 times a day with a fraction interval of 4 to 6 hours over 2.5 weeks or less. CHART was developed at Mount Vernon Hospital, United Kingdom, in the 1980s. It was designed to combine both a shortening of the overall treatment time of radiotherapy, which is analogous to the concept of dose intensification of cytotoxic chemotherapy, and a reduction in dose per fraction. The rationale was to overcome accelerated repopulation of the tumor during the course of radiotherapy, which may lead to local failure, and to reduce normal tissue toxicities that depend on the dose per fraction. After the results of a randomized trial that showed survival benefits of CHART over conventional

Table 2. Hematologic toxicities (n = 30)*

	Grade					
	0	1	2	3	4	≥Grade 3 (%)
Leukopenia	1	3	8	16	2	18 (60)
Neutropenia	3	0	2	6	19	25 (83)
Thrombocytopenia	20	7	1	2	0	2 (7)
Anemia	1	10	16	3	0	3 (10)

* National Cancer Institute–Common Toxicity Criteria version 2.

Table 3. Nonhematologic toxicities (n = 30)*

	Grade						
	0	1	2	3	4	5	≥Grade 3 (%)
Acute toxicity							
Nausea	7	16	4	3	0	0	3 (10)
Vomiting	23	3	4	0	0	0	0
Infection	20	3	2	5	0	0	5 (17)
Esophagitis	1	11	13	4	1	0	5 (17)
Pneumonitis	18	4	5	1	0	2	3 (10)
Late radiation morbidity†							
Esophagus	26	1	0	0	0	0	0
Heart	26	0	1	0	0	0	0
Lung	9	13	5	0	0	0	0
Subcutaneous tissue	17	6	2	2	0	0	2 (7)
Bone	26	0	0	0	1	0	1 (3)

* National Cancer Institute–Common Toxicity Criteria version 2.
† Three patients died within 90 days of the beginning of radiotherapy.

radiotherapy (9), the Department of Health in the United Kingdom recommended CHART as the radiotherapy schedule of choice in inoperable NSCLC, and a CHART implementation group was formed to facilitate its introduction throughout the United Kingdom (14). There were difficulties in changing departmental working hours and a lack of sufficient financial support in UK hospitals to introduce CHART into routine practice (15), although it was suggested that CHART gave more benefit than any sequential combination of conventional radiotherapy and chemotherapy with minimally increased toxicity. To make the accelerated regimen more widely applicable, Continuous Hyperfractionated Accelerated Radiotherapy Week-End Less (CHARTWEL) and HART were introduced and were found to be as effective as CHART. Both CHARTWEL and HART showed improved survival over conventional radiotherapy, but the local tumor control was still unsatisfactory. Radiation dose escalation and use of chemotherapy combined with CHARTWEL/HART were also investigated to improve the local control and survival. Saunders *et al.* (16) reported on CHARTWEL combined with induction chemotherapy (17). In that study, 113 patients were enrolled, and dose escalation from 54 Gy to 60 Gy with or without chemotherapy was successfully achieved. Locoregional control at 2 years was 37% and 55% for CHARTWEL 54 Gy and 60 Gy alone, respectively, compared with 72% in those treated with 60 Gy and induction chemotherapy. These results suggested that chemotherapy improved locoregional control, but unfortunately they failed to show a statistically significant survival advantage, because of the relatively small number of patients and imbalanced tumor characteristics enrolled in each arm. The advantage of dose-escalated CHARTWEL against conventional radiotherapy is currently being investigated in a German Phase

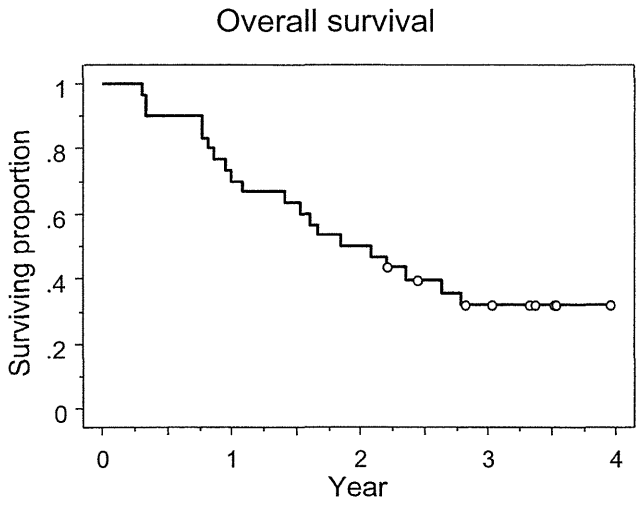

Fig. 1. Overall survival for all patients enrolled in this study.

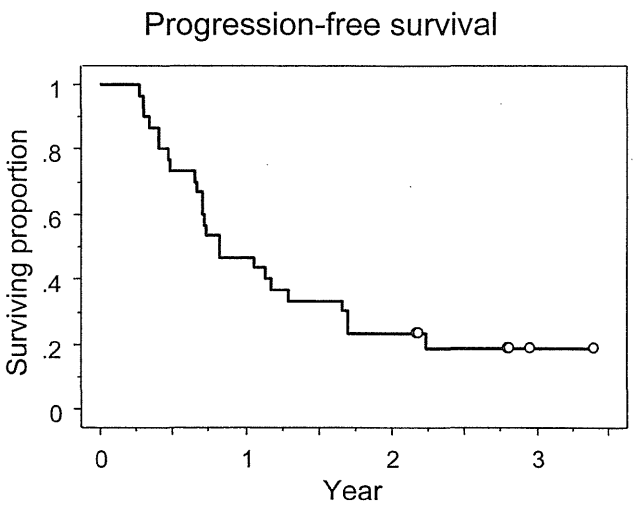

Fig. 2. Progression-free survival for all patients enrolled in this study.

III trial (18). Belani *et al.* reported the results of a randomized Phase III trial (19) that compared conventional radiotherapy with HART after induction chemotherapy (ECOG 2597). This study randomized 119 patients and unfortunately was closed because of slow accrual, but the results were provocative: The median survival time and the 2-year survivals for conventional radiotherapy and HART were 13.7 months and 33% vs. 22.2 months and 48%, respectively. These results seemed to be reliable despite the modest number of patients, because the median survival time of 13.7 months for the conventional radiotherapy arm was similar to that of a sequential chemoradiotherapy trial (2). The optimum chemotherapy regimen in combination with radiotherapy has not yet been determined, and we used a CDDP/VNR regimen instead of the carboplatin/paclitaxel regimen used in the ECOG 2597 trial. Both regimens are standards for advanced-stage NSCLC (20, 21). The compliance and toxicity profiles of chemotherapy in our study were acceptable, the incidence of esophagitis after HART was less than we expected, and the survival figure was nearly identical to that of the ECOG 2597 trial. This suggests that HART after induction CDDP/VNR or carboplatin/paclitaxel can achieve reproducible and promising results.

The pattern of failure in our study showed that local failure was still high (17 of 30, 57%) compared with distant metastasis (9 of 30, 30%), and further improvement of local control is needed. Future directions may include further dose intensification of radiotherapy and introduction of molecular-targeted agents. Recent innovation of information technology has made it possible to use sophisticated three-dimensional conformal radiotherapy (3DCRT). This can deliver intensified radiation doses to the tumor while minimizing the doses to the normal tissues that prevented further dose escalation using conventional two-dimensional radiotherapy. There have been several reports evaluating dose-intensified 3DCRT (22–25), and the technique is now under investigation in combination with cytotoxic chemotherapy in the Radiation Therapy Oncology Group trial (RTOG L-0117). Currently, molecular-targeted agents are being investigated most enthusiastically in Phase II and Phase III trials (26–29). It will be determined in the near future whether or not the combination of these agents has a survival impact. However, the optimal combination of these agents, newer cytotoxic agents, radiation fractionation, and 3DCRT will still need to be determined. Further investigation employing dose-intensified radiotherapy will be necessary to make a great leap in the treatment of locally advanced NSCLC.

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ORIGINAL REPORT

Epidermal Growth Factor Receptor Gene Mutations and Increased Copy Numbers Predict Gefitinib Sensitivity in Patients With Recurrent Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
To evaluate epidermal growth factor receptor (*EGFR*) mutations and copy number as predictors of clinical outcome in patients with non–small-cell lung cancer (NSCLC) receiving gefitinib.

Patients and Methods
Sixty-six patients with NSCLC who experienced relapse after surgery and received gefitinib were included. Direct sequencing of exons 18 to 24 of *EGFR* and exons 18 to 24 of *ERBB2* was performed using DNA extracted from surgical specimens. Pyrosequencing and quantitative real-time polymerase chain reaction were performed to analyze the allelic pattern and copy number of *EGFR*.

Results
Thirty-nine patients (59%) had *EGFR* mutations; 20 patients had deletional mutations in exon 19, 17 patients had missense mutations (L858R) in exon 21, and two patients had missense mutations (G719S or G719C) in exon 18. No mutations were identified in *ERBB2*. Response rate (82% [32 of 39 patients] *v* 11% [three of 27 patients]; $P < .0001$), time to progression (TTP; median, 12.6 *v* 1.7 months; $P < .0001$), and overall survival (median, 20.4 *v* 6.9 months; $P = .0001$) were significantly better in patients with *EGFR* mutations than in patients with wild-type *EGFR*. Increased *EGFR* copy numbers (≥ 3/cell) were observed in 29 patients (44%) and were significantly associated with a higher response rate (72% [21 of 29 patients] *v* 38% [14 of 37 patients]; $P = .005$) and a longer TTP (median, 9.4 *v* 2.6 months; $P = .038$). High *EGFR* copy numbers (≥ 6/cell) were caused by selective amplification of mutant alleles.

Conclusion
EGFR mutations and increased copy numbers were significantly associated with better clinical outcome in gefitinib-treated NSCLC patients.

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INTRODUCTION

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase of the *ErbB* family that has been implicated in cell proliferation and survival and is frequently overexpressed in many solid tumors, including non–small-cell lung cancer (NSCLC). Gefitinib (Iressa; AstraZeneca, Osaka, Japan) is an orally active, selective EGFR tyrosine kinase inhibitor that binds to the adenosine triphosphate–binding pocket of the EGFR kinase domain and blocks downstream signaling pathways. Two phase II studies, IRESSA Dose Evaluation in Advanced Lung Cancer 1 and 2 (IDEAL 1 and 2), have demonstrated that gefitinib monotherapy exerts an antitumor activity in patients with advanced NSCLC who had previously received platinum-based chemotherapy.[1,2] Gefitinib was approved in Japan for the treatment of inoperable or recurrent NSCLC in July 2002.

The IDEAL trials and retrospective studies have revealed that women, never smokers, patients with adenocarcinoma, and Japanese patients have higher response rates to gefitinib.[1-4] Among patients with adenocarcinoma, histologic subtypes have been studied; one study showed that responses were more frequent in patients with bronchioloalveolar carcinoma (BAC) features (38% *v* 14%; $P < .001$),[3] whereas another study showed that the response rate was higher in patients with a papillary-dominant subtype (76% *v* 21%; $P = .002$).[5]

Although no predictive molecular markers had been identified at the time of approval, somatic mutations in the kinase domain of *EGFR* have been subsequently linked to gefitinib sensitivity. According to three initial reports, 20 of 24 gefitinib-responsive tumors contained *EGFR* mutations, whereas 19 nonresponsive tumors did not contain any mutations.[6-8] The mutations were detected in exons 18 to 21 of *EGFR*, close to the region coding the adenosine triphosphate–binding pocket of the kinase domain, and most of them were observed in two hotspots: in-frame deletions including amino acids at codons 747 to 749 in exon 19 and an amino acid substitution at codon 858 (L858R) in exon 21. Analyses of surgically resected NSCLC tumors revealed that such mutations were more frequent among women, never smokers, patients with adenocarcinoma, and Japanese or East Asian patients,[7-13] consistent with the known clinical predictors of gefitinib sensitivity.

To evaluate the exact predictive value, we studied consecutive patients with recurrent NSCLC who received gefitinib therapy. To insure high-quality genetic analyses of the archived tissues, we used methanol-fixed, paraffin-embedded surgical specimens, which are known to preserve DNA better than formalin-fixed tissues,[14] and performed laser capture microdissection (LCM).

Recently, some other biomarkers of NSCLC have been studied. The *EGFR* and chromosome 7 copy numbers in NSCLC were assessed using fluorescence in situ hybridization (FISH), and more than 3.0 *EGFR* copies per cell (balanced polysomy or gene amplification) were detected in 39 (22%) of 183 patients.[15] A correlation between an increased *EGFR* copy number and gefitinib sensitivity was also proposed in another study.[16] In yet other studies, mutations in the kinase domain of *ERBB2 (HER2)*, a gene coding another receptor tyrosine kinase of the ErbB family, were detected in 16 (3.6%) of 445 patients with lung adenocarcinoma.[17,18] In the current study, we also analyzed the *EGFR* copy number and the presence of *ERBB2* mutations to assess their impact on clinical outcome.

The expression of EGFR and related proteins has been more widely studied using immunohistochemistry. Some studies suggested that high expression of phosphorylated Akt[19,20] or low expression of phosphorylated mitogen-activated protein kinase[20,21] was associated with better outcome in gefitinib-treated patients, but in general, methods, criteria, and results were inconsistent among studies. We thought that protein expression should be analyzed in another exploratory study, and in the current study, we focused on the genetic analyses.

PATIENTS AND METHODS

Patients

After searching the pharmaceutical records of the National Cancer Center Hospital, 279 patients with NSCLC who had begun receiving gefitinib monotherapy (250 mg/d) between July 2002 and May 2004 were identified. Seventy-three of these patients had undergone surgical resection of primary NSCLC at the hospital and subsequently relapsed. Recurrences were not necessarily confirmed pathologically but were diagnosed clinically. Seven patients were ineligible for inclusion in this study because methanol-fixed tissues were not available (n = 5) or their informed consent to the genetic analysis was not obtained (n = 2); consequently, 66 patients were included.

Genetic Analyses of EGFR and ERBB2

On a protocol approved by the institutional review board of the National Cancer Center, we performed mutational analyses of exons 18 to 24 of *EGFR* and exons 18 to 24 of *ERBB2* and analyzed the *EGFR* copy number. Methanol-fixed, paraffin-embedded surgical specimens of primary NSCLC were collected retrospectively, and DNA was extracted from bulk tumor tissue, laser capture microdissected tumor tissue, and normal lung tissue from each patient. LCM was performed using a PixCell II LCM system (Arcturus Engineering Inc, Mountain View, CA) according to a previously described method.[22] If appropriate, tumor cells were captured separately from two areas with different histologic subtypes, such as an area with a BAC subtype and another area with stromal invasion. Nested polymerase chain reaction (PCR) was performed to amplify exons 18 through 24 of *EGFR* using previously described primers,[6] and standard PCR was used to amplify exons 18 through 24 of *ERBB2*. Direct sequencing of the PCR products was performed using ABI PRISM 3700 and 3100 DNA Sequencers (Applied Biosystems, Foster City, CA). All sequencing reactions were performed in both forward and reverse directions, and single nucleotide substitutions, insertions, and deletions were detected using an application program named NAMIHEI.[23] Pyrosequencing was performed to verify the sequencing data of the hotspots of *EGFR* and to assess the proportion of mutant alleles in the laser-captured tumor cells using a Pyrosequencing PSQ 96MA (Pyrosequencing, Uppsala, Sweden).[24] On the basis of the proportion of mutant alleles, *EGFR* mutations were divided into two patterns: balanced heterozygous (BH) pattern (< 60%) and mutant-allele-dominant (MD) pattern (≥ 60%). The cutoff level of 60% was decided because if more than 60%, the superiority of the mutant over the wild-type sequences was obvious on the direct sequencing chromatograms. Quantitative, real-time, TaqMan duplex PCR was performed to analyze the *EGFR* copy number using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The *EGFR* primers were 5′-GGAGGACCGTCGCTTGGT-3′ and 5′-AACACCGCAGCATGTCAAGA-3′; the probe (5′-CACCGCGACCTGGCAGCCA-3′) was labeled with the reporter dye 6-carboxyfluorescein (FAM). RNaseP was coamplified in the same reaction mixture as the endogenous reference gene using TaqMan RNaseP Control Reagents (6-carboxyrhodamine [VIC] dye; Applied

Biosystems). The average *EGFR* copy number per cell was calculated from the differences in the threshold amplification cycles between *EGFR* and RNaseP. Peripheral-blood samples obtained from healthy volunteers were analyzed as normal controls. Decreased, normal, moderately increased, and highly increased *EGFR* copy numbers were defined as less than 1.5, 1.5 to 3.0, 3.0 to 6.0, and ≥ 6.0 copies per cell, respectively.

Pathologic Evaluation

We reviewed the histologic features of the 66 patients using hematoxylin and eosin–stained slides of tumor samples. Two board-certified pathologists (K.T. and Y.M.) who were unaware of the patients' outcome and mutational status examined all the specimens independently; in case of discrepancy, final diagnoses were established by consensus. Adenocarcinoma was categorized in two ways. The first categorization was based on the WHO's classification of lung tumors,[25] which includes four major subtypes of adenocarcinoma: papillary, acinar, BAC, and solid; the dominant subtype in the total tumor mass of each case was documented. The second categorization was based on a report from the Memorial Sloan-Kettering Cancer Center,[26] in which adenocarcinomas were classified into adenocarcinoma without BAC features (Ad), adenocarcinoma with BAC features (AwBF), BAC with focal invasion (BwFI), and pure BAC (PBAC). If two or more tumors were present in one patient, the diagnosis of the most invasive tumor in each case was documented.

Radiologic Evaluation

In patients who had measurable lesions, imaging studies were performed at baseline, approximately 4 weeks after the initiation of gefitinib treatment, and periodically thereafter throughout the treatment. One board-certified radiologist (U.T.) who was unaware of the patients' mutational status reviewed the baseline, first follow-up, and confirmatory imaging studies and classified the tumor responses into complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) using standard bidimensional measurements.[27] Responders were defined as patients with CR or PR. In this study, SD was subdivided into minor response (MR) and no response. MR was defined as a ≥ 25% decrease in the sum of the products of the perpendicular diameters of all measurable lesions at any point during gefitinib treatment. Time to progression (TTP) was defined as the time from the start of gefitinib administration to confirmed disease progression or death.

Statistical Analysis

The associations among mutational status, *EGFR* copy number, patient characteristics, and tumor response to gefitinib were assessed using a χ^2 test. The differences in TTP and overall survival (OS) according to the patient subgroups were compared using Kaplan-Meier curves and log-rank tests. Multivariate analyses using logistic regression models and Cox proportional hazard models were performed to assess the association between the biomarkers and clinical outcome while adjusting for the baseline patient characteristics. All analyses were performed using the SPSS statistical package (SPSS version 11.0 for Windows; SPSS Inc, Chicago, IL).

RESULTS

Patient Characteristics

The patient characteristics are listed in Table 1. All of the patients were Japanese. The proportions of women (39%), never smokers (47%), and patients with adenocarcinoma (94%) in this study were higher than those in a database of more than 1,000 patients with advanced or recurrent NSCLC treated at our hospital during the four most recent years (27%, 27%, and 73%, respectively). Twenty-two patients (33%) had been included in our phase II trial for first-line gefitinib therapy for patients with recurrent NSCLC, and the others had been treated with gefitinib in clinical practice settings. The operations for primary NSCLC were performed between February 1994 and August 2003, and the median time from the operations to the start of gefitinib was 2.3 years (range, 0.6 to 9.1 years).

Table 1. Patient Characteristics

	Patients (n = 66)	
	No.	%
Age, years		
Median	65	
Range	32-80	
Sex		
Female	26	39
Male	40	61
Smoking history*		
Never smokers	31	47
Former smokers	12	18
Current smokers	23	35
Histologic diagnosis		
Adenocarcinoma	62	94
Papillary/acinar/BAC/solid†	30/18/9/5	45/27/14/8
Ad/AwBF/BwFI/PBAC	15/45/2/0	23/68/3/0
Squamous cell carcinoma	3	5
Pleomorphic carcinoma	1	2
Performance status		
0/1	22/28	33/42
2/3	12/4	18/6
Prior chemotherapy regimens		
0	37	56
1	14	21
≥ 2	15	23

Abbreviations: BAC, bronchioloalveolar carcinoma; Ad, adenocarcinoma without BAC features; AwBF, adenocarcinoma with BAC features; BwFI, BAC with focal invasion; PBAC, pure BAC.
*Never smokers were defined as subjects who have never had a smoking habit, and former smokers were defined as subjects who had stopped smoking at least 1 year before diagnosis.
†Dominant subtype.

Clinical Outcome

Sixty-four patients had measurable lesions at the start of gefitinib administration. CR and PR were observed in two and 32 patients, respectively. MR was observed in three of nine patients with SD. Twenty-one patients had PD, including six patients who died before the first follow-up imaging studies. Two patients had only unmeasurable bone lesions at baseline; one patient showed rapid symptom improvement and continued to receive gefitinib therapy without progression for 13.8+ months, whereas the other

patient developed new lesions and died on day 71. These patients were included in the analysis as a responder and a nonresponder, respectively. The overall response rate was 53%. Forty-one patients died, and the median follow-up time for the 25 survivors was 14.6 months (range, 10.3 to 32.3 months). Eleven patients were still receiving gefitinib without progression at the time of the analysis. The median TTP and the median survival time (MST) for all patients were 5.2 and 16.3 months, respectively.

EGFR *and* ERBB2 *Mutations*

Forty-three mutations in the *EGFR* tyrosine kinase domain were detected in 39 (59%) of the 66 patients. All the mutations detected in this study are shown in Table 2. Twenty patients had deletional mutations in exon 19, and 17 patients had missense mutations (L858R) in exon 21. In exons 18 and 20, five types of missense mutations were detected. Two of them (G719S and G719C) occurred at a codon considered to be a third hotspot.[6,7,9-12] The others (L703V, E709K, and S768I) were detected in patients who also had mutations at the hotspots. Because these mutations were not detected in the normal lung tissues from the same patients, they were considered to be somatic mutations. No somatic mutations were detected in exons 22 to 24. Silent single nucleotide polymorphisms were identified at nucleotides 2361 (G/A; Q787Q), 2370 (G/A; T790T), and 2457 (G/A; V819V) in exon 20, and at nucleotide 2709 (C/T; T903T) in exon 23, but the association between these polymorphisms and the somatic mutations was not observed. In this study, no mutations and no polymorphisms were detected in exons 18 to 24 of *ERBB2*.

All 43 mutations were detected in LCM samples, but 11 (26%) of these mutations were not detected in the bulk tumor samples. In 13 patients, LCM was performed at separate areas with different histologic subtypes, but no heterogeneity was identified; the same mutations were detected in nine patients, and no mutations were detected in four patients. Mutational analyses of synchronous double lung cancers were performed in two patients; one patient had a tumor with wild-type *EGFR* and a more invasive tumor with L858R + S768I, and the other patient had a tumor with a 9-bp deletion (del L747-E749) and a more invasive tumor with a 15-bp deletion (del E746-T751insA) + L703V.

Among the 39 patients with *EGFR* mutations, the proportion of mutant alleles ranged from 29% to 94%. Nineteen patients showed a BH pattern and 20 patients showed an MD pattern.

EGFR *Copy Number*

The *EGFR* copy number in the laser-captured tumor cells ranged from 1.27 to 31.2 per cell, and increased *EGFR* copy numbers (≥ 3.0 per cell) were observed in 29 patients (44%). The relation between the copy number and the proportion of mutant alleles is shown in Figure 1. Increased copy numbers were observed more frequently in patients with *EGFR* mutations than in patients with wild-type *EGFR* (56% [22 of 39 patients] *v* 26% [seven of 27 patients]; $P = .014$). High copy numbers (≥ 6.0 per cell) were observed only in patients with an MD pattern of mutations. The copy number and the proportion of mutant alleles among patients with *EGFR* mutations was positively correlated (Spearman correlation coefficient = 0.643; $P < .001$), implying that the mutant alleles were selectively amplified in patients with an MD pattern. One patient with an MD pattern had a tumor with only approximately one copy per cell, indicating a hemizygous mutation with a loss of wild-type allele. No alterations in the gene copy number were observed in normal lung tissues.

Table 2. *EGFR* Mutations Detected in the Present Study

Exons	Amino Acids	Nucleotides	No. of Patients
19	del E746-A750	del 2235-2249	12
	del E746-A750	del 2236-2250	5
	del E746-T751insA	del 2237-2251	1
	del L747-E749	del 2239-2247	1
	del E746-S752insV	del 2237-2255 + ins T	1
21	L858R	T → G at 2573	17
18	G719S	G → A at 2155	1
	G719C	G → T at 2155	1
	L703V	C → G at 2107	1*
	E709K	G → A at 2125	1†
20	S768I	G → T at 2303	2‡

Abbreviations: *EGFR*, epidermal growth factor receptor; del, deletion; ins, insertion.
*A patient with del E746-T751insA.
†A patient with L858R.
‡A patient with L858R and a patient with G719C.

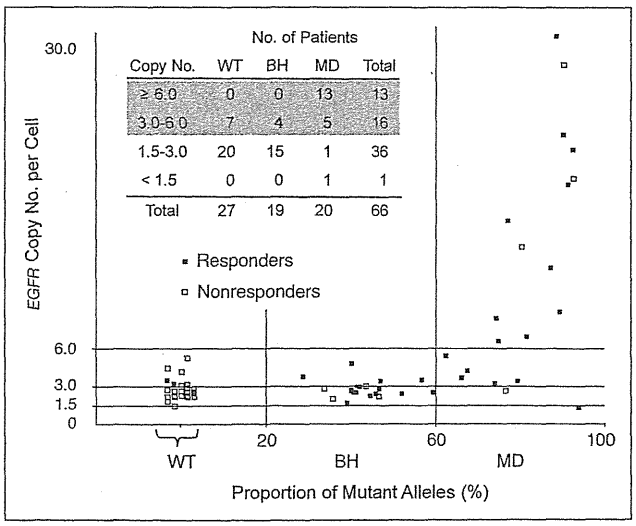

Fig 1. Relation between the epidermal growth factor receptor (*EGFR*) copy number and the proportion of mutant alleles. WT, patients with wild-type *EGFR*; BH, patients with a balanced heterozygous pattern of *EGFR* mutations; MD, patients with a mutant-allele-dominant pattern of *EGFR* mutations.

EGFR *Mutations,* EGFR *Copy Number, and Clinical Outcome*

The tumor responses to gefitinib according to the mutational status of *EGFR* are shown in Table 3. The response rates of patients with mutant and wild-type *EGFR* were 82% and 11%, respectively ($P < 10^{-7}$). Seven patients with *EGFR* mutations were nonresponders; three patients had PD at 0.3 (early death), 2.3, and 2.3 months, and four patients had SD. Three of the four patients with SD had MR (TTP, 2.5, 5.2, and 6.9 months), and the other patient continued to receive gefitinib therapy without progression for 24.2 months, whereas all SD tumors with wild-type *EGFR* progressed within 5 months without MR. Meanwhile, three patients with wild-type *EGFR* exhibited PR, and two of these patients were still receiving gefitinib therapy without progression at 10.9+ and 21.1+ months. The Kaplan-Meier plots of TTP and OS according to the presence of the *EGFR* mutations are shown in Figures 2 and 3, respectively. Patients with *EGFR* mutations had a significantly longer TTP and OS compared with those with wild-type *EGFR*.

Univariate analyses were performed to assess the correlations among patient characteristics, *EGFR* mutations, *EGFR* copy number, and clinical outcome (Tables 4 and 5). The response rates were significantly higher in women, never/former smokers, and patients with BAC features and were marginally higher in patients with a papillary-dominant subtype. The response rates among these subgroups were approximately consistent with the rates of *EGFR* mutations. An increased *EGFR* copy number was also significantly associated with a higher response rate and a longer TTP.

The results of multivariate analyses among 62 patients with adenocarcinoma are shown in Table 6. The presence of *EGFR* mutations was strongly associated with a higher response rate, a longer TTP, and a longer OS. An increased *EGFR* copy number was also a significant or marginally significant predictor of a higher response rate and a longer TTP. These results did not change substantially if any combinations of variables were included in the models.

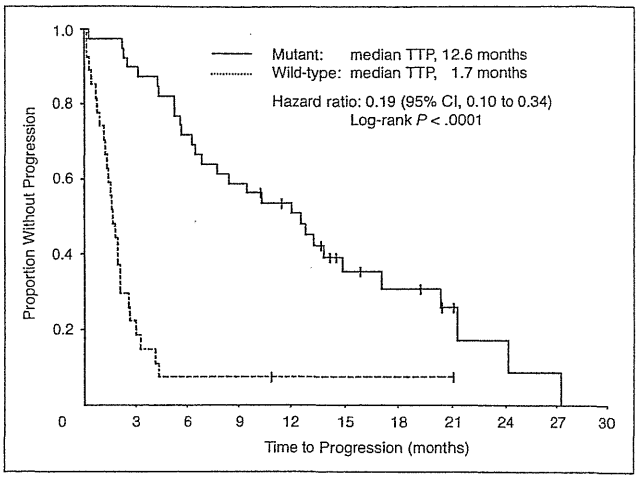

Fig 2. Kaplan-Meier plot of time to progression (TTP) according to epidermal growth factor receptor (*EGFR*) mutation status.

Among patients with wild-type *EGFR*, TTP was significantly longer in patients with increased *EGFR* copy numbers (median, 3.0 *v* 1.4 months; log-rank $P = .021$), and both of the two long-term responders had tumors with moderately increased *EGFR* copy numbers (3.20 and 3.45/cell). Among patients with *EGFR* mutations, TTP and OS were not significantly different according to the types of mutations, the presence of additional mutations, the proportion of mutant alleles, or the *EGFR* copy number (data not shown).

DISCUSSION

This study strongly implies that the mutational status of *EGFR* is a major determinant of gefitinib sensitivity in patients with NSCLC. The response rate was 82%, the median TTP was 12.6 months, and the MST was 20.4 months in gefitinib-treated patients with *EGFR*-mutant NSCLC. *EGFR* mutations might be a good prognostic factor independent of treatment, but these remarkable results suggest a

Table 3. *EGFR* Mutations and Tumor Response to Gefitinib

	Responders		Nonresponders			Responders/Total Patients	Response Rates (%)
	CR	PR	MR	SD	PD		
Mutant	2	30*	3	1	3†	32/39	82
DEL	0	18*	2	0	0	18/20	90
L858R	2	11	1	1	2†	13/17	76
G719	0	1	0	0	1	1/2	50
Wild-type	0	3	0	5	19	3/27	11
Total	2	33	3	6	22	35/66	53

Abbreviations: *EGFR*, epidermal growth factor receptor; CR, complete response; PR, partial response; MR, minor response; SD, stable disease without MR; PD, progressive disease; DEL, deletional mutations in exon 19; G719, G719S, or G719C.
*Including a clinical responder without measurable lesions.
†Including a patient who had no measurable lesions at baseline.

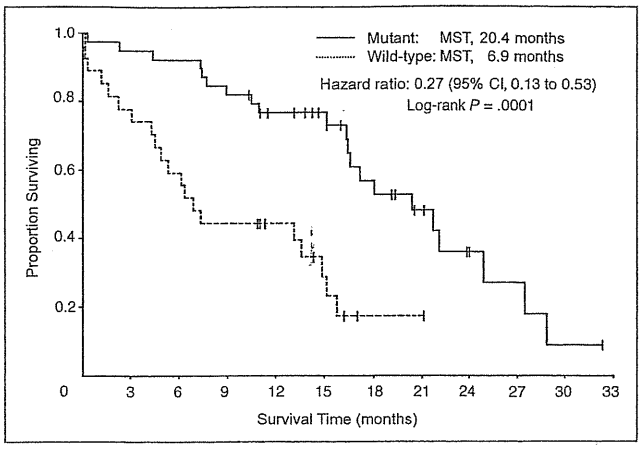

Fig 3. Kaplan-Meier plot of overall survival according to epidermal growth factor receptor (*EGFR*) mutation status. MST, median survival time.

survival benefit from gefitinib therapy in patients with *EGFR* mutations. Four of seven nonresponders with *EGFR* mutations also seemed to experience some clinical benefits because they had MR or a long SD (≥ 6 months). Among nine patients with SD, MR, or a long SD was observed only in patients with *EGFR* mutations. Although the sample size was too small to draw a firm conclusion, this finding suggests that *EGFR* mutations are also associated with clinical benefits in SD.

Table 4. *EGFR* Mutations Among Patient Subgroups

	EGFR Mutations		
	No. of Patients	%	*P*
Total	39/66	59	
Sex			.18
Female	18/26	69	
Male	21/40	53	
Smoking history			.003†
Never smokers	21/31	68	
Former smokers	10/12	83	
Current smokers	8/23	35	
Histologic diagnosis			—
Adenocarcinoma	38/62	61	
Squamous cell carcinoma	0/3	0	
Pleomorphic carcinoma	1/1	100	
Dominant subtype*			.059‡
Papillary	22/30	73	
Acinar	10/18	56	
BAC	5/9	56	
Solid	1/5	20	
BAC features*			.002
Yes	34/47	72	
No	4/15	27	

Abbreviations: *EGFR*, epidermal growth factor receptor; BAC, bronchioloalveolar carcinoma.
*Only patients with adenocarcinoma (n = 62).
†Comparison between never/former smokers and current smokers.
‡Comparison between patients with papillary-dominant adenocarcinoma and patients with other adenocarcinoma.

The *EGFR* mutations detected in this study were concentrated in three hotspots, deletions around codons 747 to 749, L858R, and G719S (or G719C), similar to the results of previous reports.[6-13] Some genetic variations existed among these mutations. Together with one of the hotspot mutations, additional missense mutations in exons 18 or 20 were detected in four patients. Among the 39 patients with *EGFR* mutations, an MD pattern was observed in 20 patients. Because the *EGFR* copy number in their tumor cells increased as the proportion of mutant alleles increased, this pattern was assumed to be caused not by homozygous mutations but by the selective amplification of the mutant alleles. Because one patient had a hemizygous mutation without amplification, the loss of wild-type alleles was also thought to be responsible for the pattern. The moderately increased copy number in patients with a BH pattern or wild-type *EGFR* can be explained by *EGFR* amplification and/or polysomy of chromosome 7.

Among the patients with *EGFR* mutations, three patients had PD and eight of the other 36 patients had tumor regrowth within 6 months. This suggests the presence of other factors associated with intrinsic or acquired resistance to gefitinib. Although any genetic alterations of *EGFR*-mutant tumors at the time of primary surgery were not significantly associated with clinical outcome, that might be because further alterations occurred after the primary surgery or after gefitinib administration. Recently, a secondary mutation (C → T at nucleotide 2369; T790M) in exon 20 was detected in patients with *EGFR*-mutant NSCLC who had tumor regrowth during gefitinib therapy after exhibiting an initial response to the agent; this mutation was thought to be associated with acquired resistance.[28,29] To elucidate the determinants and the mechanism of resistance to gefitinib, genetic analyses of tumor samples obtained after gefitinib treatment are needed.

In this study, three (11%) of the 27 patients with wild-type *EGFR* responded to gefitinib. Various explanations for this result are possible: (1) the mutational analyses of the responders were false-negative, (2) the *EGFR* mutations occurred in their tumors after the primary surgery, (3) the recurrent tumors originated from a source other than the analyzed tumor cells, or (4) other determinants of gefitinib sensitivity were present.

The results of multivariate analyses suggest that the *EGFR* copy number is another independent predictor of gefitinib sensitivity. It is noteworthy that an increased *EGFR* copy number was observed in two of the three responders with wild-type *EGFR*, and was significantly associated with a longer TTP among patients with wild-type *EGFR*. Because patients with *EGFR* mutations had favorable clinical outcome regardless of *EGFR* copy numbers, the impact of increased copy numbers on *EGFR*-mutant NSCLC was unclear. In the overall population, an increased *EGFR* copy number was significantly associated with a higher response

Table 5. Clinical Outcome Among Patient Subgroups (univariate analyses)

	Response Rate			Time to Progression		Overall Survival	
	No.	%	*P*	Median (months)	Log-Rank *P*	Median (months)	Log-Rank *P*
Total	66	53		5.2		16.3	
Sex			.033		.35		.30
Female	26	69		6.2		16.5	
Male	40	43		3.3		15.1	
Smoking history			.007		.026		.37
Never/former smokers	43	65		6.9		16.4	
Current smokers	23	30		2.6		15.1	
Dominant subtype*			.070		.28		.65
Papillary	30	67		7.7		16.4	
Others	32	44		4.2		15.7	
BAC features*			.012		.12		.19
Yes	47	64		6.5		16.5	
No	15	27		2.1		15.7	
Performance status			.77		.012		< .0001
0-1	50	52		5.2		17.1	
2-3	16	56		3.1		6.1	
EGFR mutations			< .0001		< .0001		.0001
Yes	39	82		12.6		20.4	
No	27	11		1.7		6.9	
EGFR copy number			.005		.038		.33
≥ 3.0	29	72		9.4		16.4	
< 3.0	37	38		2.6		15.7	

Abbreviation: BAC, bronchioloalveolar carcinoma; *EGFR*, epidermal growth factor receptor.
*Only patients with adenocarcinoma (n = 62).

rate and a longer TTP, but not with a longer OS, which might be because an increased copy number had an unfavorable impact on prognosis, as suggested by another study.[15] In chronic myeloid leukemia, as well as *BCR-ABL* mutations that were structurally corresponding to T790M in *EGFR*, an increased *BCR-ABL* gene copy number was reported as a determinant of resistance to imatinib, a BCR-ABL tyrosine kinase inhibitor.[30] Therefore, we should consider the possibility that an increased *EGFR* copy number is associated with not only sensitivity but also resistance to gefitinib.

Among adenocarcinomas, the presence of BAC features was significantly associated with gefitinib sensitivity and *EGFR* mutations, but the BAC component was relatively small in most of the responders. The dominant subtype associated with a higher response rate was not BAC but papillary; both of the two patients with BwFI had PD, and all three patients with pure papillary adenocarcinoma without BAC features had PR. The association between pathologic features and gefitinib sensitivity or *EGFR* mutations is also the subject of further investigation.

Table 6. Univariate and Multivariate Analyses of the Association Between Biomarkers and Clinical Outcome in Patients With Lung Adenocarcinoma (n = 62)

	Odds Ratios for Response		Hazard Ratios for TTP		Hazard Ratios for OS	
	Univariate	Multivariate*	Univariate	Multivariate*	Univariate	Multivariate*
EGFR mutations, yes *v* no	31.0	27.9	0.21	0.13	0.30	0.16
95% CI	7.2 to 134	3.7 to 209	0.11 to 0.38	0.06 to 0.29	0.15 to 0.62	0.06 to 0.39
P	< .001	.001	< .001	< .001	.001	< .001
EGFR copy number, ≥ 3.0 *v* < 3.0	4.0	4.6	0.57	0.42	0.80	0.59
95% CI	1.4 to 12	0.84 to 25	0.32 to 1.0	0.21 to 0.84	0.42 to 1.5	0.26 to 1.4
P	.011	.079	.050	.014	.49	.22

Abbreviations: TTP, time to progression; OS, overall survival; *EGFR*, epidermal growth factor receptor.
*In the multivariate analyses, age (continuous variable), sex (women *v* men), smoking history (never/former smokers *v* current smokers), dominant subtype (papillary *v* others), bronchioloalveolar carcinoma features (yes *v* no), performance status (0 to 1 *v* 2 to 3), prior chemotherapy (yes *v* no), *EGFR* mutations (yes *v* no), and *EGFR* copy number (≥ 3.0 *v* < 3.0) were included as factors.

In never/former smokers, both the *EGFR* mutation rate and the response rate were significantly higher than in current smokers. We speculate that *EGFR* mutations occur equally throughout the entire population, regardless of smoking history, and account for smoking-unrelated carcinogenesis. Because many other genetic alterations, like *KRAS* mutations, occur and induce lung adenocarcinoma more frequently in smokers, the *EGFR* mutation rate seems to be relatively lower in smokers with lung adenocarcinoma.

The response rate of 53% and the *EGFR* mutation rate of 59% observed in this study were higher than previously reported rates. These results can partially be attributed to the fact that the physicians tended to select patients with characteristics known to be predictive for gefitinib sensitivity: women, never-smokers, and patients with adenocarcinoma. Consequently, this cohort was not necessarily representative of unselected NSCLC populations in Japan. However, other recent studies have also shown relatively high frequencies (32% to 55%) of *EGFR* mutations in Japanese or East Asian patients with lung adenocarcinoma who underwent surgical resection.[7,9-11,13] The reason why such somatic mutations occur selectively in East Asian people remains unknown. Environmental or genetic factors common among East Asian populations should be investigated to answer this question.

Recently, no significant survival benefit of gefitinib was reportedly observed in the initial analysis of the IRESSA Survival Evaluation in Lung Cancer (ISEL) trial, a phase III trial comparing gefitinib monotherapy to a placebo as a second- or third-line treatment for patients with advanced NSCLC.[31] Because subgroup analyses of the trial suggested survival benefits in never smokers or Asian patients, the selection of patients is thought to be crucial when considering gefitinib treatment. Because the present study showed that the *EGFR* mutation status is a major determinant of gefitinib sensitivity, mutational analyses in patients with advanced NSCLC should be considered before deciding on a course of treatment.

In this study, we performed LCM and direct sequencing using methanol-fixed surgical specimens to obtain high-quality data. If we had analyzed only bulk tumor samples without LCM, nine of the 39 patients with *EGFR* mutations would have been misjudged as having wild-type *EGFR*. Thus such procedures with LCM are presently recommended for the detection of *EGFR* mutations. However, obtaining appropriate tumor samples is often difficult in patients with advanced NSCLC, and performing LCM and direct sequencing in all patients is not practical. Thus more practical methods for detecting the major *EGFR* mutations using small tumor samples contaminated with normal tissue should be developed and validated.

Other than *EGFR* mutations, some candidate predictive biomarkers have been studied. The *EGFR* copy number is the leading candidate, and it can also be detected by FISH. Practicality and accuracy should be assessed comparing FISH and quantitative real-time PCR. The impact of *ERBB2* mutations on clinical outcome remains to be investigated because we could not detect any mutations in *ERBB2* in the present study. Protein expression analyses by IHC are easier to perform than the genetic analyses, but their significance is still controversial. Further studies are required to evaluate the predictive values of these biomarkers and to determine whether they are independent predictors of gefitinib sensitivity or surrogate markers of *EGFR* mutations.

In conclusion, this study indicates that *EGFR* mutations and increased copy numbers predict better clinical outcome in patients with NSCLC treated with gefitinib. Further research and clinical trials are needed to incorporate these markers into clinical practice appropriately.

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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CORRESPONDENCE

Erlotinib in Lung Cancer

TO THE EDITOR: Shepherd and colleagues (July 14 issue)[1] report that erlotinib prolongs survival in non–small-cell lung cancer, as compared with placebo, after the failure of first-line or second-line chemotherapy. One disturbing aspect of this trial is that some patients underwent only one prior chemotherapy regimen before randomization. These same authors previously reported that docetaxel is superior to best supportive care after first-line chemotherapy.[2] Subsequent studies have confirmed the efficacy of docetaxel and shown that pemetrexed achieves similar results.[3] Did Shepherd and colleagues think that random assignment to placebo after the failure of first-line chemotherapy was ethically justifiable? The only patients for whom one could justify the assignment to placebo were those with a performance status of 3, who made up only 8.6 percent of all patients. Contrary to the authors' claim that inclusion of a placebo group was ethical, we believe that some patients were denied a therapeutic option known to be effective. Furthermore, the overall survival in the erlotinib group was inferior to that in published results with docetaxel and pemetrexed, suggesting that erlotinib should be used as third-line chemotherapy.

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1. Shepherd FA, Rodrigues Pereira JR, Ciuleanu T, et al. Erlotinib in previously treated non–small-cell lung cancer. N Engl J Med 2005; 353:123-32.
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TO THE EDITOR: Shepherd et al. and Tsao et al.[1] (July 14 issue) report an important study (BR.21) showing a survival benefit of erlotinib, but the results of the molecular analysis confused us. Recent East Asian studies[2-4] have strongly suggested that the mutational status of the epidermal growth factor receptor (EGFR) is the major determinant of tumor response and survival in patients with non–small-cell lung cancer who are treated with gefitinib, another EGFR tyrosine kinase inhibitor. Response rates among patients with an EGFR mutation were consistently higher than 80 percent in those studies. However, in the BR.21 study, the response rate among such patients was only 16 percent, and mutational status had no significant effect on survival, although the EGFR copy number correlated with responsiveness and survival. In our study,[2] the EGFR copy number was associated with gefitinib sensitivity, but we consider it to be a surrogate marker for EGFR mutations, rather than a true determinant.

THIS WEEK'S LETTERS

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