Table 3 Patient characteristics of all registered patients ($n = 28$)

Characteristics	No. of patients (%)
Age	
Median	68
Range	49–89
Performance status	
0	11 (39)
1	13 (47)
2	4 (14)
Sex	
Male	10 (36)
Female	18 (64)
Histology	
Adenocarcinoma	27 (96)
Squamous cell carcinoma	1 (4)
Large cell carcinoma	0 (0)
Adenosquamous carcinoma	0 (0)
Other	0 (0)
Smoking status	
Never	19 (68)
Current/former	9 (32)
Stage	
IIIA*	1 (3)
IIIB	5 (18)
IV	22 (79)
Prior cancer therapy	
Chemotherapy	
No	17 (61)
One regimen (adjuvant)	4 (14)
One regimen (not adjuvant)	5 (18)
Two regimens	2 (7)
Recurrence after surgery	11 (39)
Radiation	1 (4)

*Unresectable, no indication for thoracic radiation because of a large radiation field.

Table 4 Response rate ($n = 28$)

Response	No. of patients	Response rate (%)	95% CI
Complete response	1	3.6	
Partial response	20	71.4	
Stable disease	6	21.4	
Progressive disease	0	0.0	
Not evaluable*	1	3.6	
Overall response	21	75.0	57.6–91.0
Disease control rate	27	96.4	87.0–96.4

CI = confidence interval. *One patient was not evaluable because of a poor evaluation of efficacy.

Safety and toxicity

Toxicity was evaluated in all eligible patients (Table 5). The most frequent adverse events were rash, dry skin, diarrhoea, stomatitis and elevated AST/ALT levels. Two patients experienced grade 3 rash and one patient experienced grade 3 keratitis; however, these patients all achieved a PR, and the adverse effects subsided after pausing gefitinib treatment for around 2 weeks. Four patients experienced grade 3 hepatotoxicity; three of these patients had to discontinue treatment for this reason.

One patient developed interstitial lung disease (ILD) (Ando *et al*, 2006). Ground-glass opacity was detected in the right upper lobe 19 days after the start of gefitinib administration, resulting in the cessation of treatment. However, the lesion enlarged into bilateral lung fields on day 25, and steroid therapy was initiated. Nonetheless, the patient died of respiratory failure on day 48. Two patients also experienced grade 1 ILD. They recovered without steroid administration.

Subsequent treatment after disease progression

Of the 14 patients who become refractory to gefitinib and exhibited disease progression, 10 received chemotherapy as their first treatment regimen after gefitinib (Table 6); 5 patients received platinum doublets and 1 patient received vinorelbine as a second-line treatment; and 3 received docetaxel and 1 received platinum doublet as a third-line treatment. In all, 4 out of the 10 patients (40%) had a PR. Of the nine patients who become refractory to the first treatment regimen after gefitinib, six received chemotherapy as their second regimen after gefitinib, including one who received gemcitabine, one who received docetaxel, and one who was re-treated with gefitinib as a third-line therapy; two other patients received docetaxel and one was re-treated with gefitinib as a fourth-line therapy. Two of the six patients (33%) had a PR. The two patients who received gefitinib re-treatment both had SD.

BAC features, *EGFR* amplification and T790M mutation in exon 20

A total of 110 tissue samples were available for pathological review, of which 90 were from adenocarcinoma; 33 of these specimens (37%) revealed proportional BAC components in the specimen. Among them, 15 were considered extensive and the remaining 18 were found to have minor BAC components. The 39 surgical specimens included 36 from adenocarcinomas. The *EGFR* mutations were detected in 12 out of the 36 adenocarcinoma specimens. None of the samples with a BAC component, micropapillary pattern or mucin production was associated with an *EGFR* mutation (Table 7).

Data on *EGFR* gene copy numbers were available in only 12 samples. We used the criteria for defining a high *EGFR* gene copy number (gene amplification or high polysomy, as determined using FISH) that were described in a previous report (Cappuzzo *et al*, 2005). A total of 7 out of the 12 samples had a high gene copy number (FISH positive), and 6 (3 with *EGFR* mutations) out of the 7 samples had proportional BAC components. In all, 5 out of the 12 samples were FISH negative, only 1 (with no *EGFR* mutation) of which had a BAC component. Two patients that were FISH negative, BAC negative and *EGFR* mutation positive had SD when treated with gefitinib.

Another *EGFR* mutation, T790M in exon 20, has been reported to be associated with resistance to gefitinib (Kobayashi *et al*, 2005; Pao *et al*, 2005). We checked for this mutation in six patients who did not respond to gefitinib; however, the mutation could not be identified in any of the patients.

DISCUSSION

We performed a multicentre phase II study examining the use of gefitinib for advanced NSCLC in patients with *EGFR* mutations, prospectively recruiting patients at the time of genetic screening and avoiding a selection bias. All patients were registered in a central database. All tissues were delivered from the local participants to the central facility, where they were reviewed by a pathology specialist and the *EGFR* mutation status was evaluated. The median time for the *EGFR* mutation detection analysis was 12 days, which is probably an acceptable time lag before the start of treatment for advanced NSCLC. However, a shorter period would clearly be desirable for routine clinical practice. Indeed, 4 out of the 32 *EGFR*-positive patients were dropped from the study because of disease progression before their actual registration

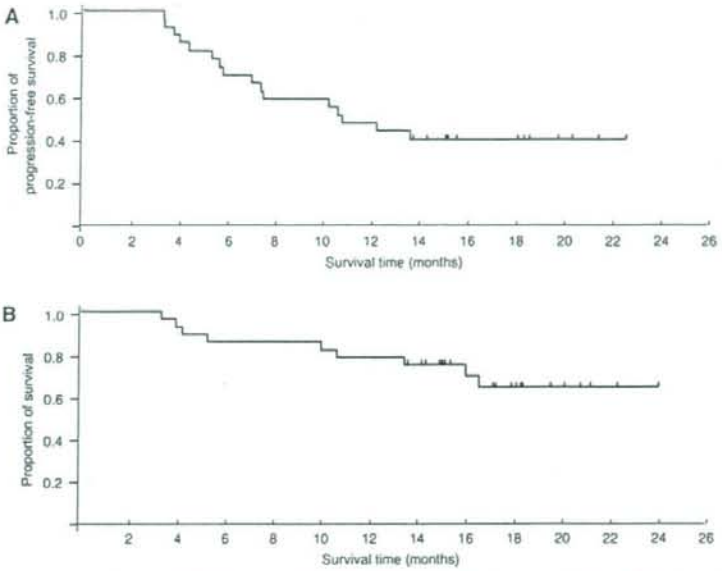

Figure 1 (**A**) Progression-free survival (PFS) and (**B**) overall survival (OS) of all eligible patients ($n = 28$). The median PFS was 11.5 months. The median OS has not yet been reached. The 1-year survival rate was 79%.

Table 5 Common adverse events ($n = 28$)

	No. of patients (%)			
Adverse events	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Haematologic				
Anaemia	12 (43)	3 (11)	0 (0)	0 (0)
Leucopaenia	4 (14)	1 (4)	2 (7)	0 (0)
Neutropaenia	4 (14)	1 (4)	1 (4)	0 (0)
Thrombocytopaenia	3 (11)	0 (0)	0 (0)	0 (0)
Nonhaematologic				
Rash	10 (36)	11 (39)	2 (7)	0 (0)
Dry skin	9 (32)	10 (36)	0 (0)	0 (0)
Nail changes	5 (18)	2 (7)	0 (0)	0 (0)
Keratitis	0 (0)	0 (0)	1 (4)	0 (0)
Fever	0 (0)	1 (4)	0 (0)	0 (0)
Fatigue	3 (10)	3 (10)	3 (10)	0 (0)
Diarrhoea	7 (25)	1 (4)	0 (0)	0 (0)
Constipation	1 (4)	0 (0)	0 (0)	0 (0)
Stomatitis	8 (29)	1 (4)	0 (0)	0 (0)
Gastritis	1 (4)	0 (0)	0 (0)	0 (0)
Anorexia	2 (7)	1 (4)	0 (0)	0 (0)
Nausea	3 (11)	1 (4)	0 (0)	0 (0)
Vomiting	2 (7)	2 (7)	1 (4)	0 (0)
Dyspnoea	2 (7)	0 (0)	1 (4)	0 (0)
ILD	2 (7)	0 (0)	0 (0)	1 (4)*
Vertigo	1 (4)	1 (4)	0 (0)	0 (0)
Dysgeusia	0 (1)	1 (4)	0 (0)	0 (0)
Elevated AST/ALT	10 (36)	2 (7)	4 (14)	1 (4)*
Elevated creatinine	2 (7)	1 (4)	2 (7)	0 (0)

ALT = alanine transaminase; AST = aspartate transaminase; ILD = interstitial lung disease. *Same patient.

could occur. Yatabe *et al* (2006) has developed a rapid assay to detect *EGFR* mutations, and we have decided to use this assay in a phase III trial. The *EGFR* mutation rates in transbronchial biopsy samples were found to be the same as those in surgical specimens, suggesting that this assay can also accommodate stage IV NSCLC. We detected the two characteristic types of *EGFR* mutations (in exons 19 and 21) in 44 and 56% of the patients, respectively (Table 1); these percentages are identical to those in previous reports from Japan (Shigematsu *et al*, 2005; Asahina *et al*, 2006; Inoue *et al*, 2006; Yatabe *et al*, 2006; Yoshida *et al*, 2007). In summary, we confirmed the feasibility of using the *EGFR* detection assay in daily practice.

The overall response rate was 75%, which was comparable to those of other phase II studies of gefitinib in patients with *EGFR* mutations (Asahina *et al*, 2006; Inoue *et al*, 2006), despite our study permitting the entry of patients who had previously received up to two chemotherapy regimens. The DCR of 96% was relatively high, and the median PFS of 11.5 months and 1Y-S of 79% were also very promising. In a Korean study, Lee *et al* (2006) also reported a very promising response rate (56%) and 1Y-S (76%) for gefitinib in a prospective study of selected NSCLC patients with adenocarcinoma and never/light smokers, defined as having smoked no more than 100 cigarettes during one's lifetime. In the screening process for the present study, *EGFR* mutations were significantly more frequent in women, patients with adenocarcinoma and those who had never smoked. However, among the patients who were selected according to their *EGFR* mutation status, no differences in response were observed between never smokers and current/former smokers or between chemotherapy-naive and postchemotherapy patients. In a retrospective study, Han *et al* (2006) directly compared clinical predictors (smoking history, gender and histology) and the *EGFR* mutation status for their ability to predict response and survival. They showed that female never smokers with adenocarcinoma (three clinical predictors) had a 33% response rate, whereas patients with a positive *EGFR* mutation status had a 62% response rate. Furthermore, in a multivariate analysis, only a positive *EGFR* mutation status was associated with an improved OS, suggesting that the *EGFR* mutation status should be analysed whenever possible to optimise response predictions based on clinical

Table 6 Subsequent treatments after failure to respond to gefitinib ($n = 28$)

Gefitinib treatment	No. of Patients	1st regimen after gefitinib	No. of patients	2nd regimen after gefitinib	No. of patients
1st line	17	Plt doublet	5	Gem or Doce	2
				Gefitinib[a]	1
		VNR	1	—	-
2nd line[b]	4	Doce	2	Doce	1
		Plt doublet	1	Doce	1
2nd line	5	Doce	1	Gefitinib[a]	1
3rd line	2	—	—	—	—
Total	28		10		
Response			4/10		2/6

Doce = docetaxel; Gem = gemcitabine; Plt = platinum; VNR = vinorelbine. [a]Both patients had an SD response after gefitinib re-treatment. [b]First regimen as systemic chemotherapy after adjuvant treatment.

Table 7 Bronchial alveolar carcinoma (BAC) features and *EGFR* mutation status

	EGFR mutation		
	+	−	*P*-value
Surgically resected adenocarcinoma case	12	24	
BAC component			
Yes	8	17	1.0
No	4	7	
Micropapillary pattern			
Yes	4	12	0.48
No	8	12	
Mucin production			
Yes	1	5	1.0
No	11	19	

EGFR = epidermal growth factor receptor.

background factors. In the present study, *EGFR* mutations were detected in 16 out of 40 (40%) female never smokers with adenocarcinoma who underwent the screening process, and 14 out of these 16 patients (88%) achieved a response after undergoing gefitinib therapy. We could not compare the predictive powers of clinical predictors and the *EGFR* mutation status with regard to the clinical benefits of gefitinib in this study. Thus, the need for *EGFR* mutation testing among clinically favourable patients remains uncertain. Decisions regarding the first-line therapy of choice for patients with *EGFR* mutations or a clinically favourable profile (nonsmoker with adenocarcinoma) must also await the results of an ongoing randomised phase III study in an Asian population (IPASS: Iressa Pan-Asian Study) comparing platinum doublets with gefitinib.

In contrast, 50% of the men, 67% of the smokers and 63% of the men who were smokers achieved a PR in this study. Furthermore, one female nonsmoker with squamous cell carcinoma also responded to gefitinib. The histological type of this tumour was reassigned by a pulmonary pathologist, and the tumour was finally confirmed to be a squamous cell carcinoma. Squamous cell carcinoma harbouring an *EGFR* mutation is rarely seen but has been previously reported (Asahina *et al*, 2006). In a Japanese phase II trial of gefitinib for unselected chemotherapy-naive patients (Niho *et al*, 2006), the response rates among smokers, men, and patients with nonadenocarcinoma were 19, 13 and 10%, respectively. Thus, NSCLC patients who are either smokers, men or have a nonadenocarcinoma histology are unlikely to receive gefitinib treatment as a first-line treatment instead of standard chemotherapies (platinum doublets), which yield a response rate of about 30% (Schiller *et al*, 2002). Therefore, *EGFR* mutation screening may have a higher impact on the selection of responders to gefitinib treatment among these kinds of Asian patient subset (for example, smokers with adenocarcinoma, and nonsmoking men or women with nonadenocarcinoma).

The benefit of chemotherapy in general among patients with *EGFR* mutations, compared with *EGFR* mutation-negative patients, remains uncertain. Previous studies (Bell *et al*, 2005) have suggested that patients with *EGFR* mutations tend to be more sensitive to chemotherapy than those with wild-type *EGFR*. In the present study, 40 and 33% of the patients responded to first- and second-line chemotherapy regimens after gefitinib, respectively. These relatively high response rates for refractory NSCLC suggest that patients with an *EGFR* mutation-positive status are generally sensitive to chemotherapy. Large-scale multivariate analyses, using pooled data from prospective phase II or III trials in which the *EGFR* mutation status was clearly confirmed, are needed to clarify this point.

The toxicities observed in the present study were mostly tolerable. Most of the common adverse events, like rash, diarrhoea or hepatotoxicity, were mild and subsided after gefitinib administration was paused for a short period. One male smoker with adenocarcinoma died of ILD. Thus, even among patients who are selected based on their *EGFR* mutation status, men or smokers may still be at risk for developing ILD; therefore, biomarkers to predict ILD are needed.

Patients with exon 19 mutations tended to have a higher response rate than those with a missense mutation in exon 21, consistent with the findings of previous reports (Jackman *et al*, 2006; Riely *et al*, 2006). The Spanish Lung Cancer Group also reported on a prospective phase II study of erlotinib in advanced NSCLC patients with *EGFR* mutations (Paz-Ares *et al*, 2006). The overall response rate was 82%. They also showed a difference in response rates between patients with mutations in exons 19 and 21 (95 and 67%, respectively). Exon 11 c-kit mutations are more closely correlated with a good prognosis in patients with gastrointestinal stromal tumour, who may benefit from lower doses of imatinib, whereas patients with exon 9 mutations may require higher doses (Debiec-Rychter *et al*, 2006). In the case of *EGFR*, functional differences between mutation types may also exist.

We found no discernible associations between the *EGFR* mutation frequency and the presence of a BAC component. Several reports, including that of Hirsch *et al* (2005) suggest that a higher *EGFR* copy number is correlated with BAC histological features. We also found an association between a high *EGFR* copy number and the presence of a BAC component, even though the number of specimens examined was relatively small. In a study on erlotinib, the presence of a BAC component was clearly associated with *EGFR* amplification. As the *EGFR* mutation rate is lower in western populations than in Asian populations, the *EGFR* gene copy number might be a more useful biomarker in western populations, especially with regard to the use of erlotinib.

In conclusion, gefitinib treatment for patients with advanced NSCLC harbouring an *EGFR* mutation demonstrated a promising activity in patients with a good performance status. Patient screening according to *EGFR* mutation status may be a useful tool in daily practice and will likely have a great impact on the selection of patients who are likely to benefit from gefitinib treatment.

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clinical Studies

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ORIGINAL ARTICLE

Predictors of Survival in Patients With Bone Metastasis of Lung Cancer

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Abstract The prognosis of patients with bone metastasis from lung cancer has not been well documented. We assessed the survival rates after bone metastasis and prognostic factors in 118 patients with bone metastases from lung cancer. The cumulative survival rates after bone metastasis from lung cancer were 59.9% at 6 months, 31.6% at 1 year, and 11.3% at 2 years. The mean survival was 9.7 months (median, 7.2 months; range, 0.1–74.5 months). A favorable prognosis was more likely in women and patients with adenocarcinoma, solitary bone metastasis, no metastases to the appendicular bone, no pathologic fractures, performance status 1 or less, use of systemic chemotherapy, and use of an epithelial growth factor receptor inhibitor. Analyses of single and multiple variables indicated better prognoses for patients with adenocarcinoma, no evidence of appendicular bone metastases, and treatment with an epithelial growth factor receptor inhibitor. The mean survival period was longer in a small group treated with an epithelial growth factor receptor inhibitor than in the larger untreated group. The data preliminarily suggest treatment with an epithelial growth factor receptor inhibitor may improve survival after bone metastasis.
Level of Evidence: Level IV, prognostic study. See the Guidelines for Authors for a complete description of levels of evidence.

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Each author certifies that his or her institution has approved or waived approval for the human protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

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Introduction

Metastatic bone tumors occur at particularly high rates in cancers of the breast, prostate, lung, and kidney, accounting for 75% of all patients [16]. Many patients with lung cancer are in advanced stages of the disease at the time of diagnosis. The 5-year survival rate for patients with lung cancer is 10% to 20%, as reported by Stanley [15] and Freise et al. [4], indicating a poor prognosis. Although it is reported bone metastasis from lung cancer occurs in 14% to 40% of patients, its clinical features have not been clearly described [9].

When treating skeletal metastasis, it is important to know the prognostic factors and prognosis after bone metastasis. Tokuhashi et al. [17] proposed six factors that predicted survival for tumors metastatic to the spine: general condition, number of extraspinal bone metastases, number of metastases in the vertebral body, metastases to major internal organs, primary site of the cancer, and severity of spinal cord palsy. The grade of malignancy of the primary tumors, visceral metastasis to vital organs, and

number of bone metastases are reportedly important prognostic factors [18, 19]. In a report of 350 patients with bone metastasis, the primary site, performance status (PS), number of bone metastases, metastasis to organs, and previous chemotherapy were important prognostic factors, with lung cancer being the poorest [10]. The Scandinavian Sarcoma Group [5] examined prognostic factors in 460 patients undergoing surgery for bone metastasis and reported poor prognoses in patients with lung cancer as the primary site, pathologic fracture, and metastasis to organs. In another report of 342 patients with vertebral metastasis, the important prognostic factors included PS, metastasis to organs, and the primary site [20]. Prognosis in bone metastasis from lung cancer also was reported as poor. However, these studies [5, 10, 17–20] reported on bone metastasis from various cancers and did not focus on lung cancer alone. Therefore, the prognostic factors and survival rates of patients with bone metastasis from lung cancer remain unclear.

Several recent reports suggest an epithelial growth factor receptor (EGFR) inhibitor has been effective in treating lung cancer [6, 11, 12, 21]. The EGFR inhibitor is a new molecule-targeted agent for lung cancer that is reported to have a considerable effect on females and nonsmokers, especially those with adenocarcinoma [6, 12]. However, its effectiveness in patients with bone metastasis from lung cancer is unknown.

We first assessed the survival rates and explored various prognostic factors of 118 patients with bone metastasis from lung cancer. We then preliminarily ascertained in a small group of patients whether treatment with an EGFR inhibitor had the potential to influence survival.

Materials and Methods

We retrospectively reviewed 1157 patients with lung cancer treated at Aichi Cancer Center Hospital between January 1, 1999, and December 31, 2002. Of these, 121 patients (10.4%) were treated for lung cancer that had metastasized to bone. We excluded three patients because of incomplete information; this left 118 patients (77 men, 41 women) who had bone metastasis from lung cancer (Table 1). Fifty-two of the 118 patients met criteria (see below) for administering an oral selective EGFR inhibitor and it was administered to 14 of the 52 patients. It was not administered to the remaining 38 patients because the use of EGFR inhibitor was not available before June 2002 in Japan. Apart from determining survival, our primary outcome was survival in patients receiving an EGFR inhibitor. Based on survival in our hospital [12], the power would be approximately 70% using a two-side type I error of 5% to detect a 30% difference in 1-year survival among the 52 patients who met the criteria for administering EGFR inhibitor.

Table 1. Distribution of patients with skeletal metastases of lung cancer (n = 118)

Prognostic factor	Subgroups	Number of patients
Age (years)	≥ 60	67 (57%)
	< 60	51 (43%)
Gender	Female	41 (35%)
	Male	77 (65%)
Performance status	0,1	67 (57%)
	2, 3, 4	51 (43%)
Subtype	Adenocarcinoma	83 (70%)
	Nonadenocarcinoma	35 (30%)
Surgery for lung cancer	Yes	36 (31%)
	No	82 (69%)
Number of bone metastases	Solitary	19 (16%)
	Multiple	99 (84%)
Appendicular bone metastasis	Yes	21 (18%)
	No	97 (82%)
Pathologic fracture	Yes	15 (13%)
	No	103 (87%)
Brain metastasis	Yes	48 (41%)
	No	70 (59%)
Liver metastasis	Yes	16 (14%)
	No	102 (86%)
Chemotherapy	Yes	67 (57%)
	No	51 (43%)
Radiation	Yes	61(52%)
	No	57 (48%)
Gefitinib	Yes	14 (12%)
	No	104 (88%)

The mean age of the 118 patients at the time of bone metastasis was 59.6 years (standard deviation [SD], 10.2 years; range, 28–85 years). Lung cancer diagnosis was confirmed by computed tomography, fiberscope examination, and biopsy. Presence or absence of bone metastasis was confirmed by radiography or bone scintigraphy. All patients provided informed consent for participation in this study.

Among the 118 patients, 308 sites with bone metastasis were determined. Sites with high incidence included the rib, vertebra, and pelvis, where there is a high concentration of red marrow (Fig. 1). When bone metastasis was first confirmed by radiography or scintigraphy, 19 patients (16%) had a solitary site of metastasis and 99 patients (84%) had multiple sites. Eight (42%) of the 19 patients had a solitary bone metastasis that developed in other new sites. The remaining 11 patients (58%) remained with a solitary site of metastasis at followup. The minimum followup was

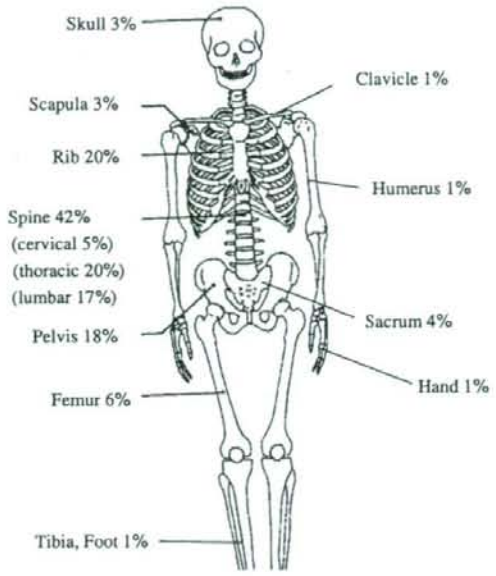

Fig. 1 Anatomic localization of skeletal metastases from lung cancer is shown (n = 118).

0.2 months (mean ± SD, 12.8 ± 14.6 months; range, 0.2–54.0 months)

The time from lung cancer diagnosis to bone metastasis was less than 1 month in 54 patients (46%), of which 12 patients initially had been diagnosed with an unknown primary cancer. For the remaining patients, the time from diagnosis to bone metastasis was 1 to 6 months in 23 patients, 6 months to 1 year in 11 patients, and 1 to 2 years in 10 patients. There were 20 patients (17%) whose lung cancer metastasized to the bone longer than 2 years after diagnosis, among whom the primary site was excised in 19 cases.

The major histologic type of the primary site was adenocarcinoma (83 patients), followed by squamous cell carcinoma (17 patients), small cell and large cell carcinoma (seven patients), and adenosquamous carcinoma (four patients). The primary site already had been excised at the time of bone metastasis in 36 patients (31%), but not in the remaining 82 patients (69%). After bone metastasis, 44 patients (37%) had brain metastasis, 12 patients (10%) had liver metastasis, and four patients (4%) had metastasis to the brain and liver. Approximately 50% of the patients had brain or liver metastasis.

Performance status was evaluated using the method devised by the Eastern Cooperative Oncology Group [14]. Patients with PS 0 are fully active and have no limitation in daily life; patients with PS 1 are restricted in physically strenuous activity but are ambulatory and able to do work of a light or sedentary nature; patients with PS 2 are ambulatory and capable of all self-care but are unable to do work activities; patients with PS 3 are capable of only limited self-care, are confined to bed or chair for greater than 50% of working hours; and patients with PS 4 are completely disabled, cannot do any self-care, and are totally confined to a bed or chair. Seventeen patients had PS 0, 50 patients had PS 1, 17 patients had PS 2, 17 patients had PS 3, and 17 patients had PS 4. Pathologic fractures during the course occurred in 15 patients, among which five underwent surgery for femoral pathologic fractures. Three patients were treated by intralesional

Table 2. Data for patients with adenocarcinoma and performance status 1 or less (n = 14) with user of gefitinib

Patient number	Age (years)	Gender	Performance status	Metastasis to appendicular bone	Pathologic fracture	Solitary or multiple	Radiation	Chemo therapy	Outcome	Survival period (days)
1	42	Female	0	–	–	Multiple	–	+	Dead	898
2	72	Female	1	–	–	Solitary	–	+	Alive	736
3	57	Male	0	–	–	Multiple	+	+	Dead	467
4	68	Female	0	–	–	Multiple	–	+	Alive	903
5	56	Male	1	–	–	Solitary	–	+	Alive	251
6	72	Male	1	+	–	Multiple	+	+	Dead	531
7	66	Male	0	–	–	Multiple	–	+	Dead	427
8	65	Male	0	–	–	Multiple	+	+	Dead	387
9	72	Female	1	–	–	Solitary	+	+	Dead	431
10	69	Male	1	–	–	Multiple	+	+	Alive	491
11	62	Female	1	+	–	Solitary	+	+	Dead	448
12	66	Female	1	–	–	Multiple	–	+	Dead	310
13	66	Male	1	–	–	Multiple	–	+	Alive	621
14	54	Female	1	–	–	Solitary	+	–	Alive	577

Table 3. Data for patients among patients with adenocarcinoma and performance status 1 or less (n = 38) without use of gefitinib

Patient number	Age (years)	Gender	Performance status	Metastasis to appendicular bone	Pathologic fracture	Solitary or multiple	Radiation	Chemotherapy	Outcome	Survival period (days)
1	52	Male	0	–	–	Multiple	+	+	Dead	460
2	61	Male	1	–	–	Multiple	–	–	Alive	48
3	59	Male	0	–	–	Multiple	–	+	Dead	294
4	39	Female	0	–	–	Multiple	+	+	Dead	365
5	28	Female	0	–	–	Multiple	–	+	Dead	336
6	56	Male	0	–	–	Multiple	–	+	Dead	369
7	51	Female	0	–	–	Multiple	+	+	Dead	201
8	61	Male	1	–	–	Solitary	+	–	Dead	188
9	57	Male	1	–	–	Multiple	–	+	Dead	28
10	49	Female	1	–	–	Multiple	+	+	Alive	303
11	49	Male	1	–	–	Multiple	+	+	Dead	243
12	63	Male	1	–	–	Solitary	–	+	Alive	390
13	59	Male	1	–	–	Multiple	–	+	Dead	144
14	54	Female	0	–	–	Multiple	+	+	Alive	285
15	51	Female	1	+	–	Multiple	+	–	Dead	61
16	57	Female	1	–	–	Multiple	+	–	Dead	53
17	63	Female	1	–	+	Multiple	–	+	Dead	244
18	65	Male	1	–	–	Multiple	+	+	Dead	166
19	62	Male	0	–	–	Multiple	–	+	Alive	470
20	55	Female	1	–	–	Solitary	+	+	Alive	207
21	42	Male	0	–	–	Multiple	+	–	Dead	36
22	56	Male	1	+	–	Multiple	+	+	Alive	316
23	28	Female	0	–	–	Multiple	–	+	Alive	308
24	56	Female	1	–	–	Multiple	+	+	Dead	351
25	60	Female	1	+	–	Multiple	+	+	Dead	196
26	63	Female	1	–	–	Multiple	–	–	Dead	68
27	47	Female	1	+	–	Multiple	+	+	Dead	163
28	66	Female	1	–	–	Multiple	–	+	Dead	393
29	45	Female	1	–	–	Multiple	–	+	Dead	345
30	41	Male	1	+	–	Multiple	+	+	Dead	306
31	55	Male	1	–	–	Solitary	–	+	Alive	1619
32	69	Male	1	–	–	Multiple	+	–	Dead	164
33	50	Male	1	–	+	Multiple	–	–	Dead	18
34	57	Female	1	–	–	Multiple	–	+	Alive	855
35	42	Female	1	–	–	Multiple	+	+	Dead	366
36	60	Female	1	–	–	Multiple	–	–	Dead	156
37	51	Male	1	–	–	Multiple	+	+	Dead	387
38	59	Female	1	–	–	Solitary	+	+	Alive	1416

resection with prosthesis implantation and two patients were treated with compound plate osteosynthesis. The remaining 10 patients had spinal compression fractures, of which two patients had complete paralysis of the lower extremities after pathologic fracture.

Regarding treatment of the primary site, radiotherapy was performed in 61 patients and systemic chemotherapy was administered to 67 patients. The administered regimens varied among patients, which included gemcitabine hydrochloride and vinorelbin ditartrate (11 patients), cisplatin and vinorelbin ditartrate (seven patients), carboplatin and vinorelbin ditartrate (six patients), carboplatin and paclitaxel (six patients), carboplatin and etoposide (four patients), carboplatin and etoposide (four patients), cisplatin and paclitaxel (four patients), cisplatin and etoposide (two patients), cisplatin and irinotecan hydrochloride (two

patients), cisplatin and gemcitabine hydrochloride (one patient), carboplatin and docetaxel hydrate (one patient), and unknown (19 patients). Systemic chemotherapy was not given to the remaining 51 patients.

We examined the cumulative survival rate after bone metastasis and prognostic factors for patients with bone metastasis from lung cancer (Table 1) and then calculated overall survival based on absence or presence of an EGFR inhibitor (Tables 2, 3).

Gefitinib (Iressa®; AstraZeneca, Osaka, Japan), an oral selective inhibitor of EGFR, was administered to patients with adenocarcinoma and PS 1 or less. In this study, there were 52 patients with adenocarcinoma and PS 1 or less. Gefitinib was administered to 14 of these patients (seven men, seven women; mean age ± SD, 63.4 ± 8.5 years; range, 42–72 years) (Table 2) and not administered to the remaining 38 patients (18 men, 20 women; mean age ± SD, 53.6 ± 9.5 years; range, 28–69 years) (Table 3).

We estimated patient survival using the Kaplan–Meier survival method, considering the relevant time scale for analysis to begin at the time of bone metastasis. Patients were censored on the basis of whether they were alive. The univariate log rank test was used to evaluate the prognostic importance of age, gender, PS, histologic type, condition of the primary site, number of bone metastases, site of bone metastasis, pathologic fractures, metastasis to the brain or liver, chemotherapy or radiotherapy for the primary site, and use of an EGFR inhibitor (gefitinib). Subsequent multivariate analysis was performed to detect factors independently associated with survival using a Cox proportional hazard survival model [4]. Multivariate regression analysis was performed by including all clinical characteristics that independently predicted 1-year survival. The results are reported as a hazard ratio and 95% confidence interval. As for the influence of gefitinib on survival, we used the Kaplan–Meier curve of overall survival based on absence or presence of gefitinib treatment among patients with adenocarcinoma and PS 1 or less. The log rank test was used to evaluate a difference. For all analyses, a p value of 0.05 or less was considered significant. We used SPSS 11.0 (SPSS Inc, Chicago, IL) software to conduct Kaplan–Meier survival analysis and the Cox proportional hazard survival model.

Results

The overall cumulative survival rate after bone metastasis for all 118 patients was 59.9% for 6-month survival, 31.6% for 1 year, and 11.3% for 2 years. The mean survival period was 9.7 months (SD, 10.3 months; median, 7.2 months; range, 0.1–74.5 months) (Fig. 2). Although

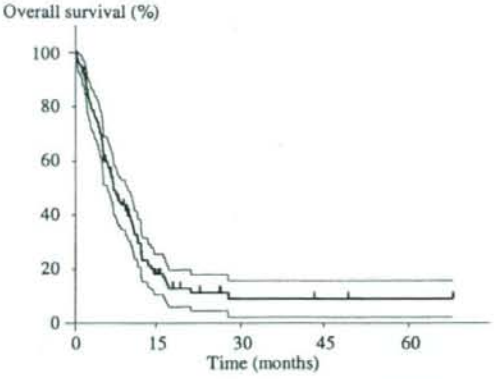

Fig. 2 A Kaplan–Meier curve of overall survival for all patients is illustrated. The dotted lines indicate the 95% confidence interval. The overall cumulative survival rates after bone metastasis for all 118 patients are 59.9% for 6 months, 31.6% for 1 year, and 11.3% for 2 years.

the prognosis in patients with bone metastasis was generally poor, seven patients survived for at least 2 years (6%).

We identified eight prognostic factors: gender, PS, histologic type, number of bone metastases, site of bone metastases (bone metastasis to the appendicular bone), pathologic fracture, systemic chemotherapy, and gefitinib use (Table 4). A favorable prognosis was more likely in women and in patients with PS 1 or less, adenocarcinoma, solitary bone metastasis, no metastases to the appendicular bone, no pathologic fractures, use of systemic chemotherapy, and use of gefitinib.

The presence of adenocarcinoma, evidence of appendicular bone metastases, and use of gefitinib independently predicted survival (Table 5). The prognosis was poorer ($p = 0.03$) in patients with metastasis to the appendicular bone (mean, 6.5 months; range, 0.1–17.7 months) than in patients without metastasis (mean, 10.4 months; range, 0.2–74.5 months) (Fig. 3). The mean survival was longer ($p = 0.005$) in the group treated with gefitinib (17.8 months; range, 8.4–30.1 months) than in the group without gefitinib (10.8 months; range, 0.6–54.0 months) among 52 patients with adenocarcinoma and PS 1 or less (Fig. 4).

Discussion

It is important to know the prognosis after bone metastasis when treating bone metastasis from lung cancer. Primary site, PS, presence or absence of metastasis to organs, and number of bone metastases have been reported as important prognostic indicators in patients with bone metastasis from various cancers [5, 10, 20]. However, we are unaware of any previous reports regarding the prognostic factors of

Table 4. Univariate analysis of 1-year survival rates in patients with skeletal metastases of lung cancer (n = 118)

Prognostic factor	Subgroup	Survival (months)	1-year survival rate (%)	p Value
Age (years)	≥ 60	9.1 (1.3)	27.1 (5.6)	0.38
	< 60	10.4 (1.5)	32.6 (7.2)	
Gender	Female	13 (2.1)	39.3 (8.1)	0.02
	Male	7.9 (0.9)	25.8 (5.2)	
Performance status	0,1	11.6 (1.2)	44.8 (6.4)	< 0.0001
	2, 3, 4	7.1 (1.5)	13.3 (5.0)	
Subtype	Adenocarcinoma	11.3 (1.3)	41.6 (5.7)	< 0.0001
	Nonadenocarcinoma	5.8 (0.8)	8.9 (4.9)	
Surgery for lung cancer	Yes	11.0 (2.0)	27.8 (7.5)	0.89
	No	9.1 (1.1)	32.0 (5.6)	
Number of bone metastases	Solitary	14.0 (3.3)	54.3 (12.2)	0.02
	Multiple	8.9 (0.9)	27.3 (4.7)	
Appendicular bone metastasis	Yes	6.5 (1.1)	12.6 (8.0)	0.03
	No	10.4 (1.1)	35.6 (5.1)	
Pathologic fracture	Yes	6.4 (1.2)	6.7 (6.4)	0.04
	No	10.2 (1.1)	35.7 (5.0)	
Brain metastasis	Yes	9.9 (1.4)	32.7 (7.3)	0.65
	No	9.5 (1.3)	28.9 (5.6)	
Liver metastasis	Yes	7.0 (1.3)	13.4 (8.8)	0.1
	No	10.1 (1.1)	33.3 (4.9)	
Chemotherapy	Yes	11.4 (1.2)	45.3 (6.3)	0.0009
	No	7.5 (1.5)	13.0 (4.9)	
Radiation	Yes	9.7 (1.4)	30.0 (6.2)	0.49
	No	9.6 (1.3)	31.2 (6.5)	
Gefitinib	Yes	17.8 (1.8)	84.6 (10.0)	0.0001
	No	8.6 (1.0)	22.7 (4.4)	

Values are expressed as mean, with standard error in parentheses.

bone metastasis specifically from lung cancer. We examined the survival rates and prognostic indicators after bone metastasis from lung cancer.

The major limitations of our study included the lack of control subjects for comparison. Furthermore, there was a wide range of chemotherapy regimens and a selection bias of gefitinib use among the individual patients. Therefore, we compared the survival based on absence or presence of EGFR inhibitor treatment among patients with adenocarcinoma and PS 1 or less to exclude selection bias. The numbers of patients receiving EGFR was small (14) and therefore the power of the study is limited and must be considered preliminary. However, our study represents the largest followup study of patients with bone metastasis from lung carcinoma at one institution.

Some reports suggest the mean length of survival in patients with Stage IV disease, including distant metastasis, is approximately 6 months [2, 13]. The mean survival period for patients with lung cancer with bone metastasis has been reported as 5 to 6 months [15]. We found a mean survival period after bone metastasis of 9.7 months, with a median of 7.2 months. Approximately 70% of the patients died within 1 year after bone metastasis. Although the prognosis in patients with lung cancer with bone metastasis was extremely poor, seven of the 118 patients (6%)

Table 5. Multivariate analysis of selected clinical factors in patients with skeletal metastasis of lung cancer

Prognostic factor	p Value	Hazard ratio (95% confidence interval)
Positive		
Gender (female)	0.63	1.13 (0.68–1.88)
Performance status (0, 1)	0.09	1.69 (0.93–3.08)
Adenocarcinoma	< 0.01	2.17 (1.30–3.62)
Pathologic fracture	0.33	1.39 (0.71–2.73)
Chemotherapy	0.53	1.20 (0.68–2.11)
Gefitinib	0.03	2.42 (1.09–5.32)
Negative		
Multiple bone metastasis	0.14	1.68 (0.84–3.34)
Appendicular bone metastasis	0.01	2.05 (1.18–3.56)

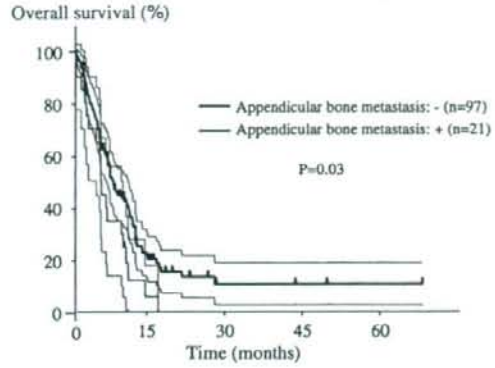

Fig. 3 A Kaplan–Meier curve of overall survival based on absence or presence of metastasis of the appendicular bone is illustrated. The dotted lines indicate the 95% confidence interval. The prognosis is poorer ($p = 0.03$) in patients with metastasis to the appendicular bone than in patients without metastasis.

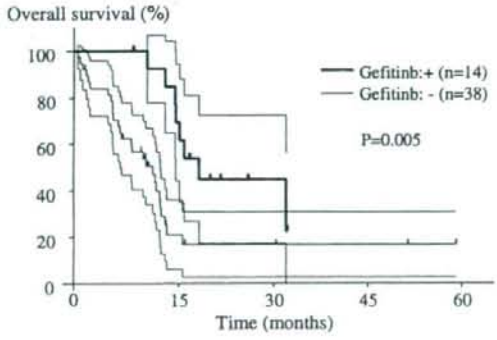

Fig. 4 A Kaplan–Meier curve of overall survival based on absence or presence of gefitinib treatment is illustrated. The dotted lines indicate the 95% confidence interval. The mean survival is longer ($p = 0.005$) in the group treated with gefitinib than in the untreated group among 52 patients with adenocarcinoma and PS 1 or less.

survived for at least 2 years. Hirano et al. [7] reported two patients with a solitary metastasis site who had extended survival by surgical resection of the metastatic site and chemotherapy. Agarwala and Hanna [1] also reported a patient with a solitary bone metastasis had apparently longer survival with aggressive treatment. Ando et al. [2] reported the grade of PS and the number of metastasized organs were important factors in patients with distal metastasis from lung cancer. In our study, the mean length of survival was substantially longer in patients with solitary-site metastasis than in patients with multiple-site metastases, and the survival rate was longer in patients with PS 1 or less than in patients with PS 2 or greater. It is suggested PS and number of bone metastases are associated with survival after bone metastasis [2].

Based on the primary site, Tofe et al. [16] reported a high incidence of metastasis in the lumbar vertebra, femur, and ilium among patients with prostate cancer; in the pelvis, vertebra, femur, and ribs among patients with breast cancer; and in the skull and vertebra among patients with thyroid cancer. We observed a high incidence of bone metastasis from lung cancer in the vertebra, rib, and pelvis, and metastasis to the femur in only 6%. The prognosis was poorer in patients with metastasis to the appendicular bone, such as the femur, than in patients with metastasis only to an axial bone, such as the vertebra, rib, or pelvis. The vertebral vein system is known as a mechanism for spread of axial bone metastasis [3]. In bone metastasis from lung cancer, metastasis may occur easily at an axial bone through the vertebral vein system [3] at an early stage and then at an appendicular bone in more advanced stages of the disease.

Among our study patients, the mean survival period was longer in the group treated with gefitinib than in the untreated group. Gefitinib, an EGFR inhibitor, is a new molecule-targeting treatment for lung cancer. It is reported to have a considerable effect on females and nonsmokers, especially those with adenocarcinoma [6, 11, 12, 21]. Analyses of single and multiple variables indicated better prognoses for patients with adenocarcinoma and patients treated with gefitinib. These findings suggest treatment with gefitinib may improve survival after bone metastasis. However, interstitial pneumonia remains a serious side effect [8]; furthermore, it is reported gefitinib is less effective in patients without the EGFR gene [12]. Therefore, indications for treatment with gefitinib should be considered carefully before improvement in survival can be expected.

We found a favorable prognosis was more likely in women and in patients with PS 1 or less, adenocarcinoma, solitary bone metastasis, no metastasis to the appendicular bone, no pathologic fracture, use of systemic chemotherapy, and use of gefitinib. Histologic subtype, no evidence of appendicular bone metastases, and use of gefitinib independently predicted survival. Our findings suggest treatment with EGFR inhibitor improves survival after bone metastasis. However, further investigations such as controlled clinical trials are needed to verify the usefulness of EGFR inhibitor.

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Epidermal Growth Factor Receptor Mutations in Small Cell Lung Cancer

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Abstract **Purpose:** The vast majority of epidermal growth factor receptor (*EGFR*) mutations occur in lung adenocarcinoma, and even rare cases of other subtypes with this mutation, such as adenosquamous cell carcinoma, are associated with adenocarcinoma histology. According to this adenocarcinoma-specific nature of *EGFR* mutation, analysis of *EGFR* mutations with small cell lung cancers (SCLC) may provide a clue to its histogenesis.

Experimental Design: The mutational status of the *EGFR* gene was accessed in a cohort of 122 patients with SCLC; all patients were from a single institute. When the *EGFR* mutated, its gene copy number was also examined.

Results: *EGFR* mutations were detected in five SCLCs (4%). The patients were mainly in the light smoker and histologic combined subtype. All but one of the tumors harbored gene amplifications. Notably, in three tumors of the combined SCLC subtype, both components of adenocarcinoma and SCLC harbored an *EGFR* mutation, whereas gene amplification was detected only in the adenocarcinoma component. A partial response was achieved in a patient (with an *EGFR* mutation) who was treated with gefitinib.

Conclusions: Although *EGFR* mutations are rare in SCLC, a combined subtype of SCLC with adenocarcinoma in light smokers may have a chance of harboring *EGFR* mutations. For patients with an *EGFR* mutation, *EGFR* tyrosine kinase inhibitor can be a treatment option. In terms of molecular pathogenesis, it is suggested that some SCLCs may have developed from pre-existing adenocarcinomas with *EGFR* mutations, but the development may not be simply linear, taking into consideration the discordant distribution of *EGFR* amplification.

The vast majority of epidermal growth factor receptor (*EGFR*) gene mutations are detected in lung adenocarcinoma. A comprehensive analysis by Shigematsu and Gazdar reported that non-adenocarcinomatous lung cancers with *EGFR* gene mutations were restricted to <5% of lung cancers (1). Although it is rare in other histologic subtypes, adenosquamous cell carcinoma showed the highest frequency among lung cancers, followed by squamous cell carcinoma and large cell carcinoma. In contrast, small cell carcinoma was not listed among *EGFR*-mutated lung cancers following a comprehensive examination of 1,380 lung tumors, which suggests a different molecular pathogenesis for this type of cancer. However, two patients (who had never smoked), recently reported having *EGFR* mutations with small cell lung cancers (SCLC; refs. 2, 3). In the first case, published in *The New England Journal of Medicine*, the patient with adenocarcinoma was initially treated with erlotinib. The recurrent tumor in the brain consisted of small cell carcinoma, which also harbored an *EGFR* mutation. Because the mutational status of the *EGFR* gene in the initial adenocarcinoma was not addressed, the clonal relationship between the two tumors was not clear. Another case was also a never-smoker who developed widespread SCLC. Mutational analysis revealed a typical *EGFR* gene deletion at exon 19. The tumor responded well to gefitinib treatment, and both primary and metastatic tumors regressed dramatically (3).

The incidence of *EGFR* mutation is quite high among the Japanese (~30-40% of non-small cell lung cancers on average) in contrast to ~10% of patients in the United States and in European countries (1, 4, 5). The clinicopathologic characteristics of patients with *EGFR* mutations include female sex, not smoking, and less frequent p53 mutation (4-6), which are very different from those of SCLC. It is therefore expected that *EGFR* mutations are very rare or absent in SCLC. A comprehensive analysis of *EGFR* mutations in SCLCs has not been reported in the literature; however, we believe it is important to determine its incidence, especially in mutation-endemic countries. In this study, we comprehensively examined a total of 122 SCLCs to address mutation incidence in SCLC.

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Translational Relevance

It is well known that epidermal growth factor receptor (*EGFR*) mutations are prevalent in female nonsmokers. However, *EGFR* mutations have recently been reported in some patients with small cell lung cancers (SCLC). In this study, we first examined a large series of SCLCs to address mutation incidence. Because the incidence of *EGFR* mutations differs between the United States and Japan, these data are important in determining the significance of ethnicity and frequency of *EGFR* mutations. As a result, a combined subtype of SCLC with adenocarcinoma in light smokers may have a chance of harboring *EGFR* mutations, although *EGFR* mutations are generally rare in SCLC. Notably, one such patient with an *EGFR* mutation achieved a partial response to gefitinib treatment. Although clinical relevance needs to be examined in more patients, *EGFR* tyrosine kinase inhibitor can be a treatment option for patients with SCLCs harboring an *EGFR* mutation.

Materials and Methods

Patients. Among 150 patients that were diagnosed with SCLC in the last 7 years at the Department of Pathology and Molecular Diagnostics, Aichi Cancer Center in Nagoya, Japan, specimens from 122 patients were available for molecular genetic analysis, and these were the subject for the current study. This series included 102 specimens obtained by biopsy, and 20 from surgically resected tumors. Histologic diagnosis of SCLC was based on the standard criteria defined by WHO classification (7). The study was a part of a comprehensive lung cancer research program, which had been approved by the institutional review board.

EGFR *mutation analysis.* All the specimens were fixed with formalin, and the *EGFR* mutation was analyzed with the method described previously, using an unstained paraffin section (8). This technique allows the detection of tumor cells constituting as little as 5% of a mixture of tumor cells with normal tissue using a single paraffin section. When frozen tissues were available, the mutational status of *EGFR* was accessed with standard reverse transcription-PCR coupled direct sequencing, as described previously (4), in addition to DNA-based analysis. In this assay, the mutational status of the L858R point mutation and the deletion of exon 19 were obtained when we examined paraffin sections, whereas direct sequencing using RNA revealed the mutational status of the whole tyrosine kinase domain.

Copy number analysis of EGFR. Gene amplification was analyzed by fluorescence *in situ* hybridization, using the LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis; Abbott Laboratories) according to the manufacturer's protocol. Fluorescence *in situ* hybridization was done on serial paraffin sections in the same tissue areas as the gene dosage analysis. A more than 4-fold increase of *EGFR* gene signals relative to CEP7 signals was considered a gene amplification. The results were confirmed by TaqMan-based gene dosage analysis as described previously (9).

Statistical analysis. Fisher's exact test for independence and unpaired *t* tests were used to show the correlation of clinicopathologic variables with EGFR mutation. $P < 0.05$ was considered statistically significant.

Results

SCLCs with EGFR *mutation.* Among 122 SCLCs examined (Table 1), we found *EGFR* mutations in five cases (4%). The mutations included L858R point mutations (three patients), a G719A point mutation (one patient), and a 15-bp deletion in exon 19 (one patient). Both frozen and paraffin tissues of 10 tumors, 2 of which harbored the above *EGFR* mutation, were available for analysis. They were examined using both reverse transcription-PCR coupled sequencing and assays for paraffin sections. The results were identical to those of the other analysis.

Clinicopathologic features of SCLCs with EGFR *mutations.* *EGFR* mutations were restricted to a very minor proportion (5 of 122; 4%) of SCLCS, and the clinicopathologic features of the patients with the mutation showed a trend similar to those of patients without the mutation. There were no significant differences in age, sex, and clinical stage at presentation. In contrast, accumulated smoking dose (pack-years) in patients with the mutation was much lower, and the difference was statistically significant (unpaired *t* test, $P = 0.02$). Indeed, three of the five patients with *EGFR* mutations were smokers with less than 40 pack-years. It is of note that one of the five patients was treated with gefitinib, and partial response was observed (case 2).

Morphologic features of SCLC with EGFR *mutations.* There are two subtypes of SCLC in the current WHO classification; thus, we examined whether the morphologic subtypes were associated with *EGFR* mutations. The combined subtype constituted a minor proportion (15 of 122, 12%) in this series, and three of them were positive for *EGFR* mutations (Table 1). Preferential mutation in the combined type were statistically significant (Fisher's exact test, $P < 0.01$). In two cases of the combined subtype (cases 1 and 3), SCLC components consisted of only a part of the nodule, and adenocarcinoma components constituted the predominant part. The representative morphologic features are displayed in Fig. 1. The other combined subtype (case 5) showed a mixture of SCLC and adenocarcinoma components throughout the tumor.

EGFR *amplification in SCLCs.* We have recently reported that *EGFR* amplification occurs in association with *EGFR* mutation (9). We therefore examined the EGFR gene copy number in the five SCLCs with *EGFR* mutations. Four of them showed gene amplification (Table 2), and the signals of the EGFR gene were loosely clustered (Fig. 2), suggesting a high degree of amplification, as is the case in homogeneously staining region patterns. Notably, three cases of combined SCLC subtypes harbored *EGFR* amplifications only in the adenocarcinoma component, but not in the SCLC component (Fig. 2).

Discussion

SCLC is a distinct neoplasm in terms of clinical aggressiveness, despite its high response to both chemotherapy and irradiation therapy. This aggressive cancer does not confer to

Table 1. Clinicopathologic features of SCLCs with and without *EGFR* mutations

	Mutated	Wild-type	*P*
No. of patients (total, $N = 122$)	5	117	
Age (median)	69	67	n.s.
Sex (female/male)	2/3	14/103	n.s.
Smoking history (median pack-years)	30	54	0.020
Disease stage (limited/extended disease)	4/1	81/33	n.s.
Histologic type (conventional/combined)	2/3	105/12	0.013

Abbreviation: n.s., not significant.

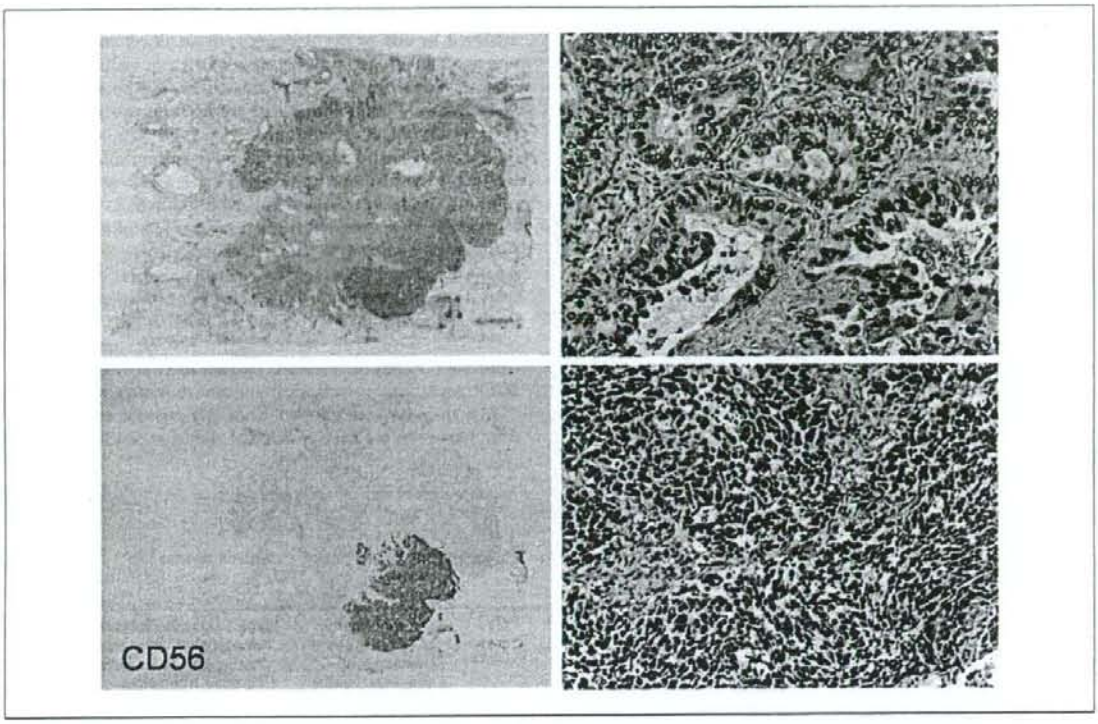

Fig. 1. Representative morphology of combined small cell carcinoma and adenocarcinoma (case 3). Approximately two-thirds of the area of the nodule (*top left*) consisted of an adenocarcinoma component, whereas the other area showed SCLC. Discrete expression of CD56 (neural cell adhesion molecule) corresponds to the component of SCLC (*bottom left*). High-power views of each component of adenocarcinoma (*top right*) and SCLC (*bottom left*). Both components harbor identical L858R *EGFR* mutations, although *EGFR* gene amplification was restricted to the adenocarcinoma component.

the lung, and it can develop in organs other than the lung, all of which share distinctive pathologic and immunohistochemical features, irrespective of their site of origin. These extrapulmonary carcinomas are characterized by frequent admixture with conventional carcinoma of the originating organ, such as adenocarcinoma in gastrointestinal tumors, and squamous cell carcinoma in head and neck cancers. This is true in SCLC. Nicholson et al. reported that 28 of 100 surgically resected SCLCs had a histologic component of non–small cell lung cancers (10). In our study, *EGFR* gene mutation was detected in 5 of 122 SCLCs. Because *EGFR* mutation was quite specific for adenocarcinoma, it is suggested that SCLCs with *EGFR* mutations are associated with adenocarcinoma. Indeed, three of the five combined SCLC had an adenocarcinoma component but not a squamous cell carcinoma component.

It has been suggested that the amine-precursor uptake and decarboxylase cells described by Pearse in 1969 (11) are the putative original cells of small carcinoma. These cells were described as comprising a neuroendocrine system in many organs, and as having ultrastructural features shared by small cell carcinomas. However, this hypothesis cannot explain the existence of combined SCLC, which is an admixture of small cell carcinoma and conventional adenocarcinoma or squamous cell carcinoma. Therefore, a multipotential cancer stem cell capable of divergent differentiation has been suggested as a putative origin of small cell carcinoma. Alternatively, the SCLC component may arise as a consequence of undifferentiated transformation from conventional carcinoma. Case 2 in the present study supported the latter scenario, because SCLC is the only component that metastasized to the lymph nodes. Furthermore, the vast majority of lung cancers harboring *EGFR* mutations are adenocarcinomas, supporting the idea that the adenocarcinomas existed prior to the development of SCLC in at least three of the cases of SCLC with *EGFR* mutations.

However, the results of *EGFR* amplification analyses support the former possibility. In three cases of combined subtype of SCLC with an *EGFR* mutation, only the adenocarcinoma component, not the SCLC component, harbored the amplification. This is in contrast to the uniform detection of *EGFR* mutations in both components. Because *EGFR* mutations in SCLC are rather rare, it is unlikely that the two components are independent of their origin. Rather, it is believed that they originated from a common ancestor. Therefore, it is suggested that the mutation occurred before a point branching off to SCLC and adenocarcinoma components, whereas gene amplification was acquired after that point. Cases 3 and 5 may be considered to have followed this scenario. However, case 1 was inconsistent with it because SCLC emerged after the therapy.

In case 1, the initial adenocarcinoma harbored both *EGFR* mutation and amplification. Subsequently, SCLC, which lacked

gene amplification, developed after the chemotherapy and gefitinib therapy. It was unlikely that the amplification was removed from cancer cells due to therapy. We have recently reported heterogeneous distribution of *EGFR* amplification in lung adenocarcinoma (9), and thus we suggested that only a clone without amplification was selected, survived, and was subsequently transformed to SCLC. The reported SCLC with *EGFR* mutation followed this pattern of progression (2, 3, 12), and lack of *EGFR* expression in SCLC may be a clue to this phenomenon. Under heavy selection pressure by gefitinib therapy, only a clone which is independent of *EGFR*-driven growth signals has a chance to expand. Transformation to SCLC fulfills this condition because *EGFR* expression in the SCLC was at a very low or undetectable level (13–15). Indeed, the SCLC component lacked *EGFR* expression, in contrast to positive expression in the initial adenocarcinoma and adenocarcinoma components (data not shown). This may be another mechanism for tolerance to the *EGFR* tyrosine kinase inhibitor, in addition to secondary genetic alterations.

Clinically, it is noteworthy that a partial response was achieved in one of the patients with an *EGFR* mutation who was treated with gefitinib. Because *EGFR* expression is at a very low or undetectable level in SCLC, it would be expected that *EGFR* tyrosine kinase inhibitors are not effective against SCLC even if the *EGFR* is mutated. However, a similar marked reduction of such cancers by *EGFR* tyrosine kinase inhibitor treatment has also been reported (2, 3). *EGFR* tyrosine kinase inhibitors may be a treatment option for SCLC with *EGFR* mutations, and a mutation test may be helpful to select such patients in addition to clinical characteristics, including the light smoker and histologic combined subtypes.

In summary, we examined 122 SCLCs and found 5 (4%) of them harboring *EGFR* mutations. The SCLCs with *EGFR* mutations were seen in the light smoker and histologic combined subtypes. Because of the specific involvement of *EGFR* mutations in adenocarcinoma, it is suggested that the SCLCs may have developed from pre-existing adenocarcinomas. However, we have concluded that this development may

Table 2. Clinicopathologic features of five SCLCs with *EGFR* mutations

Case	Sex/Age (y)	Pack-years smoking	*EGFR* mutation	*EGFR* amplification	Stage	Sample and histologic subtype	Clinical course
1	F/36	0	L858R	Amplified (>6)*	ED	Resected tumor; combined type (diagnosis of adenocarcinoma with a biopsy prior to surgery)	Stage IV adenocarcinoma was treated with CBDCA and PAC, followed by gefitinib, because of positive EGFR mutation with a biopsy specimen. Partial response was achieved but the tumor regrew. It was surgically resected, and histologically revealed to be combined small and adenocarcinoma
2	M/81	40	G719A	Amplified (>6)	ED	Biopsy specimen; conventional type	Stage IV SCLC was treated with gefitinib, because of the detection of G719A mutation using a lung biopsy specimen. A partial response was obtained
3	M/69	30	L858R	Amplified (>6)*	LD	Biopsy specimen, combined type	A lung cancer ($cT_1N_0M_0$) was surgically removed, and subsequent pathologic examination revealed combined SCLC. Adjuvant chemotherapy (CDDP and CPT-11) were administered. The patient is alive without recurrence
4	F/89	2.5	L858R	Low polysomy	LD	Biopsy specimen; conventional type	A biopsy specimen for lung cancer ($cT_2N_0M_0$) was diagnosed as SCLC. The patient refused any therapy, and was not a part of follow-up
5	M/65	67.5	Ex.19Del	Amplified (>6)*	LD	Resected tumor; combined type (cytological diagnosis of SCLC prior to surgery)	$cT_1N_1M_0$ cancer was treated with CDDP and TXT, followed by surgical resection of the tumor. Combined SCLC was revealed, and the patient was treated with adjuvant chemotherapy and irradiation. Three years later, SCLC recurred

Abbreviations: F, female; M, male; LD, limited disease; ED, extended disease; CBDCA, carboplatin; PAC, paclitaxel; CDDP, cisplatin; CPT-11, irinotecan.
* Only in the adenocarcinoma component.

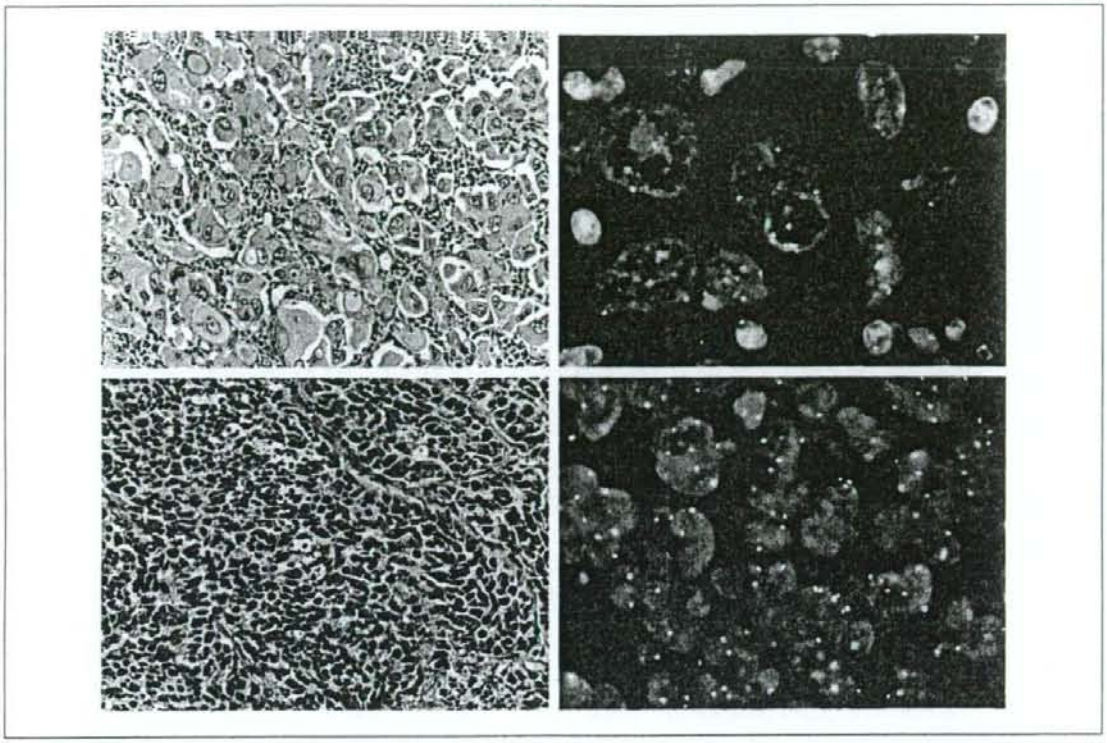

Fig. 2. *EGFR* amplification in SCLC with *EGFR* mutation (case 1). A female nonsmoker who had developed stage IV adenocarcinoma was treated with carboplatin and paclitaxel. The tumor recurred at the neck lymph node (*top left*), which was biopsied. Because molecular analysis using the tissue revealed a L858R mutation, she was subsequently treated with gefitinib. Although the tumor responded initially, rapid regrowth of the lung nodule was evident, and it was removed surgically. The SCLC component constituted most of the regrown nodule. *EGFR* mutation was detected in both adenocarcinomas in the lymph node and in the regrown SCLC. *EGFR* amplification was identified only in the adenocarcinoma but not in the regrown SCLC (*right*).

not be simply linear, considering the discordant distribution of EGFR amplification.

Disclosure of Potential Conflicts of Interest

T. Mitsudomi has a minor conflict with AstraZeneca, Chugai Pharm, Astellas, Daiichi-Sankyo, Sanofi-Aventis, Taiho Pharm, and Bristol Meyers.

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Phase I/II Pharmacokinetic and Pharmacogenomic Study of *UGT1A1* Polymorphism in Elderly Patients With Advanced Non–Small Cell Lung Cancer Treated With Irinotecan

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This phase II study investigated the recommended dose (RD) of irinotecan (CPT-11) by dose escalation in elderly (≥70 years) chemotherapy-naive Japanese patients with advanced non–small cell lung cancer. *UGT1A1*28* and **6* polymorphisms and pharmacokinetics were also investigated. Thirty-seven patients received the RD, 100 mg/m^2 of intravenous CPT-11, on days 1 and 8 of each 3-week cycle in phase II. The overall response rate was 8.1%. The median survival time was 441 days, and time to progression was 132 days. A significant correlation was observed between the incidence of grade 3/4 neutropenia and area under the time-concentration curve (AUC) values of SN-38. A reduction in AUC ratios ($AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$) and a rise in incidence of grade 3/4 neutropenia were observed with increase in polymorphism. The regimen was well tolerated and provided good disease control and promising survival effects. An analysis of the influence of *UGT1A1*28* and **6* polymorphisms provides useful information for the prediction of CPT-11-related hematological toxicity.

Lung cancer is the most common fatal cancer in Japan and in Western countries.[1] The majority of cases of advanced non–small cell lung cancer (NSCLC) are found among patients aged >65 years, and the number of such cases is predicted to rise with increases in the numbers of the elderly.[2,3]

Chemotherapy has been shown to yield better results than best supportive care in NSCLC patients in terms of survival and quality of life.[4] Platinum-based regimens containing a third-generation agent, including irinotecan (CPT-11), taxanes, gemcitabine (GEM), and vinorelbine (VNR), have been the mainstream treatment for patients with NSCLC.[5] However, these regimens have been associated with high toxicity while providing no survival benefit in elderly patients. Several prospective randomized trials have investigated optimal chemotherapy in patients aged ≥70 years with advanced NSCLC.[6–9] The regimens investigated have included VNR monotherapy,[6] GEM plus VNR vs. VNR alone,[7] VNR vs. GEM vs. VNR plus GEM,[8] and docetaxel (DOC) vs. VNR.[9] The results of the Elderly Lung Cancer Vinorelbine Italian Study (ELVIS) led to the recommendation that VNR monotherapy be used as first-line therapy in elderly patients with advanced NSCLC.[6] On the basis of these studies, and given that GEM is less active than VNR, many researchers now recommend VNR monotherapy.

CPT-11 is a semi-synthetic camptothecin derivative with topoisomerase I–inhibiting activity.[10–12] CPT-11, a prodrug, is converted to its active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin), by carboxylesterase, which is 100- to 1,000-fold more cytotoxic than CPT-11. Further hepatic metabolism by uridine diphospho-glucuronosyl-transferases (UGTs) converts SN-38 to its inactive metabolite, SN-38 glucuronide (SN-38G).[10–12]

Phase III clinical studies on CPT-11 conducted in NSCLC patients have included a comparison we made of CPT-11

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monotherapy, a cisplatin-plus-vindesine group (VDS-P), and a cisplatin-plus-CPT-11 (IP) group.[13] The response rate in the CPT-11 monotherapy group in a subset of elderly patients (aged 70–75 years) in that study was 40.0%, similar to that in the VDS-P group (43.5%). Moreover, the response rate was higher in the IP group (60.9%) than in those undergoing either of the other two regimens. Interestingly, survival time was better in the CPT-11 monotherapy group (44.3 weeks) than in the VDS-P group (35.7 weeks). As for adverse events in this subset of elderly patients, although the incidence of diarrhea tended to be higher in the CPT-11 monotherapy group, leukopenia, neutropenia, nausea/vomiting, and anorexia were all mild. Because these findings suggested that CPT-11 monotherapy might be a useful regimen in elderly patients with NSCLC, the regimen was investigated in this prospective study.

Severe CPT-11-associated diarrhea and myelosuppression have been reported as dose-limiting toxicities (DLTs).[14,15] These effects correlate significantly with the area under the time-concentration curve (AUC) values of CPT-11 and its active metabolite SN-38 and glucuronized SN-38.[14,15] Among UGT isoforms, *UGT1A1* is believed to be responsible for SN-38 glucuronidation and is also thought to be involved in the large inter-individual variations seen in SN-38 pharmacokinetics.[16] Several studies have reported a correlation between the adverse effects of CPT-11 and the presence of *UGT1A1* polymorphisms including *UGT1A1*28* and *UGT1A1*6*.[17–19] Ethnic differences have also been reported in the distribution of these polymorphisms, with higher incidences of *UGT1A1*6* occurring in Asians (including Japanese) than in Caucasians.[20–22] This suggests that *UGT1A1* polymorphism is an important determining factor in the efficacy and toxicity of CPT-11 and that pharmacogenetics-guided dosing of CPT-11 may help to individualize the dose of CPT-11 and moderate its toxicity in cancer patients.

We performed phase I and II studies involving CPT-11 monotherapy on days 1 and 8 of a 3-week cycle in elderly patients with NSCLC to determine the DLT, maximum-tolerated dose (MTD), and recommended dose (RD) and to investigate the antitumor effect and safety of the RD. Further, a prospective analysis of *UGT1A1* mutations was performed, and we investigated the relationship between the presence of these polymorphisms and the occurrence of adverse events. We also analyzed the variation in the pharmacokinetics of CPT-11 and its metabolites in elderly patients.

RESULTS

Patient characteristics

Between April 2003 and March 2006, 46 patients with stage IIIB/IV NSCLC were enrolled. In the overall study population, 76% of the patients (35 of 46) had stage IV disease, and 69.5% (32 of 46) had adenocarcinoma. Twelve patients were enrolled and treated in phase I. Six patients were treated at dose level 1 (60 mg/m^2), three patients at dose level 2 (80 mg/m^2), and three patients at dose level 3 (100 mg/m^2). DLT of persistent grade 2 leukopenia was observed in one patient at dose level 1, and an additional three patients were enrolled at this dose level. No further DLTs were observed in these patients or in patients receiving 80 or 100 mg/m^2. Therefore the MTD was not reached in this study, and the RD was set at 100 mg/m^2, in accordance with the study protocol described in "Methods."

In phase II, 34 additional patients were treated at 100 mg/m^2, making a total of 37 patients treated with the RD. **Table 1** shows the selected baseline demographics and disease characteristics of the patients treated with the RD. There were 25 men and 12 women, with a median age of 76 years (range: 71–88).

The median number of treatment cycles in phase II was 4.0 (range: 1–18); 37.8% of patients (14 of 37) received five or more cycles, and the percentage of patients with 6-month or longer treatment was ~22%. The relative dose intensity was 90.0%. Twenty-five of the 37 patients went on to second-line therapy comprising gefitinib (in 7 patients, 28%), different regimens of CPT-11 (7 patients, 28%), carboplatin/paclitaxel (4 patients, 16%), DOC (3 patients, 12%), GEM (3 patients, 12%), and S-1/cisplatin (1 patient, 4%).

Response and survival

All 37 patients (including 3 patients in phase I) who received the RD were evaluated to determine the overall response rate. The overall response rate was 8.1% (complete response (CR): 0, partial response (PR): 3; 3/37, 95% confidence interval: 1.7–21.9), and the disease control rate was 21.6% (8/37, 95% confidence interval: 9.8–38.2). The median survival time (MST) was 441 days after a median follow-up of 440 days, and the 1-year survival rate was 56.8% (**Figure 1**). The median time to progression (TTP) was 132 days.

Toxicity

In phase I, persistent grade 2 leukopenia was observed in one patient who received treatment at level 1, and the second cycle could not be started until day 30. This adverse event was therefore regarded as a DLT. Adverse events that occurred in phase II are summarized in **Table 2**. The most frequently observed hematological toxicity (grade 3/4) was neutropenia (27.0%).

Table 1 Demographics of patients treated with irinotecan 100 mg/m^2

Characteristic	No. of patients (N = 37)	%
Sex		
Male	25	68
Female	12	32
Age (years)		
Median	76.0	
Range	71–88	
Performance status		
0	11	30
1	26	70
Histology		
Adenocarcinoma	25	68
Other	12	32
Stage		
IIIB	10	27
IV	27	73