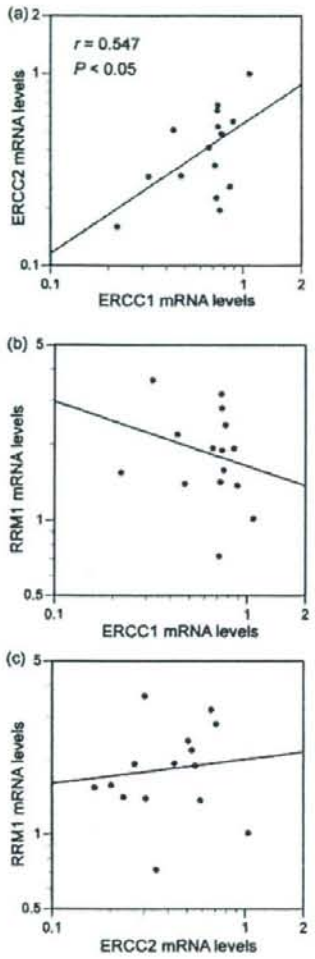

Figure 3 Correlations between (a) ERCC1 and ERCC2 mRNA expression, (b) ERCC1 and RRM1 mRNA expression and (c) ERCC2 and RRM1 mRNA expression.

analyses from clinical trials, in which the expression of biomarkers in transbronchial and percutaneous lung biopsy samples is evaluated. Thus, as one of many approaches to integrating molecular analysis with individualized chemotherapy, the *in vitro* associations between mRNA expression of the ERCC1, ERCC2 and RRM1 genes and chemosensitivity to platinum agents and gemcitabine was assessed. However, the behaviour of cell lines adapted to grow *in vitro* may differ from the *in vivo* situation, and laboratory findings may not always accurately model the clinical situation.

RRM1 expression is reported to be associated with the response to gemcitabine *in vitro*.,[23] Increased RRM1 expression has been reported in two gemcitabine-resistant NSCLC cell lines. In addition, upregulation of RRM1 has been reported in different gemcitabine-resistant cell lines,[24-26] and in a murine colon cancer model.[27] Reduced RRM1 expression has also been reported to be associated with increased sensitivity to gemcitabine in the human NSCLC H23 cell line using transfection and knockdown techniques.[7] Low levels of RRM1 expression are associated with poor survival among patients with resected NSCLC.[28] Association of increased RRM1 expression with resistance to gemcitabine was also reported in the setting of preoperative NSCLC, as well as in advanced NSCLC. In a prospective induction phase II clinical trial of chemotherapy with platinum and gemcitabine RRM1 mRNA expression was correlated with tumour response.[29] However, in the present study there was no correlation between RRM1 mRNA expression and chemosensitivity to gemcitabine, cisplatin or carboplatin. Possible explanations for the differences between this study and other *in vitro* studies are the use of tissues from different sources and the use of different assay systems, such as overexpression and/or knockdown techniques for molecular biomarkers in a limited number of cell lines. The discrepancy between this study and *in vivo* studies might be explained by possible technical limitations such as the quality of mRNA extracted from the small samples obtained by lung biopsy and the specificity of the antibody used.

The association between ERCC1 and chemosensitivity to cisplatin has been evaluated in many *in vitro* and *in vivo* studies. Increased expression of ERCC1 was associated with cisplatin resistance in ovarian cancer cells.[30] Transfection of the ERCC1 gene into an ERCC1-deficient Chinese hamster ovary (CHO) cell line conferred DNA adduct repair capability and cisplatin resistance.[31] In a human colon cancer cell line with mismatch repair deficiency, ERCC1 antisense RNA abrogated the synergistic cytotoxicity of gemcitabine and cisplatin *in vitro*.[32] The association between ERCC1 mRNA expression and chemoresponsiveness to cisplatin has been observed in primary gastric cancer and in ovarian cancer.[33-35] In the present study, there was no association between ERCC1 mRNA expression and chemoresponsiveness to either cisplatin or gemcitabine. The lack of association between ERCC1 mRNA expression and chemoresponsiveness to cisplatin is consistent with a previous *in vivo* study, of mRNA from formalin-fixed paraffin-embedded primary tumour specimens from patients with advanced NSCLC before treatment with cisplatin and gemcitabine. However, low ERCC1 mRNA expression was associated with longer survival and a trend towards a higher response rate.[16] A recent study also reported no association between ERCC1 mRNA expression and chemoresponsiveness or survival in patients with advanced NSCLC treated with platinum-based chemotherapy.[36]

ERCC1 mRNA expression in formalin-fixed paraffin-embedded tumour specimens obtained by bronchoscopic fine needle aspiration biopsy[15] is a prognostic factor in patients with resected NSCLC,[37] and patients with advanced NSCLC treated with

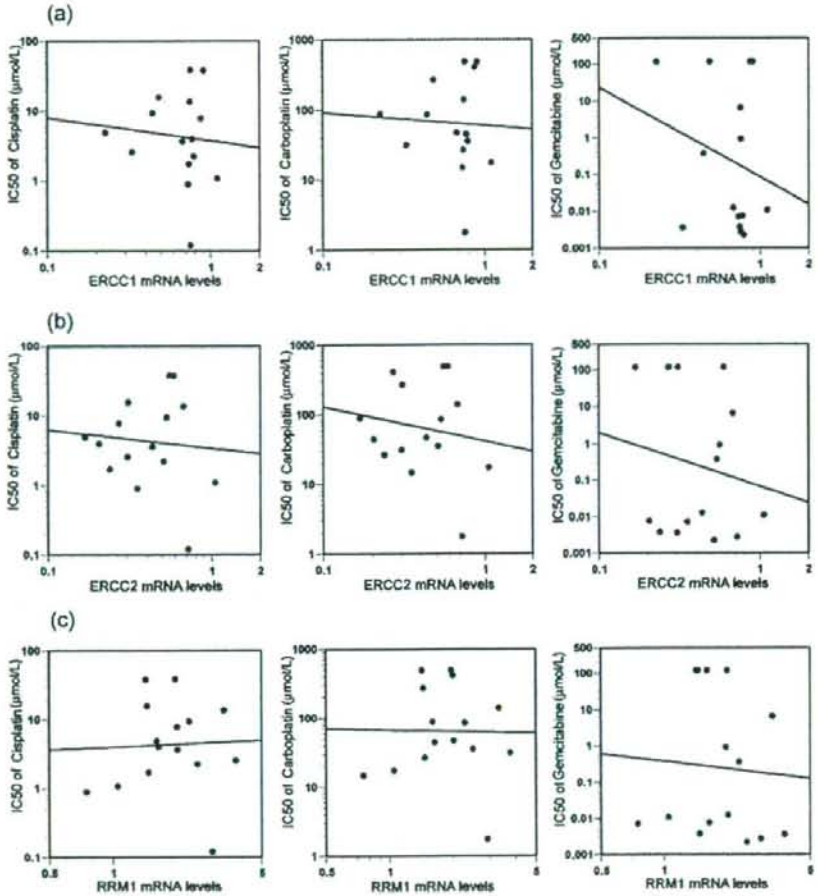

Figure 4 Associations between mRNA expression for (a) ERCC1, (b) ERCC2 and (c) RRM1 and chemosensitivities to cisplatin, carboplatin and gemcitabine.

cisplatin and gemcitabine. Furthermore, ERCC1 protein, as measured by immunohistochemical scoring, is a determinant of survival after surgical treatment of early stage NSCLC. ERCC1 protein is a prognostic factor for clinical outcome and a predictive biomarker for cisplatin-based adjuvant chemotherapy in patients with completely resected ERCC1-negative NSCLC,[8] although a problem with the specificity of the anti-ERCC1 mAb 8F1 has been reported.[22] Thus, further studies are needed to establish the role of ERCC1 in NSCLC.

The ERCC2 gene codes for a DNA helicase, which is a member of the multi-step NER pathway. The Asp312Asn polymorphism, resulting from a G/A substitution in exon 10 of the ERCC2 gene has been highly conserved through evolution, and has been reported to be a prognostic factor in patients with advanced NSCLC treated with cisplatin.[38] In addition, an *in vitro* study showed that ERCC2 overexpression leads to cisplatin resistance in a glioma cell line,[39] suggesting that expression of the ERCC2 gene may be associated with chemosensitivity to cisplatin in lung cancer cells. However, the present study failed to show associations with sensitivity to platinum agents and gemcitabine. Therefore, ERCC2 also needs further evaluation in lung cancer.

Five SCLC cell lines were included to determine whether the associations between ERCC1, ERCC2 and RRM1 mRNA expression and chemosensitivity to platinum agents and gemcitabine reported for NSCLC could be extended to SCLC. Platinum agents are key drugs and gemcitabine has modest activity in the treatment of SCLC with response rates of 11.9–13%.[40,41] However, the present study failed to show any associations. These findings are supported by a previous study, in which gene expression and the growth

inhibitory activities of various anticancer agents were similar for 19 NSCLC and 10 SCLC cell lines.[42]

There have been no *in vitro* studies examining the association between RRM1, ERCC1 or ERCC2 and chemosensitivity to platinum agents and gemcitabine, except for studies using overexpression and/or knockdown techniques. Although this *in vitro* study did not show associations in a panel of lung cancer cell lines, definitive conclusions cannot be drawn from the data, because only a limited number of cell lines were used. Exploration of the relationship between drug response phenotype and tumour genome mRNA expression profile, using cell line panels and/or tumour tissues together with cDNA and oligonucleotide arrays, would be a promising approach in the search for predictive biomarkers.[43,44] Finally, in order to validate pharmacogenetic or pharmacoproteomic candidates for lung cancer in clinical settings, further careful and more comprehensive studies using multiple approaches are warranted.

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clinical studies

Multicentre prospective phase II trial of gefitinib for advanced non-small cell lung cancer with epidermal growth factor receptor mutations: results of the West Japan Thoracic Oncology Group trial (WJTOG0403)

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The purpose of this study was to evaluate the efficacy of gefitinib and the feasibility of screening for epidermal growth factor receptor (*EGFR*) mutations among select patients with advanced non-small cell lung cancer (NSCLC). Stage IIIB/IV NSCLC, chemotherapy-naive patients or patients with recurrences after up to two prior chemotherapy regimens were eligible. Direct sequencing using DNA from tumour specimens was performed by a central laboratory to detect *EGFR* mutations. Patients harbouring *EGFR* mutations received gefitinib. The primary study objective was response; the secondary objectives were toxicity, overall survival (OS), progression-free survival (PFS), 1-year survival (1Y-S) and the disease control rate (DCR). Between March 2005 and January 2006, 118 patients were recruited from 15 institutions and were screened for *EGFR* mutations, which were detected in 32 patients – 28 of whom were enrolled in the present study. The overall response rate was 75%, the DCR was 96% and the median PFS was 11.5 months. The median OS has not yet been reached, and the 1Y-S was 79%. Thus, gefitinib chemotherapy in patients with advanced NSCLC harbouring *EGFR* mutations was highly effective. This trial documents the feasibility of performing a multicentre phase II study using a central typing laboratory, demonstrating the benefit to patients of selecting gefitinib treatment based on their *EGFR* mutation status.
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Keywords: epidermal growth factor receptor (*EGFR*) mutation; gefitinib; non-small cell lung cancer (NSCLC); multicentre prospective phase II; central laboratory

Gefitinib, a tyrosine kinase inhibitor (TKI), is an orally active small molecule that functions as a selective epidermal growth factor receptor (*EGFR*) inhibitor (Ranson *et al*, 2002). Two phase II trials (Fukuoka *et al*, 2003; Kris *et al*, 2003) for previously treated non-small cell lung cancer (NSCLC) (IDEAL-1 and -2, respectively) have documented favourable objective responses in 14–18% of patients. However, in a phase III trial (Thatcher *et al*, 2005), no survival benefit of gefitinib was observed when compared with best-supportive care (BSC) for previously treated NSCLC. In contrast, we have seen a significant survival benefit of erlotinib compared with BSC as a salvage therapy (BR21); erlotinib is also an *EGFR*-TKI and its chemical structure, which is based on quinazoline, is quite similar to that of gefitinib (Shepherd *et al*, 2005). Although we do not know whether differences between gefitinib and erlotinib were responsible for these different outcomes, appropriate patient selection to identify good responders is likely crucial for revealing the clinical benefits of the *EGFR*-TKI family.

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Patient subset analyses of these randomised phase III trials or retrospective trials (Kaneda *et al*, 2004; Miller *et al*, 2004) clearly show the existence of populations that are more likely to respond to gefitinib and erlotinib, including women, patients with adenocarcinoma (especially with bronchial alveolar carcinoma (BAC)), nonsmokers and Asian patients (compared with Caucasians). Somatic mutations in specific regions of exons 18, 19 and 21 of the ATP-binding domain of *EGFR* have recently been shown to have strong associations with sensitivity to gefitinib or erlotinib (Lynch *et al*, 2004; Paez *et al*, 2004; Pao *et al*, 2004). Consistent with these findings, the frequencies of these *EGFR* mutations were higher in women, patients with adenocarcinoma, nonsmokers and Asians, all of whom are among the more frequent responders, as mentioned above (Shigematsu *et al*, 2005). There are two characteristic types of *EGFR* mutations. One is the presence of in-frame deletions, including the amino acids at codons 746–750 in exon 19, and the other is an amino-acid substitution at codon 858 (L858R) in exon 21. Recent analyses (Bell *et al*, 2005) of phase II and III trials for *EGFR*-TKI, in which patients were not selected based on their mutation status, have suggested that *EGFR* mutations are correlated with response to therapy but are not correlated with overall survival (OS). Furthermore, *EGFR* gene amplification/copy number (Cappuzzo *et al*, 2005; Hirsch *et al*, 2005) or overexpression (Hirsch *et al*, 2003) has been shown to be a more useful prognostic marker of response to gefitinib treatment. Patient selection according to *EGFR* mutation status may yield a superior survival rate by excluding patients who are unlikely to respond to gefitinib treatment. However, other populations that might obtain a clinical benefit from gefitinib treatment, even in the absence of *EGFR* mutation, may exist.

Three Japanese groups (Asahina *et al*, 2006; Inoue *et al*, 2006; Yoshida *et al*, 2007) have reported prospective phase II studies of gefitinib for advanced-stage NSCLC that were designed to consider the *EGFR* mutation status of the patients. All of these studies have reported a high response rate and extended progression-free survival (PFS) period, compared with historical controls. However, all of these studies had a relatively short observation period, making the data preliminary. Moreover, the original sample size was calculated after patient selection, and a critical consideration of the suitability of the assay used to detect the mutations (which was performed using small paraffin-embedded specimens obtained from bronchoscopic biopsies), and the estimated *EGFR*-positive rate were lacking. Additionally, all the trials were conducted at single institutions located in one small area of Japan. Thus, the published data may not be representative of the situation found in general clinical practice throughout Japan and therefore may not directly translate to the general feasibility of gefitinib treatment in Japan.

In view of this situation, we performed a multicentre prospective phase II trial of gefitinib for advanced NSCLC harbouring *EGFR* mutations. We prospectively registered patients from 15 different institutes in Japan at the beginning of *EGFR* mutation screening using a central database. Whether or not tissue was available from a bronchoscopic biopsy or surgery was not an inclusion criterion. All the clinical samples from the registered patients were delivered to a central laboratory that then determined the *EGFR* mutation status or the histological BAC features. The analysis of the survival data was based on a minimum observation period of at least 15 months from the time of entry of the last patient.

MATERIALS AND METHODS

Eligibility criteria

Eligible patients had histologically confirmed stage III NSCLC for which thoracic irradiation was not indicated or were stage IV. Chemotherapy-naive patients or those who had previously received up to two prior chemotherapy regimens, including those performed in an adjuvant setting, were eligible. Other eligibility criteria included an age $\geqslant$20 years, measurable disease, the availability of sufficient amounts of tumour specimen for *EGFR* mutation analysis, an Eastern Cooperative Oncology Group performance status of 0–2, adequate organ function (WBC$\leqslant$3000 μl^{-1}, platelets$\geqslant$75 000 μl^{-1}, AST and ALT$\leqslant$100 IU l^{-1}, serum creatinine$\leqslant$twice the upper limit of the reference range; $P_{aO2}\geqslant$60 mm Hg). The exclusion criteria included pulmonary fibrosis, the presence of symptomatic brain metastasis, active concomitant malignancy, severe heart disease, active gastrointestinal bleeding and continuous diarrhoea. All the patients signed a written informed consent form. Approval of this study and the gene analyses were obtained from the Institutional Review Board and the Ethics Committee of each hospital.

EGFR gene analysis

Tumour specimens were obtained using bronchial fiberscope or surgical procedures. The specimens were fixed with formalin and embedded in paraffin. Four slices (4–5 μm) from the embedded block were sent to a central laboratory (Mitsubishi Chemical Safety Institute Ltd., Ibaraki, Japan) for genetic analysis. Most of the tumour specimens were available prior to the registration of this study. Genomic DNA was isolated from specimens using QIAamp Micro kits (QIAGEN KK, Tokyo, Japan). The *EGFR* mutations in exons 18, 19 and 21, as previously reported (Lynch *et al*, 2004; Paez *et al*, 2004), were determined using polymerase chain reaction (PCR) amplification and intron–exon boundary primers according to the published method. An *EGFR* registrant mutation in exon 20, which was reported by Pao *et al* (2005) was also examined using PCR and the previously reported primers. Polymerase chain reaction was performed using a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA, USA), and the PCR products were confirmed using a Bioanalyzer 2100 (Agilent Technologies Inc., Santa Clara, CA, USA), then sequenced directly using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and ABI PRISM 3100 (Applied Biosystems). All sequencing reactions were performed in both forward and reverse directions and were analysed using the Basic Local Alignment Search Tool (BLAST); all the electropherograms were reanalysed by visual inspection to check for mutations. The presence of an *EGFR* mutation was confirmed using at least three independent PCR.

All sequence data were sent from the central laboratory to Kinki University. A principle investigator then confirmed whether or not the *EGFR* mutation status was positive, and the results were sent to the West Japan Thoracic Oncology Group (WJTOG) data centre. The data centre then informed each participating centre of the results of the genetic analysis and requested that the eligibility criteria of the patients be rechecked to insure that only *EGFR*-positive subjects were registered in the trial. Each tumour was categorised according to histology by a pulmonary pathologist (JF). The percentage of area exhibiting a BAC pattern was also examined to determine the WHO pathological category.

Treatment plan

Gefitinib (250 mg day^{-1}) was administered once daily. Treatment was continued uninterrupted until disease progression or intolerable toxicity (grade 4 nonhaematological toxicities, any incidents of interstitial pneumonia or a treatment delay of more than 2 weeks because of adverse effects). Gefitinib administration was delayed if the patient's leukocyte and platelet counts were lower than 1500 and 5000 μl^{-1}, respectively, and was withheld until these counts had recovered. Gefitinib administration was also delayed if grade 3 or greater nonhaematological toxicities without nausea, vomiting or alopecia occurred and was withheld until recovery to grade 2.

Routine clinical and laboratory assessments and chest X-ray assessments were performed weekly or biweekly, where possible; CT examinations of the target lesion were performed every month, and magnetic resonance imaging of the whole brain and a bone scan were performed every 3 months. The objective responses of the patients were evaluated every month using the Response Evaluation Criteria in Solid Tumours (RECIST) guidelines (Therasse *et al*, 2000). Tumour response was centrally evaluated by independent reviewers at an extramural conference and was performed for the intent-to-treat population. All adverse effects that occurred during gefitinib treatment were reported, and the severity of the effects was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Statistical analyses

The primary end point of this study was the response rate. A one-stage design using the binominal probability was used to determine the sample size. Assuming that a response rate of 50% would indicate potential usefulness, whereas a rate of 25% would be the lower limit of interest, and with $\alpha = 0.10$ (two side) and $\beta = 0.20$, the estimated accrual number was 23 patients. Estimating that the *EGFR*-positive rate would be about 20%, the screening number required to accrue 23 *EGFR*-positive patients was 115. After assuming an inevaluability rate of $<10\%$, the final required screening number was 125.

The secondary end points of this study were toxicity, OS, PFS, 1-year survival (1Y-S) and the disease control rate (DCR). Survival analyses were conducted on the intent-to-treat population using follow-up data available as of 30 April 2007. The survival curves were estimated using Kaplan–Meier plots.

RESULTS

Patient characteristics

Between March 2005 and January 2006, 118 patients were prospectively screened from 15 institutions; 117 of them underwent *EGFR* mutation analysis (tumour tissue was not available for one patient). The median time required for the *EGFR* mutation analysis was 12 days (range: 7–28 days). Among the 117 patients, *EGFR* mutations were detected in 32 patients (27%), 14 of whom had a deletion in or near E746-A750 (including one del E746-T751 ins A, two del L747-T751 and one del L747-T753 ins S) in exon 19. A further 17 had L858R, and one had a L861Q point mutation in exon 21 (Table 1).

Tissue samples from 17 patients (53%) were obtained by transbronchial biopsy. The *EGFR* detection rates for the surgical specimens and the bronchoscopic biopsy specimens were similar (30 *vs* 25%). The *EGFR* mutations were significantly more frequent in women ($P \leqslant 0.02$), in patients with adenocarcinoma ($P = 0.001$) and in people who had never smoked ($P < 0.001$) (Table 2). Finally, 28 patients (14 with deletions in exons 19 and 14 with point mutations in exon 21) were actually registered and received treatment with gefitinib, whereas four patients were dropped from the study as they became ineligible because of tumour progression during the time required for the mutation analysis.

Patient characteristics are listed in Table 3. In the initial screening, there were 56 female patients (48%), 97 patients (83%) with adenocarcinoma and 53 (45%) who had never smoked. The frequency of these characteristics was higher among the patients with *EGFR* mutations who were actually registered; namely, 18 patients (64%) were women, 27 (96%) had adenocarcinoma and 19 (68%) had never smoked. The median age of the 28 actually registered patients was 68 years; 24 patients (86%) had a good performance status (0–1), 22 (79%) had stage IV diseases and 17 (61%) were chemotherapy naive. Thoracic irradiation was contraindicated in one patient with stage IIIA disease because of the large irradiation field that would have been required. All five patients with stage IIIB diseases had malignant effusions. Four patients had received adjuvant therapies; five had received platinum doublets or a combination of gemcitabine and vinorelbine as their first-line therapy. Two patients had received two regimens of platinum doublets followed by docetaxel or pemetrexed. One patient had received local radiation for pain control.

Table 1 Type of *EGFR* mutations ($n = 32$)

Characteristics	No. of patients	%
Exon 18	0	0
Exon 19	14	44
del E746-A750	10	32
del E746-T751 ins A	1	3
del L747-T751	2	6
del L747-T753 ins S	1	3
Exon 21	18	56
L858R	17	53
L861Q	1	3

EGFR = epidermal growth factor receptor.

Table 2 Relationship between patient characteristics and *EGFR* mutation status

	EGFR mutation positive ($n = 32$)		*EGFR* mutation negative ($n = 85$)		
Characteristics	**No. of Patients**	**%**	**No. of Patients**	**%**	***P***
Sex					
Male	11	34	50	59	
Female	21	66	35	41	<0.02
Histology					
Adenocarcinoma	31	97	66	78	
Nonadenocarcinoma	1	3	19	22	$=0.001$
Smoking status					
Never	21	66	31	36	
Current/former	11	34	54	64	<0.001

EGFR = epidermal growth factor receptor.

Response and survival

The objective tumour responses are listed in Table 4. The overall response rate and DCR were 75% (95% CI: 57.6–91.0%) and 96% (95% CI: 87.0–96.4%), respectively. Five out of ten male patients (50%), six out of nine smokers (67%) and five out of eight male smokers with adenocarcinoma (63%) achieved a PR. One female nonsmoker with squamous cell carcinoma also achieved a PR. Among the registered patients with *EGFR* mutations, the response rate was no different between current/former smokers and those who had never smoked (67 *vs* 79%) or between chemotherapy-naive and postchemotherapy patients (77 *vs* 73%). Female and patients with a mutational deletion in exon 19 tended to have a higher response rate than male (89 *vs* 50%) and patients with a missense mutation in exon 21 (86 *vs* 64%), respectively.

The median follow-up time was 18.6 months (range: 13.8–23.4 months). The median PFS time was 11.5 months (95% CI: 7.3 months to -) (Figure 1A). The median OS has not yet been reached, and the 1Y-S was 79% (95% CI: 63.4–93.8%) (Figure 1B).

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

Characteristics	No. of patients (%)
Age	
Median	68
Range	49–89
Performance status	
0	11 (29)
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

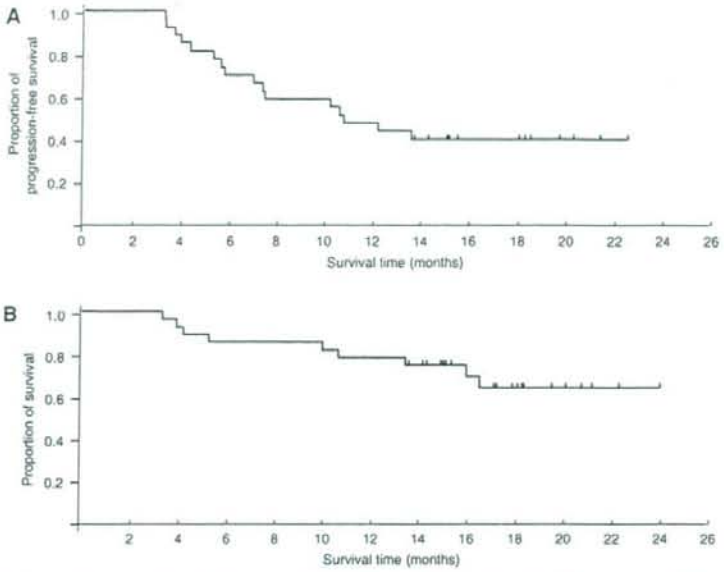

Figure 1 (**A**) Progression-free survival (PFS) and (**B**) overall survival (OS) of all eligible patients ($n = 28$). The median PFS was 11.5 months. The median OS has not yet been reached. The 1-year survival rate was 79%.

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 (0)	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 ($n = 22$)

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 a 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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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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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.

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%)

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.

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

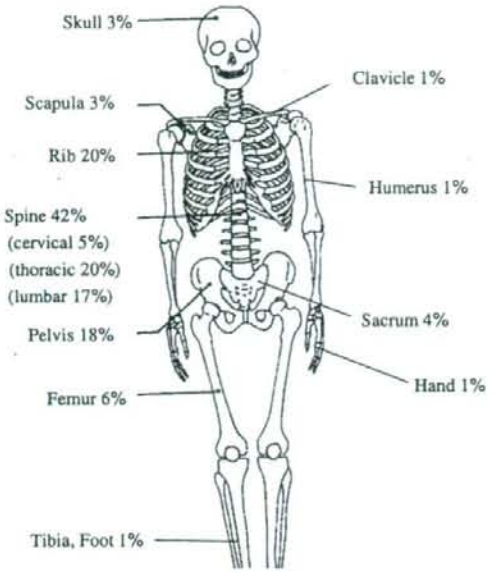

Fig. 1 Anatomic localization of skeletal metastases from lung cancer is shown (n = 118).

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 (Irresa®; 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

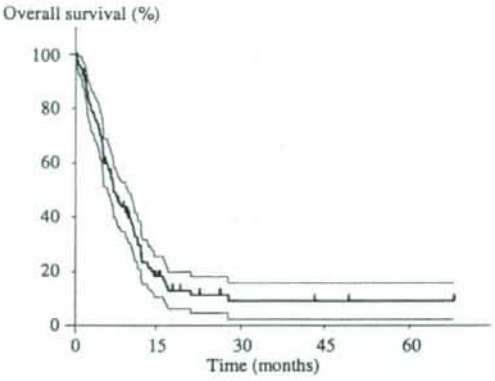

Fig. 2 A Kaplan–Meier curve of overall survival for all patients is illustrated. The dotted lines indicate the 95% confidence interval. The overall cumulative survival rates after bone metastasis for all 118 patients are 59.9% for 6 months, 31.6% for 1 year, and 11.3% for 2 years.

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)

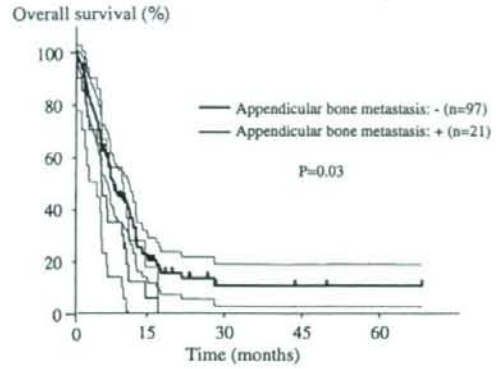

Fig. 3 A Kaplan–Meier curve of overall survival based on absence or presence of metastasis of the appendicular bone is illustrated. The dotted lines indicate the 95% confidence interval. The prognosis is poorer ($p = 0.03$) in patients with metastasis to the appendicular bone than in patients without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

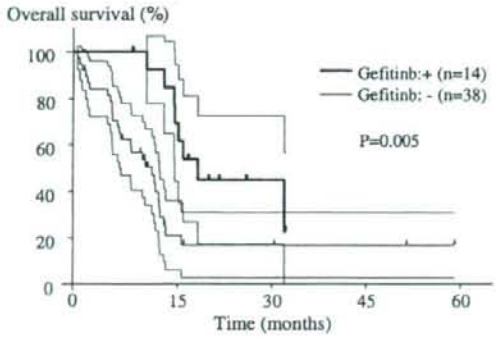

Fig. 4 A Kaplan–Meier curve of overall survival based on absence or presence of gefitinib treatment is illustrated. The dotted lines indicate the 95% confidence interval. The mean survival is longer ($p = 0.005$) in the group treated with gefitinib than in the untreated group among 52 patients with adenocarcinoma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