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Impact of metastatic status on the prognosis of *EGFR* mutation-positive non-small cell lung cancer patients treated with first-generation EGFR-tyrosine kinase inhibitors

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Abstract. The aim of the present study was to analyze the impact of metastatic status on the prognosis of epithelial growth factor receptor (*EGFR*) mutation-positive patients with non-small cell lung cancer (NSCLC) treated with first-generation EGFR-tyrosine kinase inhibitors (TKIs). A total of 178 *EGFR* mutation-positive patients with stage IIIB-IV and relapsed NSCLC who were treated with gefitinib or erlotinib as the first-line treatment were enrolled in the present study. Metastatic status, progression-free survival (PFS), overall survival (OS) and treatment-response rates were investigated. The association between the number of metastatic organ sites and patient prognosis was also investigated. The median age at the time of treatment was 72 (range, 39-91) years. A total of 168 patients had adenocarcinoma; 156 were treated with gefitinib. Patients with brain metastases, bone metastases, liver metastases and pleural effusion exhibited a significantly reduced PFS and OS time in the univariate analysis, compared with patients without each of these symptoms. In the multivariate analysis, bone metastasis was associated with a poorer PFS (hazard ratio, 2.11; 95% confidence interval, 1.44-3.09; P<0.001) and brain metastasis was associated with a poorer OS (hazard ratio, 2.41; 95% confidence interval, 1.46-3.95; P<0.001). No association was observed between metastatic status and treatment response rates. Higher numbers of different sites of organ metastases were associated with significantly poorer PFS and OS. Bone, brain metastasis and higher numbers of metastatic organ sites are negative prognostic factors for *EGFR* mutation-positive NSCLC patients treated with first-generation EGFR-TKIs.

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Abbreviations: EGFR, epidermal growth factor receptor; CI, confidence interval; HR, hazard ratio; NSCLC, non-small cell lung cancer; OS, overall survival; PE, pleural effusion; PFS, progression-free survival; TKI, tyrosine kinase inhibitor

Key words: bone metastasis, brain metastasis, lung cancer, epithelial growth factor receptor, tyrosine kinase inhibitors, prognosis

Introduction

Globally, numerous patients succumb to lung cancer (1). The use of cytotoxic chemotherapy remains a major means of treating patients with unresectable non-small cell lung cancer (NSCLC). Nevertheless, the effectiveness of cytotoxic chemotherapy may be limited in certain patients, with a response rate of 20-35% and a median survival time of 10-12 months (2,3). For treating such patients, gefitinib and erlotinib, the first generation orally administered epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) were developed. At first, the effect of EGFR-TKIs was limited due to treating unselected patients with NSCLC (4-7). However, previous studies have revealed that the presence of EGFR mutation may be associated with increased responsiveness to EGFR-TKIs in patients with NSCLC (8-11). In previous studies, the response rate was 62.1-83.0%, with a median progression-free survival (PFS) and overall survival (OS) of 9.2-13.1 months and 19.3-30.5 months, respectively (8-12). The toxicities of EGFR-TKIs are decreased compared with those of cytotoxic drugs and patients can achieve a good quality of life while using them (8,12). In patients treated with first generation EGFR-TKIs, brain, bone and liver metastasis and pleural effusion (PE) predicted a poorer prognosis compared with patients without these metastasis (13-17). However, few reports concern the association between the site of metastasis and prognosis (18,19). Understanding which metastatic organ sites influence the prognosis of patients treated with EGFR-TKIs and the prognostic significance of the number of metastatic organ sites is crucial in explaining the condition to patients and aiding them in tolerating the treatment.

Therefore, the present study was a retrospective cohort study conducted to analyze the association between the site and number of metastases, and the prognosis of EGFR-TKI-treated *EGFR* mutation-positive patients with NSCLC.

Materials and methods

Patients. Pathology reports from the National Hospital Organization Kinki-chuo Chest Medical Center (Osaka, Japan) were retrospectively reviewed between January 2009 and December 2014 and 533 patients were identified as having *EGFR* mutation-positive NSCLC. Patients with stage IA-IIIA disease, based on the 7th TNM staging system (20), and SCLC were excluded. All participants provided written informed consent for their data to be included. The study protocol was approved by the Institutional Review Board (approval no. 561; October 20, 2016) of the National Hospital Organization Kinki-chuo Chest Medical Center. Research was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

Data collection. Clinical data, including age, sex, type of *EGFR* mutation, TNM stage, smoking status, treatment history, PFS, OS and metastatic status were collected at the point of first-line treatment. Clinical responses were defined according to the Response Evaluation Criteria in Solid Tumors, version 1.1 (21). PFS was measured from the date of the commencement of primary systemic therapy to the date of disease progression or mortality from any cause. OS was measured from the date of diagnosis to the date of death, loss to follow-up or last follow-up, whichever occurred first. Patients were followed-up for disease status until February 2016.

EGFR mutation identification. Lung cancer was pathologically confirmed using tissue specimens obtained from bronchoscopy, computed tomography-guided biopsy, PE cytology, or surgical procedures. Mutational analysis of the *EGFR* gene was performed using Scorpion technology in combination with the Amplified Refractory Mutation System or polymerase chain reaction-Invader technique, as previously described (22,23).

Statistical analysis. Statistical analysis was conducted using the JMP statistical software program, version 11 (SAS Institute Inc., Cary, NC, USA) to compare clinical outcomes according to the metastatic status of the patients. Survival curves were estimated using the Kaplan-Meier method and the differences between the groups were compared using the log-rank test. Univariate and multivariate analyses were performed using the Cox proportional hazards models. Fisher's exact test was used to compare the non-parametric variables. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. Of the 533 *EGFR* mutation-positive patients with NSCLC initially recruited to the study, 355 were excluded based on the following criteria: Stage I-IIIA disease (n=228), treated with chemotherapy (n=50), treated with EGFR-TKIs and chemotherapy (n=31), received best supportive care only (n=25), treated with chemoradiotherapy (n=8), treated with second generation EGFR-TKIs (n=6), unknown TNM stage (n=3), small cell carcinoma (n=3) and treated with radiotherapy (n=1). A total of 178 patients remained, who were treated with first generation EGFR-TKIs as the first-line treatment (Fig. 1). Of these patients, 127 were female and 51 were male. The median age at the time of first-line treatment was 72 (range, 39-91) years. A total of 168 patients had adenocarcinoma, 134 patients had stage IV disease, 71 patients had a history of smoking and 156 patients were treated with gefitinib (Table I).

Survival analysis. The Kaplan-Meier method was used to assess patient survival (Fig. 2). Patients with brain metastases (8.0 vs. 13.2 months, P=0.008; Fig. 2A), bone metastases (8.8 vs. 15.4 months, P<0.001; Fig. 2B), liver metastases (6.7 vs. 12.5 months, P<0.001; Fig. 2C) and PE (10.8 vs. 12.2 months, P=0.033; Fig. 2D) at the time of first-line treatment were associated with significantly poorer PFS compared with patients without each of these metastases. Patients with brain metastases (20.2 vs. 38.0 months, P<0.0011 Fig. 2E), bone metastases (24.0 vs. 32.1 months, P=0.020; Fig. 2F), liver metastases (13.4 vs. 32.1 months, P<0.001; Fig. 2G), and PE (21.9 vs. 34.9 months, P=0.004; Fig. 2H) at the time of first-line treatment also exhibited significantly poorer OS times compared with patients without each of these metastases.

Response rate analysis. There were no significant differences in the rates of response between patients with brain metastases (58.5 vs. 60.2%, P=0.875), bone metastases (62.8 vs. 57.0%, P=0.446), liver metastases (64.7 vs. 59.0%, P=0.797) and PE (60.7 vs. 59.0%, P=0.871) at the time of first-line treatment and patients without each of these metastases (Table II).

Multivariate analysis of prognostic factors. In the multivariate analysis, bone metastasis was significantly associated with a poorer PFS time [hazard ratio (HR), 2.11; 95% confidence interval (CI), 1.44-3.09; P<0.001] and brain metastasis exhibited a trend towards a poorer PFS time, although it was not significant (HR, 2.11; 95% CI, 0.99-2.15; P=0.051; Table III). In addition, brain metastasis was significantly associated with a shorter OS time (HR, 2.41; 95% CI, 1.46-3.95; P<0.001; Table IV).

Effect of metastatic site number on prognosis. The number of metastatic organ sites per patient in the brain, bone, liver, and pleura, (0 in 43 patients, 1 in 81 patients, and ≥2 in 54 patients) was significantly associated with a reduced PFS (19.3 vs. 12.5 vs. 6.6 months, P<0.001) and OS (46.1 vs. 29.9 vs. 15.1 months, P<0.001; Fig. 2I and J) time. No significant differences in PFS (6.2 vs. 7.5 vs. 5.9 months, P=0.545) or OS (14.9 vs. 19.6 vs. 13.4 months, P=0.497) time were observed between patients with 2 (n=33), 3 (n=15) or 4 (n=6) metastatic organ sites.

Effect of EGFR exon 19 deletion and p.L858R mutations on prognosis. Patients with major *EGFR* mutations (including exon 19 deletion and p.L858R; n=158) were also evaluated. In a multivariate analysis, bone metastasis was identified to be

Table I. Patient baseline characteristics.

Characteristic	All patients	Metastasis			Pleural effusion
		Brain	Bone	Liver	
Total, n	178	65	78	17	56
Sex, n					
Male	51	15	27	5	16
Female	127	50	51	12	40
Age in years, median (range)	72 (39-91)	71 (50-89)	71 (42-89)	71 (50-89)	73 (39-91)
Histopathological subtype, n					
Adenocarcinoma	168	62	74	16	54
Squamous cell carcinoma	1	0	0	0	0
Not otherwise specified	9	3	4	1	2
Tumor node metastasis stage, n					
Stage IIIB	1	0	0	0	0
Stage IV	134	55	69	17	48
Postoperative recurrence	39	10	8	0	6
Post-radiotherapy recurrence	4	0	1	0	2
Smoking status, n					
Smoker	71	19	35	4	21
Non-smoker	102	45	41	12	33
EGFR mutation type, n					
Exon 19 deletion	80	34	32	8	20
p.L858R	78	23	33	6	29
Other	20	8	13	3	7
EGFR-tyrosine kinase inhibitor therapy					
Gefitinib	156	52	66	15	48
Erlotinib	22	13	12	2	8

EGFR, epithelial growth factor receptor.

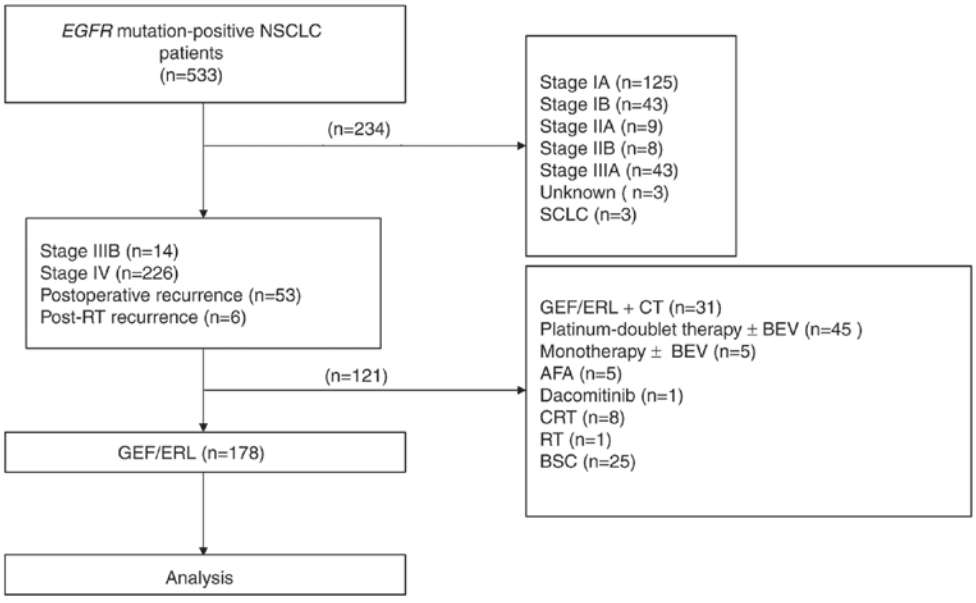

Figure 1. Study flowchart. From 533 *EGFR* mutation-positive patients with NSCLC, 178 patients treated with GEF or ERL were enrolled in the present study. A total of 65 patients had brain metastases, 78 patients had bone metastases, 17 patients had liver metastases and 56 patients had pleural effusion at the time of first-line treatment. *EGFR*, epithelial growth factor receptor; NSCLC, non-small cell lung cancer; GEF, gefitinib; ERL, erlotinib; RT, radiotherapy; CT, chemotherapy; BEV, bevacizumab; AFA, afatinib; CRT, chemoradiotherapy; BSC, best supportive care.

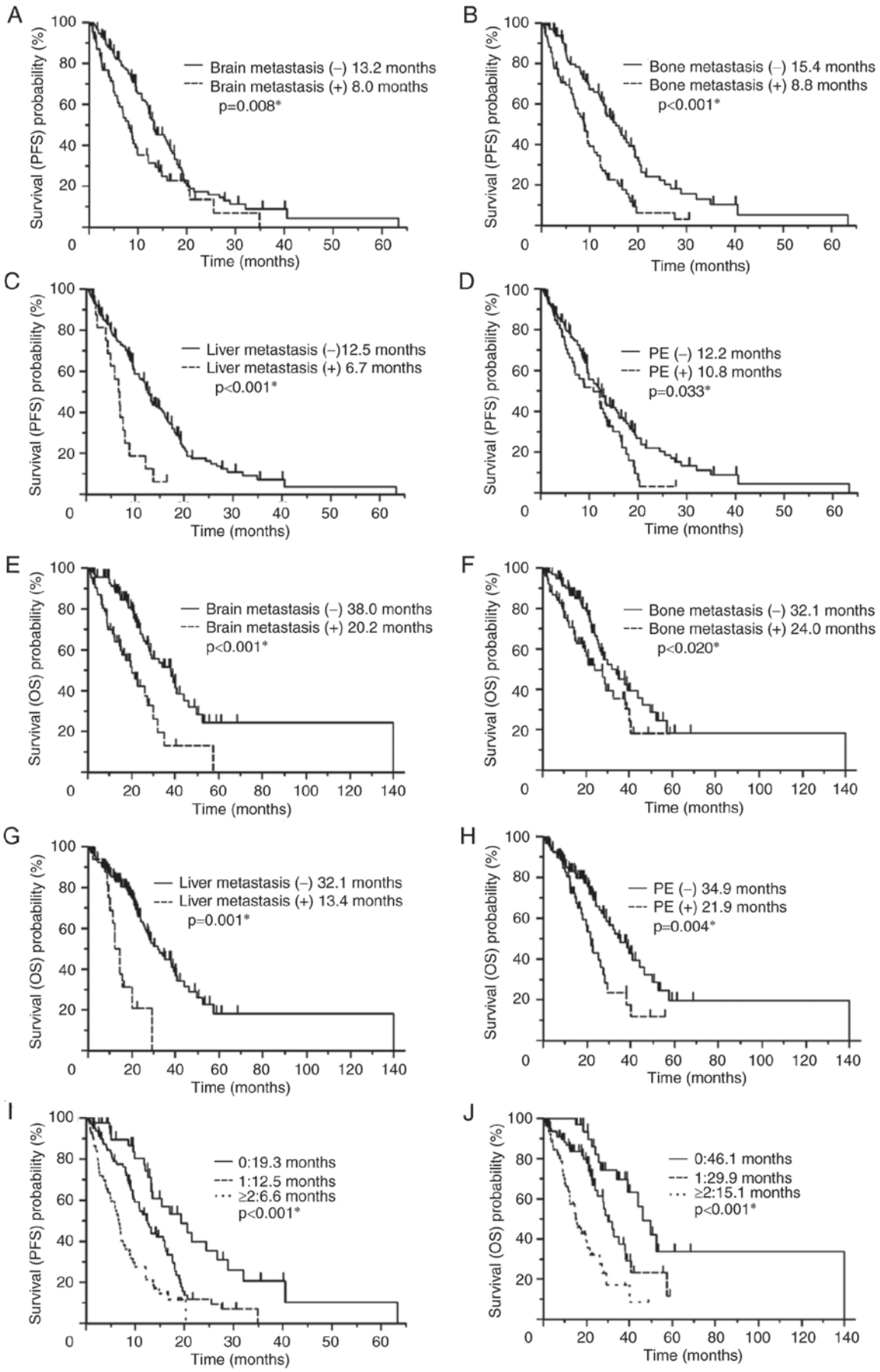

Figure 2. Survival analysis of 178 non-small cell lung carcinoma patients with epithelial growth factor receptor mutations treated with first-generation tyrosine kinase inhibitors. PFS of patients with or without (A) brain metastasis, (B) bone metastasis, (C) liver metastasis and (D) PE. OS of patients with or without (E) brain metastasis, (F) bone metastasis, (G) liver metastasis and (H) PE. (I) PFS and (J) of patients stratified by the number of metastases at these sites (0, 1 or ≥2). *P<0.05 between groups. PFS, progression-free survival; PE, pleural effusion; OS, overall survival.

Table II. Response rates to erlotinib treatment.

Characteristic	All patients	Metastasis			
		Brain	Bone	Liver	PE
Total	178	65	78	17	56
CR/PR, n	106	38	49	11	34
RR, %	59.6	58.5	62.8	64.7	60.7
P-value	-	0.875	0.446	0.797	0.871

PE, pleural effusion; CR, complete response; PR, partial response; RR, response rate.

significantly associated with a poorer PFS time. In addition, brain and liver metastases were significantly associated with a poorer OS time (Tables V and VI).

Discussion

In the univariate analysis of patients treated with first generation EGFR-TKIs, brain metastasis, bone metastasis, liver metastasis and PE were all associated with poorer PFS and OS times. Furthermore, in the multivariate analysis, bone metastasis was associated with a poorer PFS time and brain metastasis was associated with a poorer OS time. The number of metastatic organ sites was associated with a poorer PFS and OS time.

Between 30 and 40% of patients with lung cancer develop bone metastases during the course of their disease (24). To the best of our knowledge, this is the first study to assess the association between bone metastasis and a poorer PFS time in *EGFR* mutation-positive NSCLC patients. In a previous report, Fujimoto *et al* (15) revealed that bone metastasis was a significant independent negative predictive factor for OS time in *EGFR* mutation-positive patients. By contrast, in the present study, bone metastasis was not associated with OS time. One possible explanation for the association between bone metastasis and a poorer prognosis is the tumor-bone interaction that is reported to increase the malignant behavior of cancer cells. In the development of bone metastases, there is an exchange of factors from the bone matrix that are released during bone resorption, the most notable of which is transforming growth factor-β, which has been demonstrated to enhance tumor growth and the epithelial-mesenchymal transition (25,26).

Brain metastases are a frequent complication of NSCLC, with 25-40% of patients developing brain metastases during the course of their disease, often within the first 2 years following the diagnosis of the primary tumor (24,27). The risk of brain metastasis was increased in *EGFR*-mutated tumors at the time of diagnosis, as well as during the postoperative course of the disease. Compared with patients with wild-type tumors, patients with *EGFR*-mutated tumors exhibited more widespread brain lesions (28). The clinical activity of EGFR-TKIs against intracranial disease has previously been described (29-33). Furthermore, erlotinib may exhibit a superior control of intracranial disease due to the higher central nervous system penetration and drug concentrations achieved relative to gefitinib (34). However, in the present study, 80% of patients were treated with gefitinib. In patients treated with EGFR-TKIs, brain metastasis has been reported as a risk factor for poorer PFS and OS (13,35). Similarly, in the present study, brain metastasis was the only negative prognostic factor identified by multivariate analysis for OS time.

Wu *et al* (17) reported that lung adenocarcinoma patients with Stage IV disease and malignant PE at the time of diagnosis have poorer OS times than patients who develop malignant PE following disease progression. However, the difference was only statistically significant in patients with distant metastases. For patients without distant metastases, there was no significant difference. In the present study, PE was not associated with a poorer PFS or OS by univariate analysis. In addition, Wu *et al* (16) reported that *EGFR* mutation-positive stage IV lung adenocarcinoma patients with liver metastases treated with gefitinib as first-line treatment exhibited significantly poorer PFS and OS times compared with patients who did not have liver metastases. In the present study, liver metastasis was not associated with a poorer PFS or OS by multivariate analysis. However, the number of patients with liver metastases was relatively small (n=17). This may explain why the results of the present study differ from the Wu *et al* study. Additionally, in patients with major *EGFR* mutations (exon 19 deletion and p.L858R), liver metastasis was significantly associated with a poorer OS time in the multivariate analysis (HR, 3.05; 95% CI, 1.34-6.51; P<0.001; Table VI).

Higher numbers of metastatic organ sites were associated with a poorer PFS and OS time, as previously reported (18,19). It has been suggested that the prognostic significance of the number of metastatic organ sites may be due to resistance in patients with a larger tumor burden (36). As aforementioned, patients with bone or brain metastases and patients with ≥2 metastatic organ sites do not respond effectively to treatment with first-generation EGFR-TKIs. Therefore, more effective treatments are required. For *EGFR* mutation-positive NSCLC patients, novel treatment approaches have been proposed. Seto *et al* (37) reported that bevacizumab in addition to erlotinib significantly improved PFS. Tamiya *et al* (38) reported that triplet chemotherapy with gefitinib, carboplatin and tegafur/gimeracil/oteracil as a first-line treatment was efficacious and well tolerated. Furthermore, Kanda *et al* (39) reported that the addition of cisplatin and docetaxel to gefitinib treatment may have prevented the development of acquired resistance to EGFR-TKIs in *EGFR* mutation-positive patients with advanced NSCLC. Sugawara *et al* (40) reported that concurrent chemotherapy with gefitinib and carboplatin or pemetrexed was efficacious as a first-line treatment for *EGFR* mutation-positive NSCLC patients. Furthermore,Park *et al* (41) reported that afatinib significantly improved the outcome in treatment-naive patients with EGFR mutation-positive NSCLC compared with gefitinib, with a manageable tolerability profile. Such therapies may be beneficial for patients with the poor prognostic factors identified in the present study; however, no research has been conducted in a clinical setting and further systemic and clinical research is therefore warranted. Furthermore, for patients with brain metastasis, combining EGFR-TKIs and radiotherapy has potential synergistic effects; radiation permeabilizes the blood-brain barrier and TKIs exhibit radio-sensitizing effects (42).

The present study has certain limitations. First, the retrospective design means that undefined biases may have existed,

Table III. Cox proportional hazards model analysis of factors associated with progression-free survival.

	Univariate analysis			Multivariate analysis		
Factor	HR	95% CI	P-value	HR	95% CI	P-value
Brain metastasis	1.63	1.13-2.33	0.010[a]	1.48	0.99-2.15	0.051
Bone metastasis	2.22	1.55-3.20	≤0.001[a]	2.11	1.44-3.09	≤0.001[a]
Liver metastasis	2.73	1.51-4.62	0.002[a]	1.42	0.74-2.56	0.280
Pleural effusion	1.49	1.02-2.17	0.039[a]	1.48	0.99-2.16	0.053

[a]P<0.05. CI, confidence interval; HR, hazard ratio; CI, confidence interval.

Table IV. Cox proportional hazards model of factors associated with overall survival.

	Univariate analysis			Multivariate analysis		
Factor	HR	95% CI	P-value	HR	95% CI	P-value
Brain metastasis	2.60	1.64-4.10	≤0.001[a]	2.41	1.46-3.95	≤0.001[a]
Bone metastasis	1.69	1.08-2.64	0.022[a]	1.59	0.97-2.58	0.066
Liver metastasis	3.81	1.96-6.90	≤0.001[a]	1.84	0.88-3.68	0.104
Pleural effusion	1.94	1.21-3.05	0.006[a]	1.62	0.99-2.60	0.052

[a]P<0.05. CI, confidence interval; HR, hazard ratio; CI confidence interval.

Table V. Cox proportional hazards model analysis of factors associated with progression-free survival in patients with epithelial growth factor receptor exon 19 deletion and p.L858R mutations.

	Univariate analysis			Multivariate analysis		
Factor	HR	95% CI	P-value	HR	95% CI	P-value
Brain metastasis	1.69	1.12-2.52	0.012[a]	1.45	0.93-2.22	0.102
Bone metastasis	1.97	1.33-2.91	≤0.001[a]	1.78	1.16-2.65	0.008[a]
Liver metastasis	3.25	1.66-5.85	0.001[a]	1.95	0.94-3.84	0.101
Pleural effusion	1.44	0.95-2.16	0.083	-	-	-

[a]P<0.05. HR, hazard ratio; CI, confidence interval.

Table VI. Cox proportional hazards model analysis of factors associated with overall survival in patients with epithelial growth factor receptor exon 19 deletion and p.L858R mutations.

	Univariate analysis			Multivariate analysis		
Factor	HR	95% CI	P-value	HR	95% CI	P-value
Brain metastasis	2.55	1.51-4.26	≤0.001[a]	2.06	1.16-3.58	0.014[a]
Bone metastasis	1.43	0.86-2.36	0.165	-	-	-
Liver metastasis	5.00	2.29-10.05	≤0.001[a]	3.05	1.34-6.51	0.009[a]
Pleural effusion	1.89	1.12-3.16	0.018[a]	1.66	0.97-2.81	0.066

[a]P<0.05. HR, hazard ratio; CI, confidence interval.

which could have influenced the patients' clinical outcomes. Second, the data collection and analysis was performed at a single tertiary academic center, thus imposing a possible selection bias.

To conclude, bone metastasis was associated with reduced PFS time and brain metastasis was associated with reduced OS time in NSCLC patients with *EGFR* mutations treated with first-generation EGFR-TKIs. The number of metastatic organ sites was also associated with a poorer PFS and OS.

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