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and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). J Med Chem 2010;54:6342–63.
17. Chand D, Yamazaki Y, Ruuth K, Schonherr C, Martinsson T, Kogner P, et al. Cell culture and Drosophila model systems define three classes of anaplastic lymphoma kinase mutations in neuroblastoma. Dis Models & Mech 2013;6:373–82.
18. Lee CC, Jia Y, Li N, Sun X, Ng K, Ambing E, et al. Crystal structure of the ALK (anaplastic lymphoma kinase) catalytic domain. Biochem J 2010;430:425–37.
19. Zhang S, Wang F, Keats J, Zhu X, Ning Y, Wardwell SD, et al. Crizotinib-resistant mutants of EML4-ALK identified through an accelerated mutagenesis screen. Chem Biol Drug Des 2011;78:999–1005.

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Clinical Predictors of Response to EGFR Tyrosine Kinase Inhibitors in Patients with EGFR-Mutant Non-Small Cell Lung Cancer

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Key Words

Adenocarcinoma · Epidermal growth factor receptor · Erlotinib · Gefitinib · Major mutations · Non-small cell lung cancer

Abstract

Background: The presence of EGFR (epidermal growth factor receptor) mutations is a robust predictor of EGFR tyrosine kinase inhibitor (TKI) responsiveness. Predictors of EGFR-TKI responsiveness in EGFR-mutant non-small cell lung cancer (NSCLC) patients, however, have not been well investigated. The purpose of this study is to examine predictors of EGFR-TKI responsiveness in EGFR-mutant NSCLC patients. ***Patients and Methods:*** Seventy EGFR-mutant NSCLC patients who received EGFR-TKIs in our institution between April 2007 and March 2013 were analyzed retrospectively. ***Results:*** The objective response rate was 50.0% (95% confidence interval, CI, 38.6–61.4%) and the disease control rate was 91.4% (95% CI, 82.5–96.0%). The median progression-free survival (PFS) and overall survival were 9.0 (95% CI, 3.92–14.08) and 20.8 months (95% CI, 14.56–27.04), respectively. In multivariate analysis, adenocarcinoma (hazard ratio, HR, 12.25; 95% CI, 37.7–41.10; $p < 0.001$) and major mutations (deletions in exon 19 and L858R point mutation in exon 21; HR, 2.46; 95% CI, 1.14–5.28; $p = 0.022$) were significant predictors of longer PFS. ***Conclusion:*** Major mutations and adenocarcinoma histology were independent predictors of better treatment outcome in EGFR-mutant NSCLC patients who received EGFR-TKIs. Further well-controlled prospective studies are warranted to confirm our findings.

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide [1]. The identification of EGFR (epidermal growth factor receptor) mutations in non-small cell lung cancer (NSCLC) patients [2, 3] and their association with remarkable response to EGFR tyrosine kinase inhibitors (TKIs), such as erlotinib and gefitinib [2–11], have led to a paradigm shift in the management of advanced NSCLC.

Initially, East-Asian origin, female sex, adenocarcinoma histology and never-smoking history were identified as clinical predictors for a favorable response to EGFR-TKIs in advanced NSCLC patients [12–15]. Subsequent trials revealed that activating mutations of the EGFR gene were predominantly present in patients with the above-

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mentioned clinical predictors [16–19]. It is also worthy of note that the predictive value of the EGFR mutation status is superior to the other clinical predictors for EGFR-TKI responsiveness [20–22]. EGFR mutation is now regarded as the most robust predictor for clinical response to EGFR-TKIs.

Several recent studies have shown that EGFR-TKI responsiveness differs in cases of 'minor mutations' (mutations other than deletions in exon 19 and L858R point mutation in exon 21) or without adenocarcinoma histology from that in cases of 'major classical mutations' [23–26] or adenocarcinoma histology [27–29]. While some reports have shown that EGFR-TKIs are less effective for these cases, the number of EGFR-mutant patients with such characteristics is innately small. Therefore, a direct comparison between these factors and the other aforementioned well-known predictors (e.g. gender/smoking status) of EGFR-TKI responsiveness has not been reported. Furthermore, the predictors of EGFR-TKI responsiveness in EGFR-mutant patients have not been extensively investigated [6, 30]. Identification of the factors which have predominant roles in determining EGFR-TKI responsiveness in EGFR-mutant NSCLC patients would be beneficial for clinicians, because early identification of patients who will be resistant to EGFR-TKIs would be helpful in the choice of treatment.

In this retrospective study, we investigated clinical predictors associated with treatment outcome in EGFR-mutant NSCLC patients treated with EGFR-TKIs.

Methods

Patients and Clinical Characteristics

A total of 378 consecutive patients diagnosed with advanced NSCLC were screened for EGFR mutations between April 2007 and March 2013 at the Tosei General Hospital (Aichi, Japan). EGFR mutations were detected in 122 patients (32.3%), and 80 met the following inclusion criteria: (1) histologically or cytologically confirmed NSCLC at stage IIIB/IV or relapse, (2) history of treatment with either gefitinib or erlotinib, and (3) measurable disease by computed tomography (CT). Ten were excluded from the study: 3 had a history of prior treatment with EGFR-TKI; 7 discontinued EGFR-TKI treatment before the first response evaluation mainly because of symptomatic deterioration in 3 patients, death due to lung cancer in 2 and intolerable toxicity in 2. The remaining 70 patients were selected and assessed in this study. Baseline clinical characteristics were determined by retrospective chart review, including age, gender, tumor histology, EGFR mutation types, smoking history [nonsmoker (<100 cigarettes in a lifetime) vs. former or current smoker], baseline Eastern Cooperative Oncology Group (ECOG) performance status (PS), stage and treatment line (1st or 2nd line vs. ≥3rd line). Lung cancer histology was defined on the basis of the World Health Organization pathology classification. Clinical staging was decided according to the seventh edition of the tumor node metastasis classification of NSCLC. Survival status was monitored until the end of June 2013. This study was approved by the Institutional Review Board of the Tosei General Hospital.

EGFR-TKI Treatment and Response Evaluation

The initial doses of gefitinib and erlotinib were 250 and 150 mg/day, respectively, until disease progression, intolerable toxicity or patient refusal. Pretreatment evaluation before EGFR-TKIs included CT scan of the chest and/or abdomen, bone scintigram and magnetic resonance imaging of the brain. Treatment response was evaluated by CT scan every 4–8 weeks. In accordance with the Response Evaluation Criteria in Solid Tumors, version 1.1 [31], objective clinical responses to EGFR-TKIs were classified as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). SD was defined as disease control maintained for at least 6 weeks. All responses were confirmed at least 4 weeks after initial assessment. The response rate (RR) and disease control rate were defined as the best tumor responses of CR + PR and CR + PR + SD ≥6 weeks, respectively.

Analysis of EGFR and Tumor Histology

Tumor specimens were obtained by various methods: transbronchial bronchoscopic lung biopsy, ultrasound or CT-guided needle biopsy, cell blocks of malignant effusion and surgical tissue. Biopsied or surgically resected specimens were fixed with formalin and embedded in paraffin and subjected to an EGFR mutation analysis based on the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method [32]. For this study, deletions in exon 19 and L858R in exon 21 were considered major mutations. Minor mutations were defined as mutations in exons 18 and 20, and unusual mutations occurring in exons 19 and 21. When two or more different mutations were found simultaneously, the mutation types were defined as complex mutations [33]. If one of the contained mutations was a major mutation, they were classified as major mutations according to a previous study that showed gefitinib was as effective for complex mutations containing major mutations as for single major mutations [34]. Paraffin blocks were also used for immunohistochemical staining of cytokeratin 5–6 and thyroid transcription factor 1 after deparaffinization of 3- to 4-μm-thick sections.

Statistical Analyses

We used Pearson χ^2 and Fisher's exact tests to assess the correlations between clinical variables and treatment efficacy, when appropriate. Progression-free survival (PFS) and overall survival (OS) were measured from the date of the initiation of gefitinib or erlotinib until the date of disease progression or death from any cause for PFS and until the date of last follow-up, death or the final follow-up day of the study for OS. The Kaplan-Meier method was applied to estimate PFS and OS, and survival differences between groups were analyzed by the log-rank test. Cox proportional hazard regression models were used to evaluate factors in longer survival. A backward stepwise approach was adopted as our variable selection method for multivariate analyses. All statistical tests were two sided, and values of $p < 0.05$ were considered statistically significant. Statistical analyses were carried out using SPSS version 19.0 (IBM Corporation, Armonk, N.Y., USA).

Table 1. Patient characteristics (n = 70)

Characteristics	Patients	
	n	%
Age, years		
Median	68.1	
Range	43–90	
Gender		
Female	47	67.1
Male	23	32.9
Smoking history		
Former or current smoker	28	40.0
Nonsmoker	42	60.0
ECOG PS		
0	34	48.6
1	23	32.9
2	5	7.1
3	4	5.7
4	4	5.7
Histology		
Adenocarcinoma	66	94.3
Squamous cell carcinoma	3	4.3
Other	1	1.4
Tumor stage		
IIIB	10	14.3
IV	49	70.0
Relapse	11	15.7
Treatment lines		
1st line	12	17.1
2nd line	45	64.3
≥3rd line	13	18.6
EGFR mutation status		
Major mutation	62	88.6
Minor mutation	8	11.4
EGFR-TKIs		
Gefitinib	15	21.4
Erlotinib	55	78.6

Table 2. Univariate analysis of PFS

Characteristics	PFS, months	p value
Age		0.863
Gender		
Female	12.8	0.037
Male	4.0	
Smoking history		
Former or current smoker	6.9	0.080
Nonsmoker	12.8	
ECOG PS		
0–1	11.7	0.112
2–4	8.3	
Histology		
Adenocarcinoma	11.7	<0.001
No adenocarcinoma	2.1	
Tumor stage		
IIIB	7.2	0.553
IV	8.3	
Relapse	17.9	
Treatment lines		
1st or 2nd	11.7	0.038
≥3rd	7.2	
EGFR mutation status		
Major mutation	12.5	0.043
Minor mutation	4.3	
EGFR-TKIs		
Gefitinib	8.7	0.882
Erlotinib	9.7	

Results

Patient Characteristics

Baseline characteristics of the 70 consecutive patients are shown in table 1. Female patients (67.1%), never smokers (60.0%) and patients with PS 0 or 1 (81.5%) were predominant. The most common tumor histology was adenocarcinoma, which was present in 66 patients (94.3%). All patients had advanced or recurrent disease: stage IIIB: 10 patients; IV: 49 patients, and recurrence: 11 patients. Of these patients, 12 received EGFR-TKIs as 1st-line therapy, 45 as 2nd-line therapy, and 13 as ≥3rd-line therapy.

Of the 70 patients harboring EGFR mutation(s), major single mutations were found in 59 patients (deletions in exon 19 in 38 patients and L858R in exon 21 in 21 patients). On the other hand, complex mutations were found in 3 patients (deletions in exon 19 + L858R, G719S + deletions in exon 19 and G719S + L858R in 1 patient each). All 3 of the patients harboring complex EGFR mutations had either of two major mutations (deletions in exon 19 or L858R in exon 21). Therefore, they were classified as major mutations in this study. Finally, 62 patients were regarded as harboring major mutations.

Response to Treatment

Of the 70 patients, 4 had CR, 31 had PR and 29 had SD, yielding an objective RR of 50.0% (95% confidence interval, CI, 38.6–61.4%) and disease control rate of 91.4% (95% CI, 82.5–96.0%).

Analysis of Survival

At the time of analysis, the median follow-up time was 19.7 months (range 1.3–74.0 months). The median PFS was 9.0 months (95% CI, 3.92–14.08 months; fig. 1a), and the median OS was 20.8 months (95% CI, 14.56–27.04 months); 31.4% of the patients were censored at the time

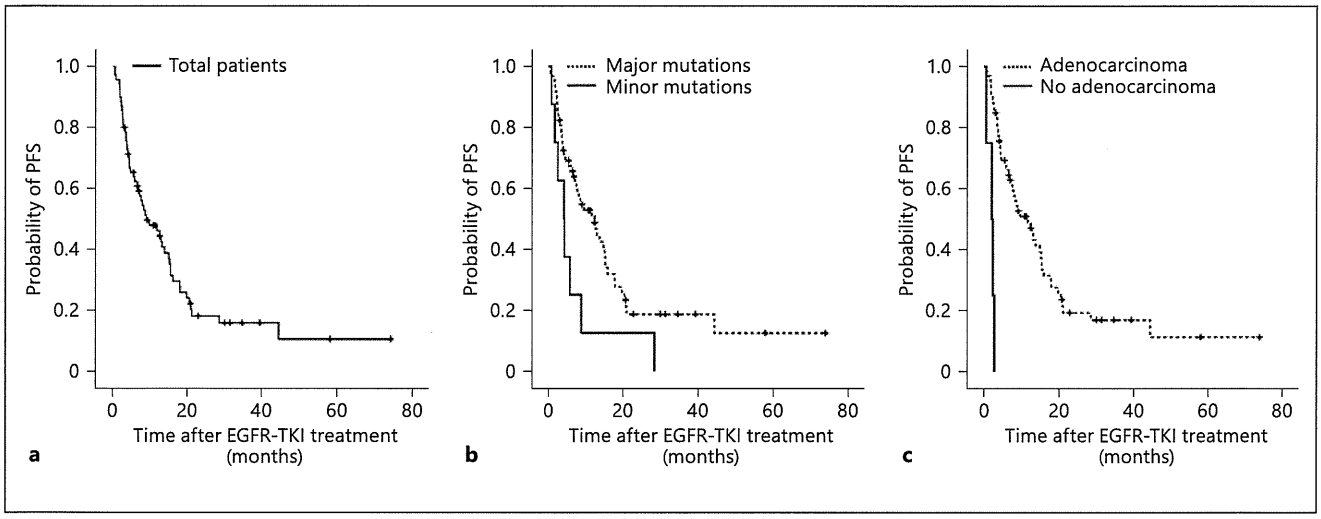

Fig. 1. Kaplan-Meier plot of survival times. **a** PFS in all patients (n = 70): 9 months. **b** PFS of patients classified according to EGFR mutation status: 62 patients with major mutations (12.5 months) and 8 patients with minor mutations (4.3 months; p = 0.043). **c** PFS of patients classified according to tumor histology status: 66 patients with adenocarcinoma (11.7 months) and 4 patients without adenocarcinoma (2.1 months; p < 0.001).

Table 3. Multivariate analysis of PFS

Characteristics	PFS		
	HR	95% CI	p value
Histology, adenocarcinoma	12.25	3.77–41.10	<0.001
EGFR, major mutation	2.46	1.14–5.28	0.022

of data cutoff. Analysis of PFS is shown in table 2. Using univariate analysis, female sex (12.8 vs. 4.0 months, p = 0.037), adenocarcinoma (11.7 vs. 2.1 months, p < 0.001), 1st- or 2nd-line therapy (11.7 vs. 7.2 months, p = 0.038) and major mutations (12.5 vs. 4.3 months, p = 0.043) were suggested to be predictors for longer PFS. There were no significant differences in PFS according to age, smoking history or ECOG PS. In the multivariate analysis using a Cox proportional hazard model, adenocarcinoma (hazard ratio, HR, 12.25; 95% CI, 37.7–41.10; p < 0.001) and major mutations (HR, 2.46; 95% CI, 1.14–5.28; p = 0.022) were significant predictors of longer PFS (table 3). Kaplan-Meier plots stratified according to EGFR mutation patterns or histological patterns are shown in figure 1b, c, respectively. In the log-rank test, the median PFS was significantly longer in patients with major mutations and adenocarcinoma than in those with minor mutations (p = 0.043) and without adenocarcinoma (p < 0.001), respectively. The characteristics of the patients with minor mutations and without adenocarcinoma are shown in tables 4 and 5, respectively.

Discussion

In this report, we retrospectively analyzed the clinical factors associated with PFS in EGFR-mutant NSCLC patients treated with EGFR-TKIs. As mentioned above, we confirmed by means of multivariate analysis that major mutations and adenocarcinoma were independent predictors of longer PFS after EGFR-TKI treatment. This result suggests that minor mutations are independent negative predictors of the efficacy of EGFR-TKI treatment, and that histology is an important additional predictor to consider when applying EGFR-TKI treatment to EGFR-mutant NSCLC patients.

Several previous studies evaluated predictors of EGFR-TKI responsiveness in EGFR-mutant NSCLC patients. For example, good PS and chemotherapy-naïve status [6], presence of deletions in exon 19 compared with L858R [35, 36], and a small number of metastatic sites at baseline [30] are reported predictors of longer survival. However, the number of such studies is still rather small, so that no firm predictors have been established yet.

Previous clinical trials investigating the efficacy of EGFR-TKIs in EGFR-mutant NSCLC patients mainly fo-

Table 4. Characteristics of patients with minor mutations

Age	Gender	Smoking	Histology	PS	Treatment line	EGFR mutation	TKI	PFS, months	Response
76	M	former smoker	Ad	1	1st	G719A	gefitinib	5.9	SD
79	F	nonsmoker	Ad	0	2nd	G719A	erlotinib	4.3	PR
54	M	former smoker	Ad	1	2nd	G719A	erlotinib	2.6	PD
67	M	former smoker	Ad	0	2nd	G719S	erlotinib	1.8	PD
64	M	former smoker	Ad	0	4th	T790M	erlotinib	0.9	PD
63	F	nonsmoker	Ad	0	2nd	L861Q	gefitinib	28.5	PR
79	F	nonsmoker	Ad	0	2nd	L861Q	erlotinib	4.4	PR
84	F	nonsmoker	Ad	3	2nd	L861Q	erlotinib	9.0	SD

M = Male; F = female; Ad = adenocarcinoma; G719A = G719A point mutation in exon 18; G719S = G719S point mutation in exon 18; T790M = T790M point mutation in exon 20; L861Q = L861Q point mutation in exon 21.

Table 5. Characteristics of patients without adenocarcinoma

Age	Gender	Smoking	Histology	PS	Treatment line	EGFR mutation	TKI	PFS, months	Response	Differentiation	CK5–6	TTF-1
75	M	former smoker	Sq	0	3rd	19 del	erlotinib	0.6	PD	well	+	–
72	M	former smoker	Sq	1	2nd	19 del	erlotinib	2.4	SD	well	+	–
54	M	former smoker	Sq	0	3rd	L858R	erlotinib	2.1	SD	well	+	–
61	M	former smoker	NSCLC	1	3rd	19 del	erlotinib	2.8	SD	poor	–	+

CK5–6 = Cytokeratin 5–6; TTF-1 = thyroid transcription factor 1; M = male; Sq = squamous cell carcinoma; NSCLC = NSCLC not otherwise specified; 19 del = deletions in exon 19; L858R = L858R point mutation in exon 21; + = immunopositive; – = immunonegative.

cused on patients harboring two major EGFR mutations (deletions in exon 19 and L858R point mutation in exon 21) [8–11, 18–22], while other minor mutations were excluded from the study cohorts. According to these trials, minor mutations are calculated to account for only about 5–20% of all EGFR mutations. The clinical prevalence and significance of minor mutations have not been completely ascertained. Recently, several studies have described EGFR-TKI responses in patients with minor mutations [24, 25]. From the results it is becoming increasingly clear that not all EGFR mutations confer sensitivity to EGFR-TKIs, and that the clinical associations of minor mutations with EGFR-TKI responsiveness are heterogeneous.

We identified minor mutations in 8 patients (11.4%) in our series, consisting of 4 G719X point mutations in exon 18, 3 L861Q point mutations in exon 21 and 1 T790M insertion in exon 20. The median PFS was 4.3 months, which was significantly worse than that of patients with major mutations. In a recent study of EGFR-mutant NSCLC patients, Wu et al. [24] investigated the clinical features of 'uncommon' EGFR mutations. They demonstrated that patients with G719X or L861Q mutations had better survival than patients with other uncommon mutations. However, compared with major mutations, survival of patients with those mutations was shorter (nonsignificant). On the other hand, T790M mutation before treatment is known as a resistance mutation to EGFR-TKI treatment [37, 38]. In the light of our results and those of recently published studies, EGFR-TKI treatment is less effective in patients with minor mutations than in those with major mutations.

Many large-scale trials have revealed adenocarcinoma histology as one of the independent predictors of outcome in NSCLC patients treated with EGFR-TKIs. However, when limited to EGFR-mutant NSCLC patients, the prognostic value of histology has not been well established. Recent studies have described the incidence of EGFR mutations in patients without adenocarcinoma to be rare, ranging from 0 to 20% [6, 10, 29, 39]. EGFR-TKI seems to be generally less effective in such patients than in EGFR-mutant adenocarcinoma patients. On the basis of a pooled analysis of 15 publications, Shukuya et al. [27] suggested

that 27 EGFR-mutant squamous cell carcinoma patients had diminished EGFR-TKI responsiveness, with a RR and median PFS of 38% and 3.1 months, respectively. Hata et al. [29] found that the incidence of EGFR mutations in patients with squamous cell carcinoma was 13.3% (33 of 249 patients). The RR and median PFS after EGFR-TKI treatment were 25% and 1.4 months, respectively. These results are clearly inferior to pivotal data for EGFR-mutant adenocarcinoma, for which, in general, the RR and median PFS have been reported to be 70–80% and 9–11 months, respectively [6–10]. Cho et al. [39] analyzed clinical outcome in EGFR-mutant NSCLC patients without adenocarcinoma who received EGFR-TKIs and compared the results with those of EGFR-mutant adenocarcinoma patients. The incidence of EGFR mutation was 8.4% (21 of 250 patients). The median PFS was 3.7 months, which was significantly lower than that of adenocarcinoma patients. In the present study, 4 of 70 patients were without adenocarcinoma (5.6%), an incidence which is in agreement with the cited studies. The median PFS in those studies was 2.1 months. This median PFS was significantly worse than that of adenocarcinoma patients. Similar to the results from recently published studies, absence of adenocarcinoma is also a significant negative predictor of EGFR-TKI responsiveness in our study.

It is noteworthy that female sex and absence of a smoking history did not show any significance in predicting longer PFS in multivariate analysis in our study, although they are well-known clinical factors for better PFS after EGFR-TKI treatment. We suppose that this is because our cohort includes only EGFR-mutant NSCLC patients. Previous studies reported the advantage of EGFR mutations in predicting the outcome of gefitinib treatment compared with gender and smoking status [20–22]. Given the superiority of the presence of EGFR mutation in predicting EGFR-TKI responsiveness, it is reasonable that these clinical factors were not retained as independent predictors exclusively in EGFR-mutant NSCLC patients.

Several limitations of this study should be mentioned. First of all, it is a retrospective, nonrandomized study in a single institution. Secondly, this study included only Japanese patients. East-Asian ethnicity is one of the well-known clinical predictors of the efficacy of EGFR-TKIs in NSCLC patients. For that reason, whether or not our results can be applied to other ethnicities should also be investigated. Thirdly, our sample size was small and the associations reported as statistically significant need validation in larger patient cohorts in the future.

In conclusion, major mutations and adenocarcinoma histology were independent predictors of better treatment outcome in EGFR-mutant NSCLC patients who received EGFR-TKIs. While our current findings provide new insights, further well-controlled prospective studies are warranted.

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References

1 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D: Global cancer statistics. CA Cancer J Clin 2011;61:69–90.

2 Scagliotti GV, Selvaggi G, Novello S, Hirsch FR: The biology of epidermal growth factor receptor in lung cancer. Clin Cancer Res 2004; 10:4227–4232.

3 Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–2139.

4 Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–1500.

5 Chou TY, Chiu CH, Li LH, Hsiao CY, Tzen CY, Chang KT, Chen YM, Perng RP, Tsai SF, Tsai CM: Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer. Clin Cancer Res 2005;11: 3750–3757.

6 Morita S, Okamoto I, Kobayashi K, Yamazaki K, Asahina H, Inoue A, Hagiwara K, Sunaga N, Yanagitani N, Hida T, Yoshida K, Hirashima T, Yasumoto K, Sugio K, Mitsudomi T, Fukuoka M, Nukiwa T: Combined survival analysis of prospective clinical trials of gefitinib for non-small cell lung cancer with EGFR mutations. Clin Cancer Res 2009;15:4493–4498.

7 Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–957.

8 Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, Takada M, Yoshioka H, Shibata K, Kudoh S, Shimizu E, Saito H, Toyooka S, Nakagawa K, Fukuoka M, West Japan Oncology Group: Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010; 11:121–128.

9 Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S, Nukiwa T, North-East Japan Study Group: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362: 2380–2388.

10 Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, Lu S, Zhang L, Hu C, Hu C, Luo Y, Chen L, Ye M, Huang J, Zhi X, Zhang Y, Xiu Q, Ma J, Zhang L, You C: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735–742.

11 Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, Porta R, Cobo M, Garrido P, Longo F, Moran T, Insa A, De Marinis F, Corre R, Bover I, Illiano A, Dansin E, de Castro J, Milella M, Reguart N, Altavilla G, Jimenez U, Provencio M, Moreno MA, Terrasa J, Muñoz-Langa J, Valdivia J, Isla D, Domine M, Molinier O, Mazieres J, Baize N, Garcia-Campelo R, Robinet G, Rodriguez-Abreu D, Lopez-Vivanco G, Gebbia V, Ferrera-Delgado L, Bombaron P, Bernabe R, Bearz A, Artal A, Cortesi E, Rolfo C, Sanchez-Ronco M, Drozdowskyj A, Queralt C, de Aguirre I, Ramirez JL, Sanchez JJ, Molina MA, Taron M, Paz-Ares L, Spanish Lung Cancer Group in collaboration with Groupe Français de Pneumo-Cancérologie and Associazione Italiana Oncologia Toracica: Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13: 239–246.

12 Haringhuizen A, van Tinteren H, Vaessen HF, Baas P, van Zandwijk N: Gefitinib as a last treatment option for non-small-cell lung cancer: durable disease control in a subset of patients. Ann Oncol 2004;15:786–792.

13 Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, Lorimer I, Zhang T, Liu N, Daneshmand M, Marrano P, da Cunha Santos G, Lagarde A, Richardson F, Seymour L, Whitehead M, Ding K, Pater J, Shepherd FA: Erlotinib in lung cancer – molecular and clinical predictors of outcome. N Engl J Med 2005;353:133–144.

14 Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, Hatooka S, Shinoda M, Takahashi T, Yatabe Y: Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. J Clin Oncol 2005; 23:2513–2520.

15 Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K: Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 2005; 366:1527–1537.

16 Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T: Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919–8923.

17 Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD, Gazdar AF: Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005; 97:339–346.

18 Riely GJ, Pao W, Pham D, Li AR, Rizvi N, Venkatraman ES, Zakowski MF, Kris MG, Ladanyi M, Miller VA: Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res 2006;12:839–844.

19 Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, Majem M, Lopez-Vivanco G, Isla D, Provencio M, Insa A, Massuti B, Gonzalez-Larriba JL, Paz-Ares L, Bover I, Garcia-Campelo R, Moreno MA, Catot S, Rolfo C, Reguart N, Palmero R, Sánchez JM, Bastus R, Mayo C, Bertran-Alamillo J, Molina MA, Sanchez JJ, Taron M, Spanish Lung Cancer Group: Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958–967.

20 Tokumo M, Toyooka S, Kiura K, Shigematsu H, Tomii K, Aoe M, Ichimura K, Tsuda T, Yano M, Tsukuda K, Tabata M, Ueoka H, Tanimoto M, Date H, Gazdar AF, Shimizu N: The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. Clin Cancer Res 2005;11:1167–1173.

21 Han SW, Kim TY, Lee KH, Hwang PG, Jeon YK, Oh DY, Lee SH, Kim DW, Im SA, Chung DH, Heo DS, Bang YJ: Clinical predictors versus epidermal growth factor receptor mutation in gefitinib-treated non-small-cell lung cancer patients. Lung Cancer 2006;54:201–207.

22 Toyooka S, Takano T, Kosaka T, Hotta K, Matsuo K, Ichihara S, Fujiwara Y, Soh J, Otani H, Kiura K, Aoe K, Yatabe Y, Ohe Y, Mitsudomi T, Date H: Epidermal growth factor receptor mutation, but not sex and smoking, is independently associated with favorable prognosis of gefitinib-treated patients with lung adenocarcinoma. Cancer Sci 2008;99: 303–308.

23 Wu JY, Wu SG, Yang CH, Gow CH, Chang YL, Yu CJ, Shih JY, Yang PC: Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. Clin Cancer Res 2008;14: 4877–4882.

24 Wu JY, Yu CJ, Chang YC, Yang CH, Shih JY, Yang PC: Effectiveness of tyrosine kinase inhibitors on 'uncommon' epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. Clin Cancer Res 2011;17:3812–3821.

25 De Pas T, Toffalorio F, Manzotti M, Fumagalli C, Spitaleri G, Catania C, Delmonte A, Giovannini M, Spaggiari L, de Braud F, Barberis M: Activity of epidermal growth factor receptor-tyrosine kinase inhibitors in patients with non-small cell lung cancer harboring rare epidermal growth factor receptor mutations. J Thorac Oncol 2011;6:1895–1901.

26 Iversen ML, Kleinberg L, Fjellbirkeland L, Helland A, Brustugun OT: Clinicopathological characteristics of 11 NSCLC patients with EGFR-exon 20 mutations. J Thorac Oncol 2012;7:1471–1473.

27 Shukuya T, Takahashi T, Kaira R, Ono A, Nakamura Y, Tsuya A, Kenmotsu H, Naito T, Kaira K, Murakami H, Endo M, Takahashi K, Yamamoto N: Efficacy of gefitinib for non-adenocarcinoma non-small-cell lung cancer patients harboring epidermal growth factor receptor mutations: a pooled analysis of published reports. Cancer Sci 2011;102:1032–1037.

28 Paik PK, Varghese AM, Sima CS, Moreira AL, Ladanyi M, Kris MG, Rekhtman N: Response to erlotinib in patients with EGFR mutant advanced non-small cell lung cancers with a squamous or squamous-like component. Mol Cancer Ther 2012;11:2535–2540.

29 Hata A, Katakami N, Yoshioka H, Kunimasa K, Fujita S, Kaji R, Notohara K, Imai Y, Tachikawa R, Tomii K, Korogi Y, Iwasaku M, Nishiyama A, Ishida T: How sensitive are epidermal growth factor receptor-tyrosine kinase inhibitors for squamous cell carcinoma of the lung harboring EGFR gene-sensitive mutations? J Thorac Oncol 2013;8:89–95.

30 Park JH, Kim TM, Keam B, Jeon YK, Lee SH, Kim DW, Chung DH, Kim YT, Kim YW, Heo DS: Tumor burden is predictive of survival in patients with non-small-cell lung cancer and with activating epidermal growth factor receptor mutations who receive gefitinib. Clin Lung Cancer 2013;14:383–389.

▶31 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–247.

▶32 Nagai Y, Miyazawa H, Huqun, Tanaka T, Udagawa K, Kato M, Fukuyama S, Yokote A, Kobayashi K, Kanazawa M, Hagiwara K: Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. Cancer Res 2005;65:7276–7282.

▶33 Hsieh MH, Fang YF, Chang WC, Kuo HP, Lin SY, Liu HP, Liu CL, Chen HC, Ku YC, Chen YT, Chang YH, Chen YT, Hsi BL, Tsai SF, Huang SF: Complex mutation patterns of epidermal growth factor receptor gene associated with variable responses to gefitinib treatment in patients with non-small cell lung cancer. Lung Cancer 2006;53:311–322.

▶34 Wu SG, Chang YL, Hsu YC, Wu JY, Yang CH, Yu CJ, Tsai MF, Shih JY, Yang PC: Good response to gefitinib in lung adenocarcinoma of complex epidermal growth factor receptor (EGFR) mutations with the classical mutation pattern. Oncologist 2008;13:1276–1284.

▶35 Won YW, Han JY, Lee GK, Park SY, Lim KY, Yoon KA, Yun T, Kim HT, Lee JS: Comparison of clinical outcome of patients with non-small-cell lung cancer harbouring epidermal growth factor receptor exon 19 or exon 21 mutations. J Clin Pathol 2011;64:947–952.

▶36 Sun JM, Won YW, Kim ST, Kim JH, Choi YL, Lee J, Park YH, Ahn JS, Park K, Ahn MJ: The different efficacy of gefitinib or erlotinib according to epidermal growth factor receptor exon 19 and exon 21 mutations in Korean non-small cell lung cancer patients. J Cancer Res Clin Oncol 2011;137:687–694.

▶37 Rosell R, Molina MA, Costa C, Simonetti S, Gimenez-Capitan A, Bertran-Alamillo J, Mayo C, Moran T, Mendez P, Cardenal F, Isla D, Provencio M, Cobo M, Insa A, Garcia-Campelo R, Reguart N, Majem M, Viteri S, Carcereny E, Porta R, Massuti B, Queralt C, de Aguirre I, Sanchez JM, Sanchez-Ronco M, Mate JL, Ariza A, Benlloch S, Sanchez JJ, Bivona TG, Sawyers CL, Taron M: Pretreatment EGFR T790M mutation and BRCA1 mRNA expression in erlotinib-treated advanced non-small-cell lung cancer patients with EGFR mutations. Clin Cancer Res 2011;17:1160–1168.

▶38 Su KY, Chen HY, Li KC, Kuo ML, Yang JC, Chan WK, Ho BC, Chang GC, Shih JY, Yu SL, Yang PC: Pretreatment epidermal growth factor receptor (EGFR) T790M mutation predicts shorter EGFR tyrosine kinase inhibitor response duration in patients with non-small-cell lung cancer. J Clin Oncol 2012;30:433–440.

▶39 Cho SH, Park LC, Ji JH, Park S, Hwang DW, Lee JY, Choi YL, Han JH, Sun JM, Ahn JS, Park K, Ahn MJ: Efficacy of EGFR tyrosine kinase inhibitors for non-adenocarcinoma NSCLC patients with EGFR mutation. Cancer Chemother Pharmacol 2012;70:315–320.

Phase II study of erlotinib for previously treated patients with EGFR wild-type non-small-cell lung cancer, following EGFR mutation status reevaluation with the Scorpion Amplified Refractory Mutation System

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Abstract. While assessing the efficacy of erlotinib in patients with epidermal growth factor receptor (EGFR) wild-type (WT) non-small-cell lung cancer (NSCLC), the sensitivity of the method used for the EGFR mutation analysis may affect the evaluation of the efficacy. We conducted a phase II study of erlotinib for previously treated patients with EGFR WT NSCLC screened by the peptide nucleic acid-locked nucleic acid (PNA-LNA) polymerase chain reaction (PCR) clamp method, which is known to be highly sensitive. The primary endpoint was the objective response rate (ORR). Preplanned reevaluation of the EGFR genotype as an exploratory endpoint was performed using the Scorpion Amplification Refractory Mutation System (S-ARMS) assay. Erlotinib was administered daily until disease progression or development of unacceptable toxicity. A total of 53 evaluable patients were enrolled. The histological subtypes were adenocarcinoma in 40 patients, squamous cell carcinoma in 9 patients and not otherwise specified NSCLC in 4 patients. Partial response (PR) was achieved in 6 patients (4 with adenocarcinoma and 2 with squamous cell carcinoma). The ORR was 11.3% [95% confidence interval (CI): 4.3-23.0]. The median progression-free survival (PFS) was 1.8 months (95% CI: 1.2-2.3). Samples from 26 of the 53 patients (49.0%) were available for EGFR mutation reanalysis with the S-ARMS assay. Of these 26 samples, only 1 sample of adenocarcinoma was found to be EGFR mutation-positive (exon 19 deletion) and the patient achieved a PR. The EGFR WT genotype was reconfirmed by the S-ARMS assay in the remaining 25 patients and 2 of these patients exhibited a PR. This study did not meet the primary endpoint, although erlotinib was found to be moderately effective in pretreated patients with EGFR WT NSCLC, even when the EGFR mutational status was confirmed by the highly sensitive PNA-LNA clamp PCR method.

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Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide. Non-small-cell lung cancer (NSCLC) is the predominant histological type of lung cancer and ~70.0% of all NSCLC patients have advanced-stage IIIB or IV disease at diagnosis. Platinum-based chemotherapy is currently the standard treatment for advanced NSCLC; however, almost all the patients treated by initial chemotherapy alone eventually develop a relapse.

Erlotinib, a selective epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI), is currently recommended as second- or third-line standard treatment in patients with NSCLC (1). The presence of activating somatic

mutations in the EGFR gene has been shown to be a predictor of the response to treatment with EGFR-TKIs (2) and first-line EGFR-TKI therapy for patients with EGFR mutation-positive NSCLC was shown to improve the progression-free survival (PFS) compared to standard platinum-based chemotherapy (3-6). However, the results of subgroup analyses in the BR21 and SATURN trials suggest that erlotinib may also be beneficial to patients with EGFR wild-type (WT) NSCLC (1,7).

While assessing the efficacy of erlotinib in patients with EGFR WT NSCLC, the sensitivity of the method(s) used for the EGFR mutation analysis may affect the results of the evaluation. Although direct DNA sequencing has been widely used for EGFR mutation analysis, several new techniques, such as the peptide nucleic acid-locked nucleic acid (PNA-LNA) polymerase chain reaction (PCR) clamp method and the Scorpion Amplification Refractory Mutation System (S-ARMS) assay are currently available (8,9). Kim *et al* (10) reported a higher sensitivity of the PNA-LNA clamp method as compared to direct DNA sequencing for the detection of EGFR mutations in patients with NSCLC. In their study, the EGFR mutation positivity rate in 240 NSCLC patients was 34.6% when assessed by the PNA-LNA clamp method, but only 26.3% when assessed by direct DNA sequencing. Therefore, it is possible that erlotinib is found to be considerably less effective in patients with EGFR WT NSCLC, when the EGFR genotype is confirmed by highly sensitive methods, such as the PNA-LNA clamp method.

In addition, the predictive value of KRAS mutations for the efficacy of erlotinib in patients with EGFR WT NSCLC has not been fully elucidated. It was previously suggested that the presence of KRAS mutations may predict a poor response to EGFR-TKI therapy in patients with NSCLC (11). However, the EGFR mutation status may be a confounding factor in the analysis of the predictive value of KRAS mutations, since KRAS and EGFR mutations exhibit a strong negative correlation and EGFR mutation is a predictor of the response to EGFR-TKI therapy. Therefore, further evaluation of the predictive value of KRAS mutations in patients with EGFR WT NSCLC is required.

Based on these findings, we conducted a multicenter phase II trial of erlotinib for previously treated patients with EGFR WT NSCLC. The primary endpoint of this study was to assess the efficacy and safety of erlotinib in patients with EGFR WT NSCLC, as confirmed by the PNA-LNA clamp method, which is a highly sensitive method for EGFR mutation analysis. Preplanned reevaluation of the EGFR and KRAS mutation status as exploratory endpoints was performed using the S-ARMS assay in this study.

Patients and methods

Study design. This study was a multicenter, open-label, single-arm, phase II trial conducted in Japan. The study protocol was approved by the Central Japan Lung Study Group (CJLSG) Protocol Review Committee and the Institutional Review Board of each center as the CJLSG 0903 trial. The study was performed in accordance with the principles laid out in the Declaration of Helsinki and is registered with the University Hospital Medical Information Network in Japan (no. 000002692). The primary endpoint was the objective response rate (ORR) and the secondary endpoints were disease control rate (DCR), PFS, overall survival (OS) and safety. Moreover, if residual samples were available, we performed a preplanned reevaluation of the EGFR mutation status and KRAS mutation analysis with the S-ARMS assay as a secondary endpoint.

Eligibility criteria. Pretreated stage IIIB/IV NSCLC patients were assessed regarding their eligibility for enrollment in this study. The main inclusion criteria were as follows: Pathologically proven NSCLC; EGFR WT genotype confirmed by the PNA-LNA PCR clamp method; history of one or two prior chemotherapies, including at least one platinum-based chemotherapy; age ≥20 years; Eastern Cooperative Oncology Group performance status (PS) of 0-2; adequate bone marrow, hepatic and renal function; at least one measurable lesion as defined by the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 (12); life expectancy of ≥3 months; and patient willingness to provide written informed consent. The main exclusion criteria were as follows: Pulmonary disorders, such as interstitial lung disease, pneumoconioses, or active radiation pneumonitis; severe eye disorders; and massive pleural or pericardial effusion.

EGFR genotype testing for eligibility. The PNA-LNA PCR clamp method was used for confirmation of the EGFR mutation status in the NSCLC patients prior to enrollment. This method is a highly sensitive and simple procedure for the detection of 13 known EGFR mutations (8). For this study, we enrolled patients with the WT allele of EGFR in all 13 mutation sites. A total of 5 tissue slides (5-μm) or pleural effusion cytology samples containing tumor cells were used for the analysis. Tissue slides were prepared from tumor cell-rich sections of formalin-fixed paraffin-embedded tumor samples. In Japan, the PNA-LNA PCR clamp method is commercially available and performed by the Mitsubishi Chemical Medience Corporation (Tokyo, Japan).

Screening of tumors for the KRAS genotype and reanalysis of the EGFR mutation status using the S-ARMS assay. Following completion of patient enrollment, the tumor samples available for KRAS mutation analysis and EGFR mutation reanalysis were collected. DNA was extracted at the laboratory of the Department of Respiratory Medicine, Nagoya University Graduate School of Medicine, using the QIAamp DNA Mini kit (Qiagen, Tokyo, Japan), followed by quantitation of the DNA. According to a previous report, the PNA-LNA PCR clamp method and the S-ARMS assay exhibit an equally high sensitivity for the detection of the EGFR mutation status (13). Therefore, we prioritized KRAS mutation screening if the amount of DNA available was not sufficient for evaluation of both the KRAS and EGFR mutation status by the S-ARMS assay. S-ARMS analysis for the detection of EGFR mutation was performed using the EGFR Mutation RGQ PCR kit (Qiagen, Manchester, UK) and S-ARMS analysis for evaluation of the KRAS mutation status was performed using the KRAS PCR kit (Qiagen, Manchester), which is able to detect 7 mutations in codons 12 and 13 of the KRAS gene.

Treatment. Oral erlotinib was administered at a dose of 150 mg daily until disease progression or development of unacceptable toxicity. The erlotinib dose was reduced (first reduction to 100 mg daily and second reduction to 50 mg daily) or treatment was interrupted in the event of any grade 3 non-hematological toxicity. Dose escalation was not permitted. In the event of development of interstitial lung disease (ILD) of any grade or any grade 4 toxicity, the protocol was discontinued.

Efficacy and safety evaluation. Tumor response was assessed in accordance with RECIST, version 1.1 (12). The baseline assessment included chest and upper abdominal computed tomography (CT), head CT or magnetic resonance imaging and bone scintigraphy or ^{18}F-fluorodeoxyglucose-positron emission tomography. Assessment of the tumor response was performed every 4 weeks during the first 8 weeks, every 8 weeks during the subsequent 40 weeks and every 12 weeks thereafter. In this study, the definition of stable disease (SD) required a duration of ≥8 weeks. PFS was defined as the time from the date of study enrollment until the date of objectively determined progressive disease (PD) or death due to any cause or the date of the last follow-up. OS was defined as the time from the date of study enrollment until death due to any cause or the date of last follow-up. Toxicity was evaluated using the Common Toxicity Criteria for Adverse Events (version 3.0).

Statistical analysis. The primary endpoint was the ORR and the sample size for the trial was calculated using Simon's two-stage design. Assuming that a response rate of 18.0% indicates potential usefulness, while a rate of 6.8% is the lower limit of interest, with α=0.05 and β=0.20, the estimated accrual number was 49 patients. In this study, the rate of the lower limit of interest was adopted based on the ORRs of docetaxel reported in previous phase III studies (14,15). Among these, ≥7 responders were required for this therapy to be considered worthy of further evaluation. We selected a target sample number of 54, to allow for 5 dropouts. The differences in ORR according to histology were analyzed using the Mantel extension test adjusted for PS and M factor (M0, M1a and M1b). A stratified log-rank test adjusted for these factors was used to evaluate the difference in PFS according to histology. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. Between February, 2010 and April, 2012, a total of 55 patients were enrolled. A review of the data indicated that 2 of the patients enrolled in this study did not fulfill the eligibility criteria listed in the study protocol and the remaining 53 patients were included in the analysis as evaluable. The characteristics of the 53 patients are summarized in Table I. The median age of the patients was 67 years (range, 47-77 years). The histological subtypes were non-squamous cell carcinoma (non-SCC) in 44 patients [adenocarcinoma, 40 patients; and not otherwise specified (NOS), 4 patients] and SCC in 9 patients. The number of prior chemotherapies was 1 in 26 patients (49.0%) and 2 in the remaining 27 patients (51.0%).

Table I. Patient characteristics.

Characteristics	Patient no. (%) (n=53)
Age, years	
Median	67
Range	47-77
Gender	
Male	43 (81.0)
Female	10 (19.0)
Smoking status	
Never	7 (13.0)
Former/current	46 (87.0)
Histology	
Adenocarcinoma	40 (75.0)
Squamous cell carcinoma	9 (17.0)
NOS	4 (8.0)
No. of prior chemotherapies	
1	26 (49.0)
2	27 (51.0)
Stage	
IIIB	2 (4.0)
IV	
M1a	16 (30.0)
M1b	35 (66.0)
ECOG PS	
0	23 (43.4)
1	24 (45.3)
2	6 (11.3)

NOS, not otherwise specified; ECOG PS, Eastern Cooperative Oncology Group performance status.

Efficacy. The median treatment duration was 51 days (range, 5-404 days). Of the 53 eligible patients, partial response (PR) was obtained in 6 patients (4 with adenocarcinoma and 2 with SCC), yielding an ORR of 11.3% (95% confidence interval (CI): 4.3-23.0). SD was observed in 9 patients and the DCR was 28.3% (95% CI: 16.8-42.3). The ORR according to the histology was 9.1% (95% CI: 2.5-21.7) in patients with non-SCC and 22.2% (95% CI: 2.8-60.0) in patients with SCC. The difference in the ORR between these two groups was not statistically significant (P=0.29, Mantel extension test). A summary of the tumor responses is provided in Table II. At the time of the analysis, 48 patients (91.0%) had developed disease progression and 34 (64.0%) had succumbed to the disease. The median PFS of the entire patient cohort was 1.8 months (95% CI: 1.2-2.3). The median PFS in the patients with non-SCC and SCC was 1.7 months (95% CI: 1.2-2.1), and 2.2 months (95% CI: 1.0-11.3), respectively, without a statistically significant difference (P=0.54, stratified log-rank test). The Kaplan-Meier survival curve for PFS is shown in Fig. 1. The median OS was 6.4 months (95% CI: 4.5-10.4) and the

Table II. Tumor response.

Type of response	Total (n=53)	Non-SCC (n=44)	SCC (n=9)
CR	0	0	0
PR	6	4	2
SD	9	7	2
PD	37	32	5
NE	1	1	0
ORR, % (95% CI)	11.3 (4.3-23.0)	9.1 (2.5-21.7)	22.2 (2.8-60.0)
DCR, % (95% CI)	28.3 (16.8-42.3)	25.0 (13.2–40.3)	44.4 (13.7-78.8)

SCC, squamous cell carcinoma; non-SCC, adenocarcinoma and not otherwise specified non-small-cell lung cancer; CR, complete response; PR, partial response; SD, stable disease (a duration of ≥8 weeks was required for the definition of SD in this study); PD, progressive disease; NE, not evaluable; ORR, objective response rate; CI, confidence interval; DCR, disease control rate.

Table III. Adverse events in the patients (n=53).

Adverse events	Grade (patient no.) 1	2	3	4	% of patients with grade 3-4 toxicity
Skin rash	11	26	6	0	11.3
Diarrhea	16	1	1	0	1.9
Anorexia	14	6	5	0	9.4
Nausea	4	2	0	0	0.0
Vomiting	1	1	1	0	1.9
Fatigue	8	7	3	0	5.7
Stomatitis	10	2	1	0	1.9
Ocular disorders	2	0	1	0	1.9
ALT increased	7	4	1	2	5.7
AST increased	10	4	1	2	5.7
Amy increased	0	1	0	1	1.9
Leukopenia	1	1	0	0	0.0
Thrombocytopenia	6	0	0	0	0.0
ILD	2	0	1	0	5.6[a] (G3-5)

[a]Grade 5 ILD was observed in 2 patients. ALT, alanine transaminase; AST, aspartate transaminase; amy, amylase; ILD, interstitial lung disease.

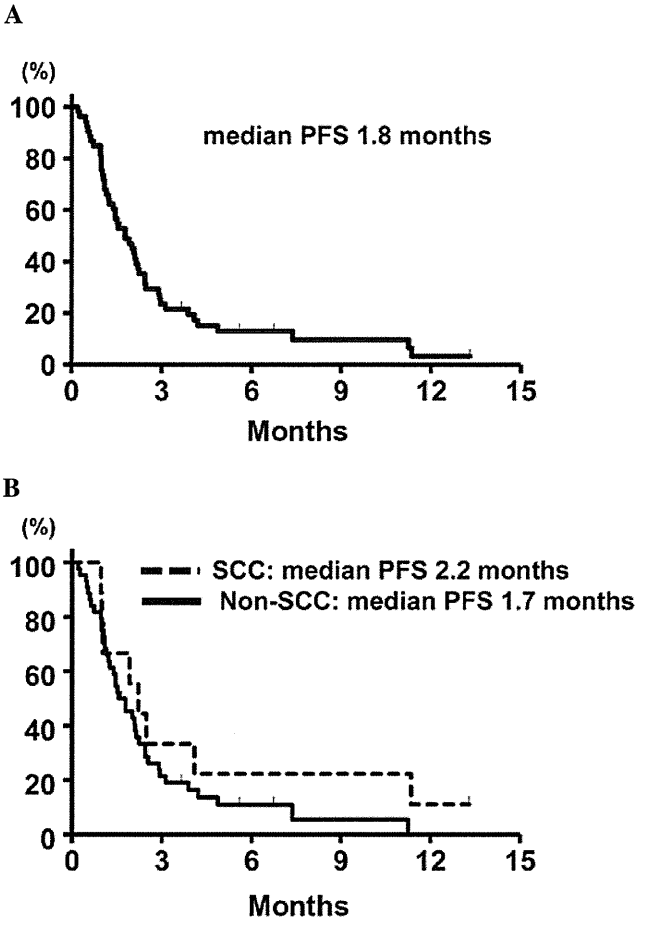

Figure 1. Kaplan-Meier survival curves for (A) progression-free survival (PFS) in the overall study population (n=53) and (B) progression-free survival in subgroups classified according to histology [squamous cell carcinoma (SCC) (n=9) vs. non-SCC (n=44)].

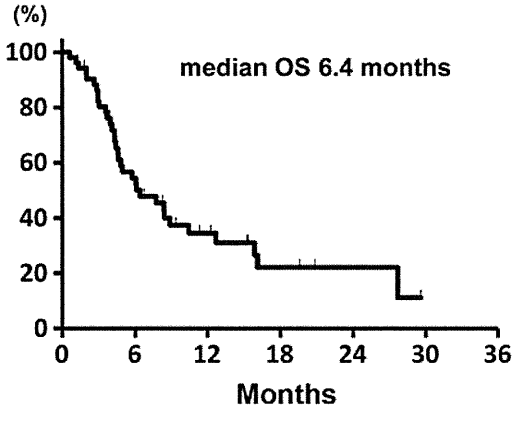

Figure 2. Kaplan-Meier survival curves for overall survival (OS) of the overall study population (n=53).

Kaplan-Meier survival curve for OS is shown in Fig. 2. The median OS in the patients with 1 and 2 prior chemotherapies was 8.5 and 5.5 months, respectively.

Safety. The adverse events are summarized in Table III. The major adverse events were rash in 81.1% of the patients (11.3% ≥grade 3) and anorexia in 47.1% (9.4% ≥grade 3). No grade 3 or 4 hematological adverse events were observed. Grade 3-5 ILD was reported in 3 patients (5.6%) and grade 5 ILD possibly related to erlotinib in 2 patients (3.8%). In the 2 patients with grade 5 ILD, the baseline chest CT revealed carcinomatous lymphangitis and lung cancer progression was concurrently detected by chest CT at the time of development of the ILD.

EGFR mutation reanalysis with the S-ARMS assay and KRAS mutation screening. Samples from 26 patients (49% of the

Table IV. KRAS mutation-positive patients.

Case	Gender	Smoking status	Smoking index	Amino acid change	Best overall response
1	Male	Former	1020	Gly12Ala (GGT>GCT)	PD
2	Male	Current	1020	Gly12Cys (GGT>TGT)	PD
3	Male	Current	1000	Gly12Ala (GGT>GCT)	PD
4	Male	Former	1520	Gly12Cys (GGT>TGT)	PD

Gly, glycine; Ala, alanine; Cys, cysteine; PD, progressive disease.

eligible patients) were available for EGFR mutation reanalysis. Of these, only 1 patient with adenocarcinoma was found to be EGFR mutation-positive (exon 19 deletion) NSCLC with the S-ARMS assay and this patient exhibited a PR. In the remaining 25 patients, EGFR WT was reconfirmed by the S-ARMS assay and two of these patients exhibited a PR. The ORR was 8.0% in the NSCLC patients with EGFR WT as confirmed by both the PNA-LNA PCR clamp method and the S-ARMS assay.

The KRAS mutation status was screened by the S-ARMS assay in samples obtained from 44 patients, of which DNA amplification was unsuccessful in 2. KRAS mutation screening was successfully performed in the samples from the remaining 42 patients (79.0% of eligible patients). Of these 42 patients, 4 (9.1%) were found to be KRAS mutation-positive. The characteristics of these 4 patients and the sites of the KRAS mutations are listed in Table IV. As regards treatment response, PD was observed in all 4 patients. By contrast, the ORR and median PFS in the patients with KRAS WT NSCLC were 6.9% and 1.9 months, respectively.

Discussion

In this study, we evaluated the efficacy and safety of erlotinib in pretreated patients with NSCLC harboring EGFR WT as confirmed by the PNA-LNA clamp method, which is reported as being highly sensitive. This study did not meet the primary endpoint based on the reported ORRs of docetaxel in previous studies, although erlotinib treatment was associated with an ORR of 11.3%.

Two recent phase III studies reported the inferiority of erlotinib compared to docetaxel regarding ORR and PFS in EGFR WT NSCLC patients (16,17). Based on these results, including the findings of our study, it appears that docetaxel should be preferred as second-line therapy, if not used as a part of first-line platinum based combination therapy.

However, there remains the clinical question of whether erlotinib should not be used for EGFR WT NSCLC in any-line setting. In our opinion, erlotinib monotherapy may be an viable option in pretreated patients with EGFR WT NSCLC following failure of docetaxel treatment for the following reasons: First, EGFR WT was reconfirmed by the S-ARMS assay in 25 of the 26 patient samples examined in this study, of which 2 (8.0%) achieved a PR. Our results suggested that erlotinib may still be effective against EGFR WT NSCLC, even when the EGFR mutation status is confirmed by two different highly sensitive methods.

Second, a discordance in the EGFR mutation status between the PNA-LNA clamp method and S-ARMS assay was observed in 1 patient in this study. Although large, tumor cell-rich samples are required for accurate EGFR mutation analysis, we cannot, in general, obtain surgically resected specimens from advanced NSCLC patients in clinical practice. Fukui *et al* (18) verified the accuracy of the EGFR mutation analysis in small samples by high-resolution melting analysis, which has also been reported to be a highly sensitive method. In that study, the results of DNA sequencing combined with laser capture microdissection in paired surgically resected specimens revealed a few false-negative results in small samples. Those data suggested that it may be difficult to determine the EGFR mutation status with complete accuracy in small tissue samples, irrespective of the sensitivity of the method used. Therefore, if we do not use erlotinib for EGFR WT NSCLC in any-line setting, we may miss the opportunity to attempt elrotinib treatment for patients with a false-negative EGFR mutation result. This may also lead to loss of the significant survival benefit obtained from EGFR-TKI therapy for EGFR mutation-positive NSCLCs.

We succeeded in obtaining 42 samples (79% of the eligible patients) for KRAS mutation screening. KRAS mutations were detected in 4 of the 42 patients screened (9.5%) and all the KRAS mutation-positive patients exhibited PD. In a phase III study conducted to compare erlotinib and pemetrexed, none of the patients with KRAS mutation-positive NSCLC responded to erlotinib treatment, which was similar to the findings of our study (19). These results should be interpreted with caution, as we could not exclude the KRAS mutation status as a potential prognostic factor. However, the presence of KRAS mutation may be useful as a negative predictive factor, at least regarding response to erlotinib therapy, in patients with EGFR WT NSCLC.

We performed a subgroup analysis according to histological subtype. In patients with EGFR mutated NSCLC, the efficacy of EGFR-TKIs for SCC appeared to be lower compared to that for non-SCC (20). However, SCC histology may not be associated with poor efficacy of erlotinib in patients with EGFR WT NSCLC based on our results. Molecular biomarkers, such as KRAS, may be required to select suitable candidates for erlotinib treatment among patients with EGFR WT NSCLC.

The toxicity profile of erlotinib in this study, in terms of the incidence/grade of skin rash, diarrhea and hematological toxicities, was consistent with previous reports. However, grade 3-5 ILD was reported in 3 patients (5.8%). In a large-scale surveillance study conducted in Japan, the incidence of ILD

was also higher compared to that reported by the BR21 and SATURN trials (1,7,21). Further studies are required to determine whether there are ethnic differences in the incidence of ILD, as suggested by a previous study (22).

In conclusion, this study did not meet the primary endpoint, although erlotinib was found to be moderately effective in pre-treated patients with EGFR WT NSCLC, even when the EGFR mutational status was confirmed by the highly sensitive PNA-LNA clamp PCR method.

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References

1. Shepherd FA, Rodrigues Pereira J, *et al*; National Cancer Institute of Canada Clinical Trials Group: Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 353: 123-132, 2005.
2. Paez JG, Janne PA, Lee JC, *et al*: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304: 1497-1500, 2004.
3. Maemondo M, Inoue A, Kobayashi K, *et al*: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362: 2380-2388, 2010.
4. Mitsudomi T, Morita S, Yatabe Y, *et al*: Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 11: 121-128, 2010.
5. Zhou C, Wu YL, Chen G, *et al*: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 12: 735-742, 2011.
6. Rosell R, Carcereny E, Gervais R, *et al*: Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 13: 239-246, 2012.
7. Cappuzzo F, Ciuleanu T, Stelmakh L, *et al*: Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. Lancet Oncol 11: 521-529, 2010.
8. Nagai Y, Miyazawa H, Huqun, *et al*: Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. Cancer Res 65: 7276-7282, 2005.
9. Kimura H, Fujiwara Y, Sone T, *et al*: High sensitivity detection of epidermal growth factor receptor mutations in the pleural effusion of non-small cell lung cancer patients. Cancer Sci 97: 642-648, 2006.
10. Kim HJ, Lee KY, Kim YC, *et al*: Detection and comparison of peptide nucleic acid-mediated real-time polymerase chain reaction clamping and direct gene sequencing for epidermal growth factor receptor mutations in patients with non-small cell lung cancer. Lung Cancer 75: 321-325, 2012.
11. Linardou H, Dahabreh IJ, Kanaloupiti D, *et al*: Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol 9: 962-972, 2008.
12. Eisenhauer EA, Therasse P, Bogaerts J, *et al*: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
13. Goto K, Satouchi M, Ishii G, *et al*: An evaluation study of EGFR mutation tests utilized for non-small-cell lung cancer in the diagnostic setting. Ann Oncol 23: 2914-2919, 2012.
14. Fossella FV, DeVore R, Kerr RN, *et al*: Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. The TAX 320 Non-Small Cell Lung Cancer Study Group. J Clin Oncol 18: 2354-2362, 2000.
15. Shepherd FA, Dancey J, Ramlau R, *et al*: Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 18: 2095-2103, 2000.
16. Garassino MC, Martelli O, Broggini M, *et al*; TAILOR trialists: Erlotinib versus docetaxel as second-line treatment of patients with advanced non-small-cell lung cancer and wild-type EGFR tumours (TAILOR): a randomised controlled trial. Lancet Oncol 14: 981-988, 2013.
17. Kawaguchi T, Ando M, Asami K, *et al*: Randomized phase III trial of erlotinib versus docetaxel as second- or third-line therapy in patients with advanced non-small cell lung cancer : Docetaxel and Erlotinib Lung Cancer Trial (DELTA). J Clin Oncol: May 19, 2014 (Epub ahead of print).
18. Fukui T, Ohe Y, Tsuta K, *et al*: Prospective study of the accuracy of EGFR mutational analysis by high-resolution melting analysis in small samples obtained from patients with non-small cell lung cancer. Clin Cancer Res 14: 4751-4757, 2008.
19. Karampeazis A, Voutsina A, Souglakos J, *et al*: Pemetrexed versus erlotinib in pretreated patients with advanced non-small cell lung cancer: a Hellenic Oncology Research Group (HORG) randomized phase 3 study. Cancer 119: 2754-2764, 2013.
20. Hata A, Katakami N, Yoshioka H, *et al*: How sensitive are epidermal growth factor receptor-tyrosine kinase inhibitors for squamous cell carcinoma of the lung harboring EGFR gene-sensitive mutations? J Thorac Oncol 8: 89-95, 2013.
21. Nakagawa K, Kudoh S, Ohe Y, *et al*: Postmarketing surveillance study of erlotinib in Japanese patients with non-small-cell lung cancer (NSCLC): an interim analysis of 3488 patients (POLARSTAR). J Thorac Oncol 7: 1296-1303, 2012.
22. Yoshioka H, Hotta K, Kiura K, *et al*; Okayama Lung Cancer Study Group: A phase II trial of erlotinib monotherapy in pretreated patients with advanced non-small cell lung cancer who do not possess active EGFR mutations: Okayama Lung Cancer Study Group trial 0705. J Thorac Oncol 5: 99-104, 2010.

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ORIGINAL ARTICLE

First-line gefitinib therapy for elderly patients with non-small cell lung cancer harboring EGFR mutation: Central Japan Lung Study Group 0901

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Abstract
Background The population of elderly patients with lung cancer is increasing worldwide. Although first-line gefitinib is one of the standard treatments for advanced non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR) mutation, few data have been reported regarding gefitinib and elderly patients.
Patients and methods Chemotherapy-naïve patients aged 70 years or older with stage IIIB or IV NSCLC harboring EGFR-activating mutation were enrolled and treated with 250 mg of gefitinib daily until disease progression. The primary end point was response rate, and secondary end points were survival, safety, and quality of life.
Results Twenty patients were enrolled, and the median age was 79.5 years (range 72–90). Overall response rate was 70 % (95 % CI 45.7–88.1 %), and the disease control rate was 90 % (95 % CI 68.3–98.7 %). The median progression-free survival and overall survival time were 10.0 and 26.4 months, respectively. The Functional Assessment of Cancer Therapy-Lung Cancer Subscale (FACT-LCS) scores improved significantly 4 weeks after the initiation of gefitinib ($P = 0.037$) and maintained favorably over a 12-week assessment period. Among the seven items of FACT-LCS, shortness of breath and cough improved

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significantly after 4 weeks of treatment ($P = 0.046$ and $P = 0.008$, respectively). The most common adverse events were rash and liver dysfunction. Although Grade 1 pneumonitis developed in one patient, no treatment-related death was observed.

Conclusion First-line gefitinib therapy is effective and feasible for elderly patients harboring EGFR mutation, and improves disease-related symptoms, especially pulmonary symptoms like shortness of breath and cough.

Keywords Non-small cell lung cancer · EGFR mutation · Elderly · Gefitinib · Quality of life · First-line treatment

Introduction

Lung cancer is the leading cause of cancer mortality. Non-small cell lung cancer (NSCLC) accounts for 85 % of lung cancer cases, with at least 40 % of the patients at an advanced stage. The population of elderly patients with lung cancer is increasing worldwide. Two-thirds of the lung cancer cases are diagnosed in patients over the age of 65, and the median age at diagnosis is 70 years [1, 2].

Aging is associated with physiologic changes in organ function and altered drug pharmacokinetics. Furthermore, the presence of comorbidities and polypharmacy is frequent in elderly populations. Elderly patients are more likely to experience severe hematologic and non-hematologic toxicity from conventional chemotherapy than their younger counterparts [3]. Before the discovery of driver mutations including epidermal growth factor receptor (EGFR) mutation, single-agent chemotherapy was considered to be a standard of care for elderly patients with advanced NSCLC [4–6]. Although carboplatin and weekly paclitaxel doublet chemotherapy improved overall survival compared with vinorelbine or gemcitabine monotherapy in the IFCT-0501 trial, accompanying toxicity such as Grade 3 or Grade 4 neutropenia, febrile neutropenia, and asthenia was more frequent in the doublet chemotherapy arm [7]. Therefore, investigations of effective treatments with less toxicity are needed for this population.

Gefitinib is an orally administered EGFR tyrosine kinase inhibitor (TKI) that blocks signal transduction pathways implicated in the proliferation and survival of cancer cells. Since EGFR somatic mutation was reported to be strongly related to the response of EGFR-TKI therapy, several studies have demonstrated the efficacy of gefitinib for NSCLC harboring EGFR-activating mutation [8–11]. Two phase III studies comparing gefitinib with platinum doublet chemotherapy as a first-line treatment for NSCLC patients with EGFR mutation showed that the gefitinib group had a higher response and longer progression-free survival than a standard chemotherapy group [12, 13]. However, these studies targeted patients aged 75 years or younger, and few data were available on the efficacy and feasibility of first-line gefitinib therapy for elderly NSCLC patients with EGFR mutation. Therefore, we started our current study of this population. The present study included the assessment of quality of life (QOL) besides the efficacy and feasibility of treatment.

Patients and methods

Patient eligibility

Patients aged 70 years or older with a histologically or cytologically proven diagnosis of non-small cell lung cancer were eligible for this study. Other eligibility criteria included the following: EGFR-activating mutation (either exon 19 deletion or L858R in exon 21); measurable disease; stage IIIB/IV or postoperative recurrence; no prior therapy including chemotherapy or radiotherapy of the primary tumor; Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; an adequate organ function defined as leukocyte count $\geq$3,000/mm^3, platelet count $\geq$100,000/mm^3, hemoglobin $\geq$9.0 g/dl, aspartate aminotransferase and alanine aminotransferase $\leq$100 IU/l, total bilirubin $\leq$1.5 mg/dl, serum creatinine $\leq$1.5 mg/dl, and PaO_2 at rest $\geq$60 mmHg. Patients with any of the following criteria were ineligible: superior vena caval syndrome; history of serious drug allergy; massive pleural or pericardial effusion or ascites that required drainage; interstitial lung disease or pulmonary fibrosis detected by conventional computed tomography of the chest; symptomatic brain metastasis; other concurrent active malignancy; pregnancy, lactation, or other concomitant serious medical conditions. All patients gave written informed consent before enrollment. The study protocol was approved by each institutional review board and was carried out in accordance with the Declaration of Helsinki 1964 (as revised 2000).

Study design and treatment

This was a single-arm, prospective, multicenter, phase II trial. Patients were treated with 250 mg of oral gefitinib daily. Therapy was continued unless there was evidence of disease progression, unacceptable toxicity, or withdrawal of consent. If Grade 3 toxicity other than pneumonitis was observed, gefitinib was discontinued for a maximum of 4 weeks. After the toxicity recovered to the level of Grade 2, gefitinib was given every other day. If toxicity further improved, gefitinib was given daily. If Grade $\geq$1 pneumonitis or Grade 4 toxicity other than pneumonitis was observed, the patient was removed from the study.

Evaluation of response and toxicity

The pretreatment baseline evaluation included a complete medical history and physical examination, complete blood cell count, blood chemistry studies, computed tomography scan of the chest and abdomen, computed tomography or magnetic resonance imaging of the brain, bone scintigraphy or positron emission tomography, arterial blood gas analysis, pulmonary function tests, and electrocardiography. Tumor response was assessed every 2 months during the first year after enrollment and every 3 months between 12 and 18 months. Thereafter, the interval was at the physician's discretion.

The Response Evaluation Criteria in Solid Tumors (RECIST) were used for response assessment [14]. Disease control rate (DCR) was defined as the rate of complete response (CR) plus partial response (PR) plus stable disease (SD). An extramural review was conducted to validate staging and response. Toxicity was evaluated according to the National Cancer Institute Common Terminology Criteria (version 3.0).

Quality of life (QOL) was assessed with the Functional Assessment of Cancer Therapy-Lung Cancer Subscale (FACT-LCS) questionnaire version 4. The maximum attainable score on the FACT-LCS was 28, with which the patient was considered to be asymptomatic. Patients were asked to complete the FACT-LCS questionnaire at the time of enrollment and at 4, 8, and 12 weeks after the initiation of treatment.

Mutational analysis of EGFR

Epidermal growth factor receptor (EGFR) genetic testing methods included either direct sequencing, PCR invader, peptide nucleic acid-locked nucleic acid PCR clamp, or the combination of fragment analysis and the Cycleave method.

Statistical analyses

The primary end point of this study was the response rate. We calculated the sample size based on Simon's two-stage design of the phase II study [15]. Assuming that a response rate of 60 % from eligible patients would indicate potential usefulness, and that a rate of 30 % would be the lower limit of interest (with a power of 0.8 at a one-sided significance level of 0.05), accrual of 17 eligible patients was required. Therefore, we planned to accrue a total of 19 patients, assuming there would be a 10 % dropout rate. The duration of survival was measured from the day of enrollment, and the overall survival curve and progression-free survival curve were calculated according to the method of Kaplan and Meier [16]. Repeated-measures analysis of variance was used to assess the differences in the FACT-LCS between baseline and each point during the treatment. Comparisons of the FACT-LCS scores with the baseline scores were adjusted for multiple comparisons using the Dunnett–Hsu test. The software SAS/Proc Mixed version 9.2 (SAS Institute Inc., Cary, NC) was used for statistical analysis. All comparisons were two-sided, and the statistical significance level was set at $P < 0.05$.

Results

Patient characteristics

Between April 2009 and March 2011, 20 patients were enrolled in this study. Sixteen patients (80 %) were aged 75 years or older, and the median age was 79.5 years (range 72–90 years old) (Table 1). All of the 20 patients had adenocarcinoma, 13 (65 %) were female, two (10 %) had an ECOG performance status of 2, and 12 (60 %) had exon 19 deletion mutations.

Tumor responses and survival

Overall response rate was 70 % (95 % CI 45.7–88.1 %), and the disease control rate was 90 % (95 % CI 68.3–98.7 %) (Table 2). Although the response of one patient who developed pneumonitis was not evaluable, progressive disease was observed in only one patient. The median progression-free survival and overall survival time were 10.0 and 26.4 months, respectively (Figs. 1, 2).

Table 1 Patient characteristics

Characteristics	$N = 20$	(%)
Age, years		
Median (range)	79.5 (72–90)	
Sex		
Male	7	35
Female	13	65
Smoking status		
Never smoker	14	70
Former/current smoker	6	30
ECOG performance status		
0	13	65
1	5	25
2	2	10
Stage		
IIIB	4	20
IV	15	75
Postoperative recurrence	1	5
Type of EGFR mutation		
Exon 19 deletion	12	60
L858R	8	40

ECOG Eastern Cooperative Oncology Group

Table 2 Response rate

Response	$N = 20$	% (95% CI)
Partial response	14	70
Stable disease	4	20
Progressive disease	1	5
Inevaluable	1	5
Overall response rate	14	70 % (45.7–88.1)
Disease control rate	18	90 % (68.3–98.7)

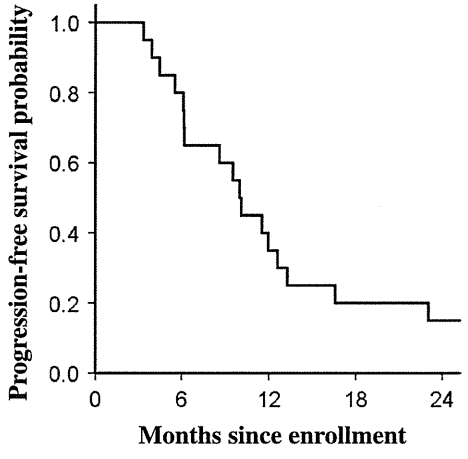

Fig. 1 Kaplan–Meier progression-free survival curve with gefitinib

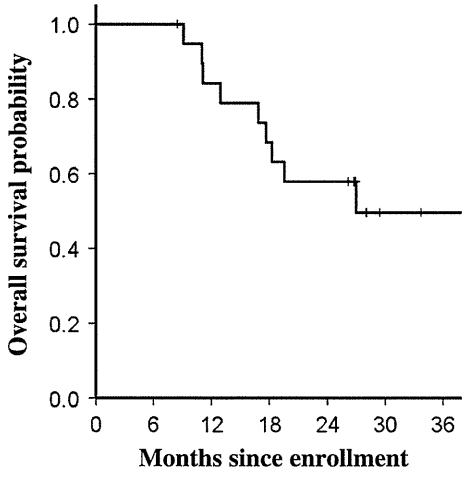

Fig. 2 Kaplan–Meier survival curve with gefitinib

Quality-of-life assessment

All 20 patients completed the FACT-LCS questionnaire at registration and after 4, 8, and 12 weeks of treatment. The adjusted mean FACT-LCS score was 22.8 ± 1.0 at baseline and 25.1 ± 0.7 at 4 weeks. The score improved

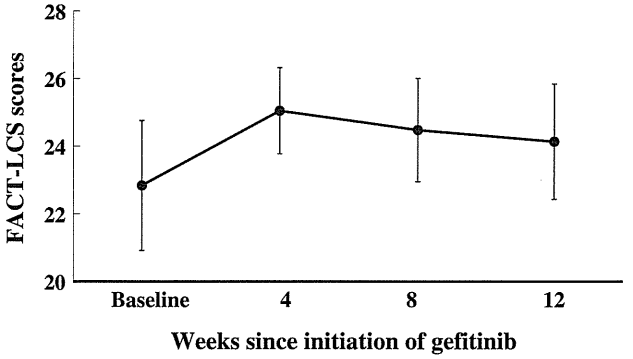

Fig. 3 FACT-LCS scores before treatment and at 4, 8, and 12 weeks after initiation of gefitinib. *Abbreviation FACT-LCS* Functional Assessment of Cancer Therapy-Lung Cancer Subscale

significantly at 4 weeks ($P = 0.037$) and maintained favorably during the 12-week assessment period (Fig. 3). FACT-LCS consisted of seven items: shortness of breath, cough, chest tightness, ease of breathing, changes in appetite, body weight loss, and disruptions to clear thinking. Among those seven items, shortness of breath and cough improved significantly after 4 weeks of treatment ($P = 0.046$ and $P = 0.008$, respectively).

Toxicity

Toxicity data for all 20 patients are listed in Table 3. Non-hematologic toxicity was the principal toxicity from gefitinib treatment and mainly consisted of liver dysfunction, skin rash, anorexia, diarrhea, and fatigue. Grade 3 or Grade 4 liver dysfunction occurred in 3 patients (15 %) but no other Grade 3 or Grade 4 toxicity was occurred. One case of Grade 1 pneumonitis developed in an 87-year-old woman. She had no specific symptoms; however, routine chest X-ray on day 14 showed an increase in density in the bilateral lower lung fields. Since subsequent chest computed tomography revealed bilateral diffuse interstitial opacities and the bronchoalveolar lavage findings were consistent

Table 3 Adverse events ($N = 20$)

	Grade 1	Grade 2	Grade 3	Grade 4	Grades 3–4
AST/ALT	8	4	2	1	3
Rash	8	10	0	0	0
Anorexia	8	2	0	0	0
Diarrhea	6	2	0	0	0
Fatigue	6	2	0	0	0
Mucositis	1	3	0	0	0
Nausea	3	0	0	0	0
Pneumonitis	1	0	0	0	0

AST aspartate aminotransferase, *ALT* alanine aminotransferase

with pneumonitis, gefitinib was discontinued and the treatment with oral prednisolone (0.5 mg/kg/day) was started. Although the pneumonitis was stable, pulmonary and brain metastases gradually progressed and she died of progression of lung cancer 6 months after the occurrence of this adverse event. No treatment-related death was observed.

Discussion

The present study evaluated the efficacy and feasibility of first-line gefitinib treatment for elderly patients harboring EGFR mutation, achieving the response rate of 70 % and disease control rate of 90 %. After we started this phase II study, three groups reported comparable results of response rates from 45.5 to 74 %, and progression-free survival of 9.7–12.9 months for similar populations [17–19]. Efficacy of the present study is also comparable to the results obtained from non-elderly phase III studies. Two prospective studies (WJTOG3405 and NEJ002) and subset analysis of EGFR-mutated patients in the IPASS showed response rates of 62.1–73.7 % and progression-free survival of 9.2–10.8 months [11–13, 20]. From these data, gefitinib treatment for elderly EGFR-mutated patients appears to be as effective as that for the younger population. A randomized trial of EGFR-TKI focusing on efficacy is needed to further improve survival of elderly patients.

We also revealed that disease-related symptoms improved significantly with gefitinib therapy. FACT-LCS score improved more than two points, which is considered a clinically meaningful change [21]. Although superior QOL results were reported with gefitinib versus chemotherapy in the IPASS and NEJ002 studies, the QOL benefit for the elderly population has not been reported [22, 23]. Among the seven items of FACT-LCS, shortness of breath and cough improved significantly. This finding is in accordance with two previous QOL analyses during gefitinib treatment. Cella et al. [24] found that more patients showed an improvement in the pulmonary items of FACT-LCS, such as shortness of breath, cough, or chest tightness than in the non-pulmonary items in the IDEAL2 study, which evaluated two doses of gefitinib for the mutation-unselected population. Oizumi et al. [23] reported that more patients showed an improvement in pain and shortness of breath in the gefitinib arm in the NEJ002 study. With regard to the speed of symptom improvement, our data demonstrated significant improvement at the first follow-up, namely at 4 weeks of treatment. A former analysis reported that the median time to symptom improvement was as immediate as 10 days with gefitinib [24]. In light of its rapid effect, gefitinib could be a good treatment option for patients suffering from pulmonary symptoms like cough or dyspnea.

Toxicity in the present study was generally mild and well tolerated. Grade 3 or Grade 4 adverse events were only in three cases of liver dysfunction. No unpredicted toxicity or treatment-related death was observed. On the other hand, a subgroup analysis of a phase III study of erlotinib treatment indicated that elderly patients experienced significantly more toxicity and tended to discontinue treatment more than their younger counterparts [25]. This difference may be partly explained by the difference in EGFR-TKIs. Gefitinib 250 mg is about one-third of the maximum tolerated dose, and erlotinib 150 mg is just the maximum tolerated dose [26, 27]. Accordingly, gefitinib may have some safety margin, especially for the frail population. In the present study, the oldest patient, aged 90 years, was able to continue gefitinib therapy for about 7 months with side effects no more severe than Grade 2 mucositis and Grade 2 rash.

Pneumonitis is one of the most serious adverse events related to EGFR-TKI therapy. In our previous study evaluating gefitinib in mutation-unselected elderly NSCLC patients, three out of 30 patients (10 %) had pneumonitis, two of them with a Grade $\geq$3 [28]. In the present study, Grade 1 pneumonitis developed in one patient (5 %). Since risk factors of pneumonitis include smoking, preexisting interstitial lung disease, and older age, careful monitoring is desirable for elderly patients [29, 30].

In conclusion, the present study revealed that first-line therapy with gefitinib is effective and feasible for elderly patients harboring EGFR mutation, and improves disease-related symptoms.

Conflict of interest Kosuke Takahashi, Hiroshi Saito, Yoshinori Hasegawa, Yasuteru Sugino, and Joe Shindoh received honoraria from AstraZeneca. Yoshinori Hasegawa received research funding for his institute from AstraZeneca.

References

1. Quoix E, Westeel V, Zalcman G, Milleron B (2011) Chemotherapy in elderly patients with advanced non-small cell lung cancer. Lung Cancer 74:364–368
2. Howlader N, Noone AM, Krapcho M, Garshell J, Neyman N, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds) (2013) SEER cancer statistics review, 1975–2010. National Cancer Institute. http://seer.cancer.gov/csr/1975_2010/, based on November 2012 SEER data submission, posted to the SEER web site, April 2013
3. Pallis AG, Karampeazis A, Vamvakas L, Vardakis N, Kotsakis A, Bozionelou V, Kalykaki A, Hatzidaki D, Mavroudis D, Georgoulias V (2011) Efficacy and treatment tolerance in older patients with NSCLC: a meta-analysis of five phase III randomized trials conducted by the Hellenic Oncology Research Group. Ann Oncol 22:2448–2455
4. The Elderly Lung Cancer Vinorelbine Italian Study Group (1999) Effects of vinorelbine on quality of life and survival of elderly