Original Article

The effectiveness of afatinib in patients with lung adenocarcinoma harboring complex epidermal growth factor receptor mutation

Shang-Gin Wu, Chong-Jen Yu, James Chih-Hsin Yang and Jin-Yuan Shih

Abstract

Background and aims: Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs) are effective against classical *EGFR* mutations in lung cancer. However, their effectiveness and the prognosis of lung cancer patients with complex *EGFR* mutations are not well delineated. Therefore, we aimed to investigate the treatment effectiveness of different EGFR TKIs in patients with complex *EGFR* mutations.

Patients and methods: From 2005 to 2020, we collected lung adenocarcinoma tissue samples for *EGFR* mutation analysis using direct Sanger sequencing. Patients with *EGFR* mutations treated with EGFR TKIs as first-line treatment were enrolled. Clinical characteristics, *EGFR* mutation status, treatment response, progression-free survival (PFS), and overall survival (OS) were analyzed.

Results: Among 2675 patients with *EGFR* mutations, 239 (8.9%) had complex *EGFR* mutations, of whom 125 received EGFR TKI treatment as first-line treatment. Multivariate analysis revealed that afatinib was a more favorable factor for PFS than gefitinib [hazard ratio (HR), 2.01; 95% confidence interval (CI), 1.11–3.62] and erlotinib (HR, 2.61; 95% CI, 1.31–5.22), especially in patients with uncommon mutation patterns. Afatinib treatment as first-line treatment was also associated with longer OS compared with erlotinib (HR, 2.48; 95% CI, 1.20–5.12). Classical mutation pattern was associated with longer PFS ($p = 0.001$) and OS ($p = 0.020$). Secondary T790M was detected in 22 of 52 (42.3%) patients who had re-biopsied tissue samples after acquiring resistance to EGFR TKIs. There was no significant difference in secondary T790M formation after acquired resistance to the three EGFR TKIs ($p = 0.261$). Furthermore, three (5.8%) patients had small-cell lung cancer transformation.

Conclusion: Afatinib is an effective first-line treatment for patients with lung adenocarcinoma harboring complex *EGFR* mutations, especially those with uncommon mutation patterns.

Keywords: afatinib, complex mutation, *EGFR* mutation, lung adenocarcinoma, progression-free survival

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Introduction

Lung cancer is the leading cause of cancer-related death worldwide.[1] Most patients have an advanced-stage disease or distant metastases at the initial diagnosis of lung cancer. The prognosis of lung cancer is poor, and the effectiveness of standard platinum-based chemotherapy is limited.[1] The advancement of targeted therapy provides a favorable survival benefit for patients with non-small cell lung cancer (NSCLC) harboring oncogenic driver mutations, such as *EGFR* and *B-Raf* mutations or *ALK* and *ROS-1* fusion genes.[2–6]

EGFR mutations are the most important predictive biomarkers of the effectiveness of tyrosine kinase inhibitors (TKIs) targeting the epidermal growth factor receptor (EGFR).[7] Deletion in exon 19 (del-19) and L858R point mutation in

exon 21 are the most common types of *EGFR* mutation and are classified as classical mutations.[8] Patients harboring tumors with these mutations have shown favorable treatment responses to EGFR TKIs.[7]

Besides classical mutations, approximately 10% of NSCLC patients with *EGFR* mutations have rare or uncommon mutations, including L861Q, G719X, and S768I. Such uncommon *EGFR* mutations are associated with a poorer prognosis and a less effective EGFR TKI treatment than classical mutations.[9] After a pooled analysis of three studies from the LUX-Lung clinical trial program (phase II LUX-Lung 2, phase III LUX-Lung 3, and phase III LUX-Lung 6), afatinib (a second-generation EGFR TKI) showed effectiveness for uncommon *EGFR* mutations based on the objective response rate, duration of response, disease control, progression-free survival (PFS), and overall survival (OS).[10] In 2018, afatinib was approved by the US Food and Drug Administration (FDA) for lung cancer with *EGFR* L861Q, G719X, and S768I mutations.[11]

Most *EGFR* mutations in NSCLC are single mutations, but sometimes a single tumor sample can have two or more different concomitant *EGFR* mutations, termed as "complex *EGFR* mutations".[12] The frequency of complex *EGFR* mutations is 3.2–6.0%.[13,14] The introduction of another mutation leads to a conformational change in the EGFR tyrosine kinase domain, affecting the affinity of the receptors and the treatment response to TKIs.[14,15] In our previous study, patients with complex classical *EGFR* mutations had the same response rate, PFS, and OS as those with single classical mutations.[16] Moreover, patients with complex *EGFR* mutations with the classical mutation pattern had a better response to gefitinib and longer PFS and OS than those without the classical mutation pattern.[16] However, it is unclear whether the different EGFR TKIs have the same effectiveness in patients with complex *EGFR* mutations.

Although there have been a few studies on complex *EGFR* mutations and EGFR TKIs, the sample sizes of these published studies are very small and the conclusions were inconsistent.[13,16] Especially, the mechanism of acquired resistance to EGFR TKIs remains unclear in patients with complex *EGFR* mutations. In this study, we aim to clarify the effectiveness of different EGFR-TKIs as the first-line therapy in lung adenocarcinoma patients with complex *EGFR* mutations.

Materials and methods

Patients and tissue procurement
This study was conducted in the National Taiwan University Hospital (NTUH) from June 2005 to March 2020. We enrolled patients if they had (1) a stage IV disease status or stage I–III lung adenocarcinoma with a subsequent systemic relapse, (2) tumors with complex *EGFR* mutations, and (3) first-line systemic treatment with first-generation (gefitinib or erlotinib) or second-generation (afatinib) EGFR TKIs. To evaluate the effectiveness of EGFR TKIs, patients who received concurrent radiotherapy or combined chemotherapy and EGFR TKI treatment were excluded from the subsequent analysis. The study was approved by the Institutional Review Board of the NTUH Research Ethics Committee (REC No. 201103013RC and 201111039RIC). All patients provided written informed consent for the collection of demographic and clinical outcome data and for molecular analysis before the collection of tissue specimens, including surgically resected lung tumors, bronchoscopy biopsy/brushing specimens, and malignant pleural effusions (MPEs) from thoracentesis. Some patients had been reported in our prior studies.[16–20]

The International Multidisciplinary Classification of Lung Adenocarcinoma, established by the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society, and European Respiratory Society, was adopted to confirm the histological classification of lung adenocarcinoma.[21] MPEs or ascites was confirmed by cytologists.

All patients underwent a complete lung cancer staging—including bronchoscopy; computed tomography (CT) of the head, chest, and abdomen; and a whole-body bone scintigraphy. The disease status was determined according to the seventh edition of the IASLC TNM staging system.[22] The patients' clinical data, including demographic information, smoking status, performance status, treatment regimens, and imaging studies, were reviewed. Patients who had smoked fewer than 100 cigarettes in their lifetime were categorized as never-smokers,[23] whereas those who had quit smoking for more than a year

were regarded as former smokers. The Eastern Cooperative Oncology Group performance status was also recorded.[24]

Evaluation of treatment response to EGFR TKIs in lung adenocarcinoma patients

The enrolled patients received single-agent EGFR TKIs daily as first-line systemic treatment, with no concurrent chemotherapy or radiotherapy for the primary lung tumor during the EGFR TKI treatment course. They underwent chest radiography every 2–4 weeks and CT of the chest (including the liver and adrenal glands) every 2–3 months as routine clinical practice and as needed for the evaluation of treatment response. The treatment response was evaluated using the Response Evaluation Criteria in Solid Tumors guidelines (version 1.1).[25] The objective responses were defined as complete response, partial response (PR), stable disease (SD), and progressive disease.[25] Acquired resistance was defined as objective treatment response, including a PR to EGFR TKI treatment or durable SD (PFS, ≥6 months), followed by disease progression. OS was calculated as the duration from the start of the first-line systemic treatment for a metastatic or disease relapse status until death from any cause. PFS was defined as the duration from the initiation of EGFR TKI treatment to disease progression or death, whichever occurred first.

EGFR mutation analysis

RNA was extracted from tissue specimens, including lung tumors, metastatic sites, and MPEs. The quantity and quality of the extracted RNA were measured by spectrophotometry. Tissue specimens were processed for *EGFR* mutation analysis as described previously.[26,27] Exons 18–21 of the *EGFR* gene were amplified by reverse transcription-polymerase chain reaction (RT-PCR) using the QIAGEN OneStep RT-PCR Kit (QIAGEN, Hilden, Germany), as described previously.[26,28]

Complex EGFR mutations with classical and uncommon mutation patterns

The mutations were defined as complex *EGFR* mutations when two or more different concomitant *EGFR* mutations were detected, as in our previous study.[16] Complex *EGFR* mutations that contained either a del-19 or L858R mutation were defined as having the "classical mutation pattern", whereas those without a del-19 or L858R mutation were defined as having the "uncommon mutation pattern".

Statistical analysis

All categorical variables were analyzed using the chi-squared test, whereas Fisher's exact test was used for small study sizes (<5). The median ages between groups were compared using the non-parametric Mann–Whitney *U* test. PFS and OS curves were plotted using the Kaplan–Meier method and compared using the log-rank test. Two-sided *p*-values of <0.05 were considered significant. Multivariate analysis was performed using the Cox regression model to evaluate the predictive factors of PFS and the potential prognostic factors of OS. All analyses were performed using SPSS software (version 17.0 for Windows; SPSS Inc., Chicago, Illinois, USA).

Results

Clinical characteristics of lung adenocarcinoma patients with complex EGFR mutations

From June 2005 to March 2020, we consecutively collected lung adenocarcinoma specimens. There were 4714 patients who had adequate tissue samples for *EGFR* mutation analysis, and 2675 patients (mutation rate, 56.7%) had tumors harboring *EGFR* mutations. Among the 2675 patients, there were 239 (8.9%) patients who had complex *EGFR* mutations, including 48 patients with *de novo* T790M of *EGFR* mutations (Figure 1). The detail of these complex *EGFR* mutations other than *de novo* T790M is shown in Supplemental Material Table 1 and Supplemental Figure 1 online. L858R was the major component of the complex *EGFR* mutations. We did not detect patients with complex *EGFR* mutations that contained exon 20 insertion in this cohort whether the patients took EGFR TKIs or not.

To evaluate the effectiveness of EGFR TKIs, we excluded patients with tumors harboring *de novo* T790M mutations due to intrinsic resistance to first- and second-generation EGFR TKIs.[29,30] Only patients who received EGFR TKIs as first-line treatment were enrolled to reduce the treatment heterogenicity and potential chemotherapy impacts. We also excluded three patients who received local curative radiotherapy as a combination therapy with EGFR TKI treatment and one patient with basal cell carcinoma who received systemic treatment. Finally, we enrolled 125 lung
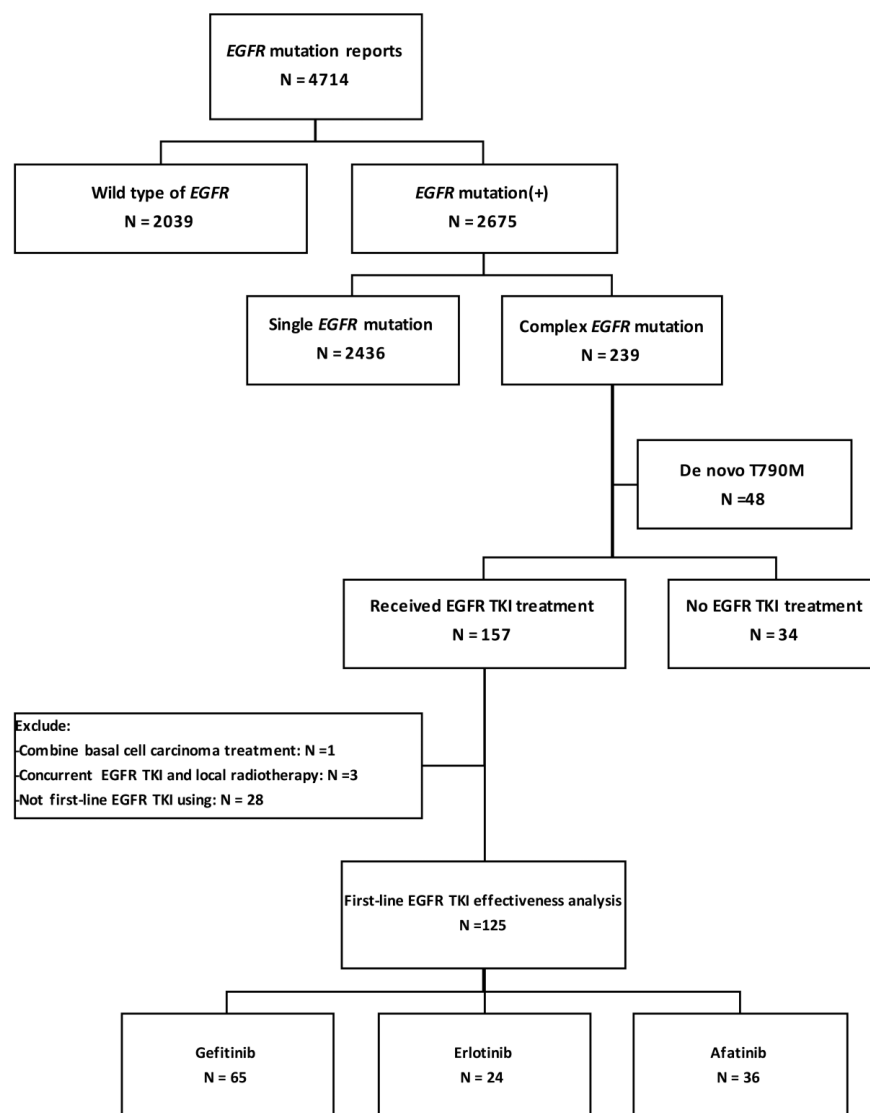
Figure 1. Flowchart for patient collection.
EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

adenocarcinoma patients with complex *EGFR* mutations who took EGFR TKIs as first-line treatment, to evaluate the effectiveness of the drugs.

Table 1 summarizes the clinical characteristics of the 125 patients who received EGFR TKIs as first-line treatment, including 65 (52.0%) gefitinib, 24 (19.2%) erlotinib, and 36 (28.8%) afatinib. The median age at diagnosis was 65.5 (range: 27.5–88.1) years old. Among them, 83 (66.4%) were female, 102 (81.6%) were never-smokers, and 83 (66.4%) had the classical mutation pattern. The proportions of the complex *EGFR* mutations are shown by Figure 2 and Supplemental Table 2. Of them, there were 68 patients (54.4%) with complex *EGFR* mutations

that contained L858R. There were no significant differences in age, smoking history, Eastern Cooperative Oncology Group performance status, disease stage [relapse, stage IV (M1a) and stage IV (M1b)] between patients treated with the different EGFR TKIs as first-line treatment. The erlotinib-treated patients had more male, and afatinib-treated patients had more patients with uncommon mutation pattern.

Treatment response to EGFR TKIs in lung adenocarcinoma patients with complex EGFR mutations

Among the 125 patients, there was no significant difference in the maximum treatment response

Table 1. Clinical characteristics of lung adenocarcinoma patients with complex *EGFR* mutations treated with EGFR TKIs as first-line systemic treatment.

	All patients	Gefitinib	Erlotinib	Afatinib	*p*
Total	125	65 (52.0%)	24 (19.2%)	36 (28.8%)	
Age, median, years (range)	65.5 (27.5–88.1)	65.5 (27.5–88.1)	63.6 (43.8–85.3)	68.7 (43.0–86.1)	0.523§
Sex					0.050
Female	83	45 (69.2%)	11 (45.8%)	27 (75.0%)	
Male	42	20 (30.8%)	13 (54.2%)	9 (25.0%)	
Smoking status					0.209
Never-smokers	102	53 (81.5%)	17 (70.8%)	32 (88.9%)	
Smokers	23	12 (18.5%)	7 (29.2%)	4 (11.1%)	
ECOG PS					0.538
0–1	104	53 (81.5%)	19 (79.2%)	32 (88.9%)	
2–4	21	12 (18.5%)	5 (20.8%)	4 (11.1%)	
Stage					0.747
Relapse†	23	14 (21.5%)	5 (20.8%)	4 (11.1%)	
IV (M1a)	30	15 (23.1%)	5 (20.8%)	10 (27.8%)	
IV (M1b)	72	36 (55.4%)	14 (58.3%)	22 (61.1%)	
Classical mutation type					0.013
Yes	83	47 (72.3%)	19 (79.2%)	17 (47.2%)	
No	42	18 (27.7%)	5 (20.8%)	19 (52.8%)	
Post-TKI chemotherapy					0.240
Yes	78	39 (60.0%)	18 (75.0%)	21 (58.3%)	
No	35	22 (33.8%)	4 (16.7%)	9 (25.0%)	
Still on TKI treatment	12	4 (6.2%)	2 (8.3%)	6 (16.7%)	

§By Kruskal–Wallis test.
†Patients with stages I–III with systemic relapse after definitive surgery.
ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor gene; EGRF TKI, epidermal growth factor receptor tyrosine kinase inhibitor

rate between the three EGFR TKIs [gefitinib *versus* erlotinib *versus* afatinib: 70.8% (46 of 65) *versus* 62.5% (15 of 24) *versus* 83.3% (30 of 36); *p* = 0.179] (Supplemental Table 3). In addition, patients with the classical mutation pattern had a higher treatment response rate than those with the uncommon mutation pattern [81.9% (68 of 83) *versus* 54.8% (23 of 42); *p* = 0.001].

Effectiveness and survival analysis of different EGFR TKIs in patients with complex EGFR mutations

To evaluate the effectiveness of the three EGFR TKIs, the PFS and OS of the patients who received the different EGFR TKIs as first-line treatment were compared. There was no significant difference in PFS between the three drugs
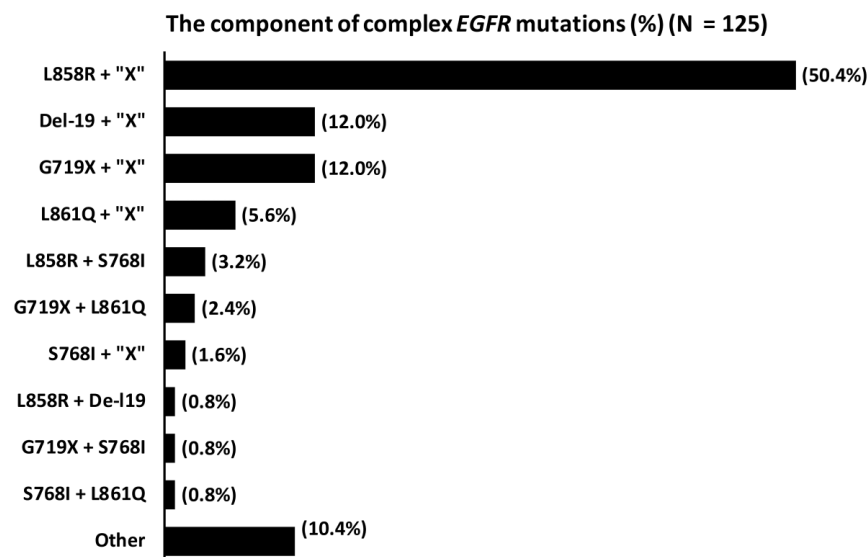
The component of complex *EGFR* mutations (%) (N = 125)

Figure 2. The proportion of different mutation components of complex *EGFR* mutations in patients who received EGFR TKIs as first-line treatment (*n* = 125). [del-19, deletion in exon 19; "X", EGFR mutation other than common (L858R and del-19) and uncommon mutations (G719X, S768I, and L861Q)].
EGFR, epidermal growth factor receptor; *EGFR*, epidermal growth factor receptor gene; TKI, tyrosine kinase inhibitor.

(gefitinib *versus* erlotinib *versus* afatinib: 7.4 months *versus* 8.1 months *versus* 9.7 months; *p* = 0.264) [Figure 3(A)]. There was also no significant difference in OS between the three drugs (gefitinib *versus* erlotinib *versus* afatinib: 18.4 months *versus* 13.3 months *versus* 26.4 months; *p* = 0.101) [Figure 3(B)].

To clarify the interaction between complex *EGFR* mutation patterns and treatment responses to EGFR TKIs, patients were stratified according to the classical and the uncommon mutation patterns. For patients with the classical mutation pattern, there were no significant differences in treatment response to the three EGFR TKIs [gefitinib *versus* erlotinib *versus* afatinib: 83.0% (39 of 47) *versus* 73.7% (14 of 19) *versus* 88.2% (15 of 17); *p* = 0.506] and in PFS (gefitinib *versus* erlotinib *versus* afatinib: 10.9 months *versus* 8.5 months *versus* 9.6 months; *p* = 0.385) [Figure 4(A)].

For patients with the uncommon mutation pattern, the second-generation EGFR TKI (afatinib) had a higher response rate [78.9% (15 of 19)] than the two first-generation EGFR TKIs [gefitinib: 38.9% (7 of 18); erlotinib: 20.0% (1 of 5)] (*p* = 0.013). There were significant differences in PFS between the three EGFR TKIs (gefitinib *versus* erlotinib *versus* afatinib: 3.0 months *versus* 0.9 months *versus* 10.5 months; *p* = 0.013). Furthermore, afatinib was associated with longer

PFS than gefitinib (*p* = 0.011) and erlotinib (*p* = 0.004) [Figure 4(B)].

The patients with the classical mutation pattern had longer PFS (9.6 months *versus* 5.2 months; *p* = 0.023) and OS (27.6 months *versus* 15.2 months; *p* = 0.039) than those with the uncommon mutation pattern [Supplemental Figure 2(A) and (B)]. These results were the same in our prior reports, which showed gefitinib had good treatment efficacy in patients with classical mutation pattern.[16]

Prognostic factors in patients with complex EGFR mutations treated with EGFR TKIs as first-line treatment

Multivariate analysis was performed using the Cox regression model to determine potential predictive factors of PFS, including age (<65 years *versus* ⩾65 years), sex, smoking, performance status, presence of the classical mutation pattern, and EGFR TKI use (Table 2). Compared with patients treated with afatinib, patients treated with gefitinib [hazard ratio (HR), 2.01; 95% confidence interval (CI), 1.11–3.62; *p* = 0.020] or erlotinib (HR, 2.61; 95% CI, 1.31–5.22; *p* = 0.007) had shorter PFS. Patients with the classical mutation pattern had significantly longer PFS than those with the uncommon mutation pattern (HR, 0.41; 95% CI, 0.24–0.69; *p* = 0.001). Being male (HR, 2.08; 95% CI, 1.16–3.73;
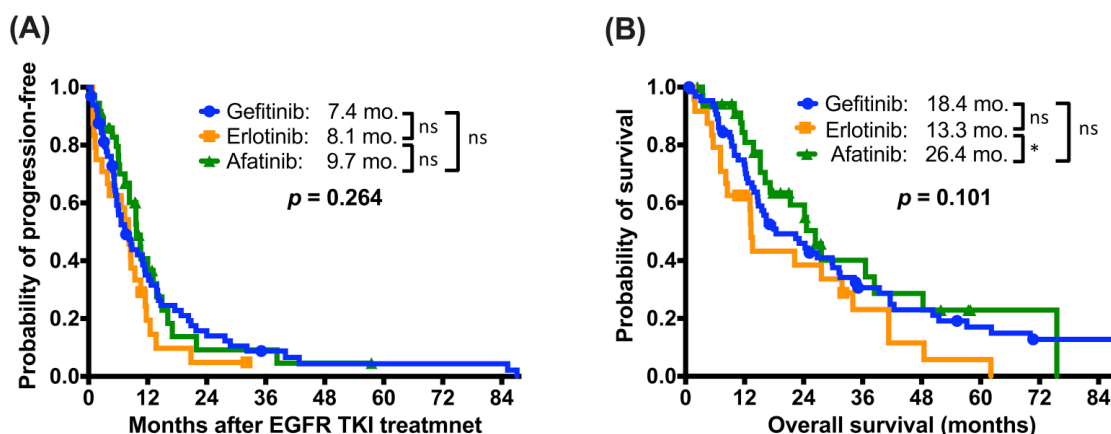
(A)

(B)

Figure 3. Kaplan–Meier survival curve of progression-free survival (PFS) and overall survival (OS) in lung adenocarcinoma patients with complex epidermal growth factor receptor (*EGFR*) mutations who took EGFR TKIs treatment as first-line treatment. (A) There were no significant differences in PFS between the three EGFR TKIs (gefitinib *versus* erlotinib *versus* afatinib: 7.4 months *versus* 8.1 months *versus* 9.7 months; $p = 0.264$, by the log-rank test). (B) There were no significant differences in OS between the three EGFR TKIs (gefitinib *versus* erlotinib *versus* afatinib: 18.4 months *versus* 13.3 months *versus* 26.4 months; $p = 0.101$, by the log-rank test).
*$p = 0.048$.
EGFR, epidermal growth factor receptor gene; mo., month; ns, not significant; TKI, tyrosine kinase inhibitor.
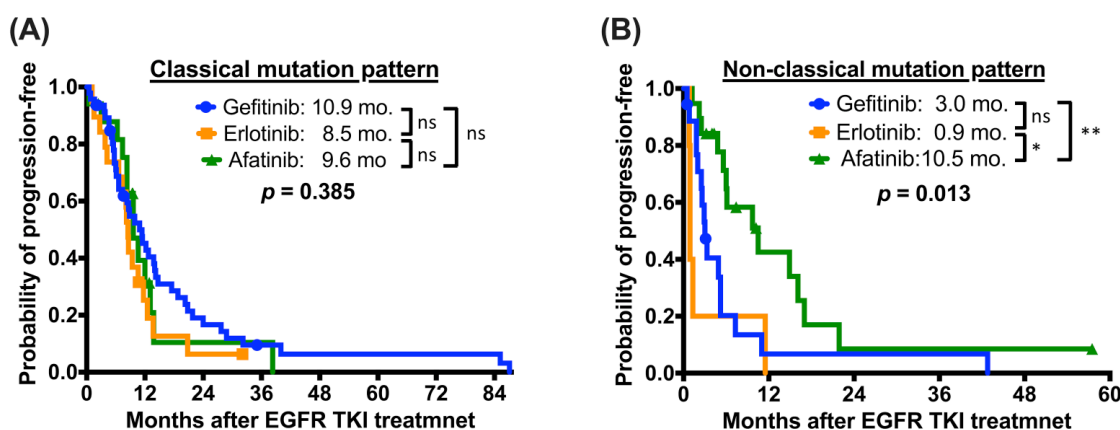
(A)

(B)

Figure 4. Kaplan–Meier survival curve of progression-free survival (PFS) in the three EGFR TKI-treated adenocarcinoma patients with complex epidermal growth factor receptor (*EGFR*) mutations. (A) For patients with the classical mutation pattern, there were no significant differences in PFS between the three EGFR TKIs ($p = 0.385$). (B) For patients with the uncommon mutation pattern, there was a significant difference that the PFS of afatinib was longer than gefitinib and erlotinib (gefitinib *versus* erlotinib *versus* afatinib: 3.0 months *versus* 0.9 months *versus* 10.5 months; $p = 0.013$).
*$p = 0.011$.
**$p = 0.004$.
EGFR, epidermal growth factor receptor gene; mo., month; ns, not significant; TKI, tyrosine kinase inhibitor.

$p = 0.014$) was significantly associated with shorter PFS. Compared with stage IV M1b, disease relapse (HR, 0.39; 95% CI, 0.21–0.72; $p = 0.003$) and stage IV M1a (HR, 0.55; 95% CI, 0.34–0.90; $p = 0.018$) at the initial diagnosis of lung cancer were favorable predictive factors of PFS.

For OS, multivariate analysis was also performed using the Cox regression model to determine the potential prognostic factors (Table 2). Compared with patients who received afatinib, patients treated with erlotinib had a significantly shorter OS (HR, 2.48; 95% CI, 1.20–5.12; $p = 0.014$), but there was no significant difference in OS from

Table 2. Multivariate analysis of predictive factors for progression-free survival in complex *EGFR* mutant patients who have taken EGFR TKIs.

Prognostic factors	Progression free survival		Overall survival	
	HR 95% CI	*p*	HR 95% CI	*p*
Male	2.08 (1.16–3.73)	0.014	2.64 (1.41–4.94)	0.002
≧65 y/o	1.46 (0.94–2.26)	0.092	1.47 (0.89–2.43)	0.130
Smokers	0.62 (0.32–1.20)	0.157	0.54 (0.26–1.12)	0.097
ECOG PS 2–4	0.80 (0.46–1.41)	0.439	1.80 (1.00–3.25)	0.049
Stage				
Relapse† *versus* IV (M1b)	0.39 (0.21–0.72)	0.003	0.47 (0.25–0.89)	0.020
IV (M1a) *versus* IV (M1b)	0.55 (0.34–0.90)	0.018	0.38 (0.21–0.67)	0.001
Classical mutation pattern	0.41 (0.24–0.69)	0.001	0.55 (0.33–0.91)	0.020
EGFR TKI				
Gefitinib *versus* afatinib	2.01 (1.11–3.62)	0.020	1.44 (0.80–2.60)	0.226
Erlotinib *versus* afatinib	2.61 (1.31–5.22)	0.007	2.48 (1.20–5.12)	0.014
Platinum using			1.11 (0.62–2.00)	0.722
Pemetrexed using			0.54 (0.31–0.94)	0.029

†Patients with stages I to III with systemic relapse after definitive surgery.
CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor gene; EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; HR, hazard ratio; y/o, years old.

patients treated with gefitinib (OR, 1.44; 95% CI, 0.80–2.60; $p = 0.226$). The classical mutation pattern (HR, 0.55; 95% CI, 0.33–0.91; $p = 0.020$) was associated with longer OS than the uncommon mutation pattern. Being male (HR, 2.64; 95% CI, 1.41–4.94; $p = 0.002$) and having stage IV MIb [compared with disease relapse (HR, 0.47; 95% CI, 0.25–0.89; $p = 0.020$) or stage IV M1a (HR, 0.38; 95% CI, 0.21–0.67; $p = 0.001$)] were poor prognostic factors of OS. Pemetrexed using (HR, 0.54; 95% CI, 0.31–0.94; $p = 0.029$) was also a good prognostic factor.

Secondary T790M mutations and small cell lung cancer transformation after acquired resistance to EGFR TKIs

A total of 80 EGFR TKI-treated patients met the criteria of acquired resistance to EGFR TKIs, and we obtained tissue samples in 52 of them after they developed acquired resistance. The clinical characteristics of these patients are listed in Table 3. Secondary T790M mutations were detected in 22 patients (42.3%) (Table 4), and there was no significant difference in T790M mutation between patients treated with the three EGFR TKIs, gefitinib [38.5% (10 of 26)], erlotinib [66.7% (6 of 9)] and afatinib [35.3% (6 of 17)] ($p = 0261$).

There was no significant difference in the detection of secondary T790M mutations between patients with the classical [46.5% (20 of 43)] and the uncommon mutation patterns [22.2% (2 of 9); $p = 0.272$], using Fisher's exact test, because of the small sample size. There were also no significant differences in PFS [T790M(+) *versus* T790M(−): 9.4 months *versus* 11.5 months; $p = 0.143$] and OS [T790M(+) *versus* T790M(−): 38.4 months *versus* 26.8 months; $p = 0.340$] between patients with and without secondary T790M mutations.

Small cell lung cancer (SCLC) transformation was detected in three (5.8%) tissue samples of with acquired resistance to EGFR TKIs (two patients

Table 3. Clinical characteristics of the patients with or without secondary T790M development after acquired resistance to EGFR TKIs.

	Patient no.	Secondary T790M		p
		Presence	**Absence**	
Total no.	52	22 (42.3%)	30 (57.7%)	
Age, mean years (range)	64.4 (43.0–85.0)	65.5 (43.8–82.9)	64.0 (43.0–85.0)	0.420§
Sex				0.685
Female	37	15	22	
Male	15	7	8	
Smoking				0.260*
Never-smokers	44	17	27	
Smokers	8	5	3	
ECOG PS				0.065*
0–1	47	22	25	
2–4	5	0	5	
Stage IV				0.497
Relapse†	11	3	8	
M1a	14	6	8	
M1b	27	13	14	
Classical mutation pattern				0.272*
Yes	43	20	23	
No	9	2	7	
EGFR TKI				0.261
Gefitinib	26	10	16	
Erlotinib	9	6	3	
Afatinib	17	6	11	

§By Mann–Whitney *U* test.
*By Fisher's exact test.
†Patients with stages I–III with systemic relapse after definitive surgery.
ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor.

for gefitinib and one for erlotinib). The three pairs of pre- and post-treatment specimens showed the same complex *EGFR* mutations: L858R + K757N, L858R + V834L and L858R + L833V. One post-treatment sample particularly had concomitant L858R + K757N + T790M mutations in addition to SCLC transformation. Two of the patients had a partial response after receiving etoposide/ cisplatin treatment for SCLC transformation. One patient received supportive care only because of poor performance status.

Discussion
Complex *EGFR* mutations are a rare subtype of *EGFR*-mutant lung cancers. The effectiveness of

Table 4. Patients who have secondary T790M after acquired resistance to EGFR TKIs.

No.	Gender	Age	Smoking	Initial stage	EGFR mutation	EGFR TKI	PFS	Max. response	3rd-G TKI/ line	Osimertinib response	3rd-G TKI PFS	OS	Status
1	F	58.9	Never	I	E709A + G719C	Gefitinib	7.3	PR	–	–		15.9	Death
2	F	65.2	Smoker	IV(M1a)	del E746_A750 + V765M	Afatinib	10.6	PR	–	–		27.6	Death
3	F	72.5	Never	IV(M1b)	del E746_A750 + H805Y	Afatinib	13.1	PR	Osi/7	PR	8.3	38.4	Death
4	F	61.8	Never	IV(M1b)	del E746_A750 + G857V	Afatinib	3.8	PD	Osi/4	PR	26.0	39.0	Alive
5	F	74.9	Never	I	del L747_T751 + E746Q	Gefitinib	14.7	PR	–	–		62.2	Death
6	M	47.6	Never	IV(M1a)	del L747_P753insS + A755G	Erlotinib	12.5	PR	HS-10296/2	SD	8.5	41.3	Death
7	M	59.7	Smoker	IV(M1b)	L858R + V689L	Gefitinib	5.7	PR	Osi/4	PR	3.0	17.9	Death
8	F	67.6	Never	IV(M1b)	L858R + E709V	Gefitinib	8.6	PR	–	–		31.4	Death
9	M	80.1	Smoker	IV(M1a)	L858R + E709G	Gefitinib	28.8	PR	CO1686/2	SD	33.2	90.9	Alive
10	F	43.8	Never	IV(M1b)	L858R + L718Q	Erlotinib	7.3	PR	Osi/6	PR	21.1	48.5	Death
11	M	56.8	Smoker	IV(M1b)	L858R + L718V	Gefitinib	24.0	PR	EGF816/2	SD	3.2	55.2	Alive
12	F	67.4	Never	III	L858R + K757N	Gefitinib	8.8	SD	–	–		29.9	Death
13	F	56.0	Never	IV(M1b)	L858R + I759M	Gefitinib	6.6	PR	–			50.3	Death
14	F	57.3	Never	IV(M1b)	L858R + A767V	Gefitinib	6.6	PR	Osi/13	PR	8.2	51.5	Death
15	F	57.4	Never	IV(M1a)	L858R + R776G	Erlotinib	11.7	PR	Osi/6	PR	6.2	31.8	Death
16	F	73.1	Never	IV(M1a)	L861R + R776G	Afatinib	5.6	PR	–	–		45.0	Alive
17	F	71.2	Never	IV(M1b)	L858R + R776H	Afatinib	13.8	PR	–	–		36.6	Death
18	M	82.9	Never	IV(M1b)	L858R + Q791H	Erlotinib	8.1	PR	Osi/3	PD	3.9	13.6	Death
19	F	78.9	Never	IV(M1b)	L858R + G810D	Afatinib	0.6	PR	Osi/3	PD	0.7	17.8	Alive
20	M	60.5	Smoker	IV(M1b)	L858R + R831Q	Erlotinib	10.6	PR	Osi/2	SD	5.0	34.0	Death
21	F	77.7	Never	IV(M1a)	L858R + L833V	Erlotinib	9.4	PR	Osi/3	SD	13.5	41.3	Death
22	F	65.9	Never	IV(M1b)	L858R + L861F	Gefitinib	5.3	PR	–	–		13.5	Death

3rd-G, third generation; *EGFR*, epidermal growth factor receptor gene; EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; F, female; M, male; Max., maximum; OS, overall survival; Osi, osimertinib; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

different EGFR TKIs in patients with complex *EGFR* mutations has not been fully elucidated. The incidence of complex *EGFR* mutations in lung adenocarcinoma has been reported to be 0.12–16.00% in all patients with *EGFR* mutations,[13,31] and the incidence in the current study was 5.1% (239 of 4714). Our cohort study comprised a comprehensive comparison of treatment effectiveness between different EGFR TKIs as first-line treatment in patients with complex *EGFR* mutations. Afatinib was a more favorable factor for PFS than the first-generation EGFR TKI, gefitinib, and erlotinib, especially in patients with uncommon mutation patterns. Compared with erlotinib, the PFS benefit of afatinib as first-line treatment also translated to overall survival benefit. Classical mutation pattern was a favorable predictive and prognostic factor of PFS and OS. The incidence of secondary T790M mutations was 42.3%. In addition to secondary T790M mutation, SCLC transformation was also one of the mechanisms of acquired resistance to EGFR TKIs among patients with complex *EGFR* mutations.

For patients with complex *EGFR* mutations with the classical mutation pattern, administration of first- and second-generation EGFR TKIs resulted in similar treatment response and PFS rates by univariate analysis. However, in patients with the uncommon mutation pattern, the second-generation EGFR TKI, afatinib, showed better effectiveness and longer PFS than the first-generation EGFR TKIs, gefitinib and erlotinib. This finding is similar to that of the LUX-Lung clinical trials, in which afatinib demonstrated clinical activity in patients with uncommon mutations L861Q, G719X, and S768I.[10] Because of the result of the previously mentioned study in 2015, clinicians of the present study might prefer to prescribed more afatinib for patients with uncommon mutation patterns. In contrast, the first-generation EGFR TKIs gefitinib and erlotinib have shown inconsistent responses in patients with uncommon *EGFR* mutations.[32–35] In *in vitro* studies, afatinib had a lower half-maximal inhibitory concentration (IC_{50}) in Ba/F3 cell lines transfected with S768I or G719X mutations than first-generation EGFR TKIs.[36,37] Afatinib also had a lower IC_{50} in Ba/F3 cells transfected with the complex *EGFR* mutations L858R + L747P, L858R + D761Y, and L858R + T854A than gefitinib and erlotinib.[38] In our study, afatinib also showed clinical activity in lung adenocarcinoma of complex *EGFR* mutations, especially with mutations of a uncommon pattern.

Although afatinib showed better effectiveness in patients with the uncommon mutation pattern, the benefit did not reflect prolonged OS when compared with gefitinib. Such findings are similar to those of the LUX-Lung 7 clinical trial, which demonstrated no significant difference in OS between afatinib and gefitinib, although the PFS and treatment response rates were significantly improved with afatinib.[39] However, patients treated with afatinib had longer OS than those treated with erlotinib. Therefore, there may be still different effectiveness of the different first-generation EGFR TKIs. In addition, it is unknown whether specific EGFR TKIs can prolong the OS of patients with specific complex *EGFR* mutation types. Therefore, further studies involving a larger number of patients with complex *EGFR* mutations are necessary to explore the effects of different EGFR TKIs, especially the novel third-generation agent osimertinib, on survival.

In our previous study, the effectiveness of gefitinib and the OS rate in patients with complex *EGFR* mutations with the classical mutation pattern were similar to those of patients with single classical *EGFR* mutations.[40] However, an *in vitro* study showed that some rare types of *EGFR* substitution mutations suggest relative gefitinib resistance when combined with the common *EGFR* mutations.[41] Complex *EGFR* mutations with different patterns may cause various conformational changes in the EGFR protein, resulting in different sensitivities to EGFR TKIs.

The present study showed that L858R was the predominant subtype contained in the complex *EGFR* mutation. For single *EGFR* mutation, the PIONEER study demonstrated that the incidences of individual mutation types were 40.6% (303 of 746) for L858R and 43.0% (321 of 746) for del-19 in Asian patients with *EGFR* mutations.[42] In Taiwan, the incidences of L858R and del-19 were 43–44% and 37–51% in patients with *EGFR* mutations, respectively.[43,44] There was no obvious difference in mutation incidences between L858R and del-19. For complex *EGFR* mutations, Reiss *et al*. and Yang *et al*. both reported that there were more patients with complex *EGFR* mutation that contained L858R than those that contained del-19.[45,46] Therefore, the incidence of complex *EGFR* mutation that contained L858R is higher than those that contained del-19.

Recently, real-time PCR methods, such as the amplification refractory mutation system real-time

PCR and Cobas systems, have been used to detect *EGFR* mutations in NSCLC. The real-time PCR has demonstrated higher sensitivity than that of Sanger sequencing, but is prone to missing mutations due to the use of a special primer design for prespecified known mutations.[47] To detect unknown or rare concomitant *EGFR* mutations, our study used Sanger sequencing, which is the gold standard for gene mutation detection. Recently, next-generation sequencing (NGS) is used to detect genetic alteration for lung cancer patients. Compared with traditional methods, NGS can comprehensively sequence complete genomes, exomes, and transcriptomes. So, rare mutation could be detected more frequently by NGS. Therefore, categorizing complex *EGFR* mutations according to their sensitivity to different EGFR TKIs is important to enable precision medicine.

Although patients with *EGFR* mutations have a favorable treatment response to EGFR TKIs initially, acquired resistance to EGFR TKI treatment can eventually develop. Various mechanisms of acquired resistance have been discovered.[48] However, T790M mutation is the most common mechanism of acquired resistance (~50%) to first- and second-generation EGFR TKIs.[30,49] In the current study, the incidence of acquired T790M mutations was 42.3% among patients with complex *EGFR* mutations. A complex conformational change due to two or more concomitant mutations may be an obstacle to developing T790M mutations. More clinical trials are still necessary to confirm the incidence of acquired T790M mutations.

Platinum-based doublet chemotherapy has been approved as the standard treatment for patients with NSCLC. In addition, pemetrexed in combination with cisplatin or carboplatin has also been approved as first-line treatment in non-squamous patients with NSCLC and a performance status of 0–1 and *EGFR*-, *ALK*-, or *ROS1*-negative disease.[50] Recently, pemetrexed combined with carboplatin and gefitinib has been shown to prolong survival, although it could not prolong a second PFS.[51] Therefore, this combination therapy is also considered as first-line treatment in patients with *EGFR* mutations. We also showed the survival benefit of pemetrexed treatment (Table 3). However, it remains unclear whether the combination therapy of EGFR TKIs and pemetrexed/platinum can benefit patients with complex *EGFR* mutations.

EGFR exon 20 insertion mutations account for 1.6–5% of all *EGFR* mutations in Asia,[52–55] and about 2–10% in Europe and USA.[56–60] Our group had also reported *EGFR* exon 20 insertions comprised 4.0% of *EGFR* mutated NSCLC patients.[61] The present study showed that no exon 20 insertion was detected in the complex *EGFR* mutations. We explored the COSMIC database, which collected 211 lung cancers with *EGFR* insertion mutations, and no complex *EGFR* mutation with exon 20 insertion was reported. In addition, Yang *et al.* pooled analysis assessed the activity of afatinib in 693 patients with tumors harboring uncommon *EGFR* mutations, and there were also no patients harboring complex *EGFR* mutation with exon 20 insertion.[46] However, there were still a few patients with complex *EGFR* mutation that contained exon 20 insertions reported from large cohorts of patients with *EGFR* mutations and using high-sensitive detection methods for *EGFR* mutations, for example, NGS.[45,52,62] For mutation analysis, low tumor contents in small lung biopsy tissue samples may cause detection limitation and pitfall. In addition, Tsai *et al.* demonstrated that RNA is a more favorable source for *EGFR* testing than genomic DNA in the highly heterogeneous specimens of MPE related to lung cancer.[27]

Osimertinib is approved for first-line treatment of lung cancers with classical *EGFR* mutations. The efficacy of osimertinib for lung cancers with complex *EGFR* mutation is rarely reported. Recently, Cho *et al.* reported that the efficacy of osimertinib for patients with NSCLC harboring uncommon *EGFR* mutations, including two G719X + S768I and two G719X + L861Q, and the maximum response were three PR and one SD.[63] In addition, Yang *et al.* reported a case with complex mutations of *EGFR* H773L + V774M, who was irresponsive to gefitinib, but had sustained disease control to osimertinib in combination with bevacizumab for 12 months.[64] Astaras *et al.* presented a patient with complex *EGFR* mutations in exon 21 (L833V + H835F), showing a response to osimertinib with a PFS of 9 months.[65] Because of the limited number of patients with complex *EGFR* mutations, the efficacy of osimertinib in this group of patients warrants future study.

The present study showed 100% of response rates to osimertinib in patients with complex *EGFR* mutations that contained del-19 (2 of 2). Especially, both of the two patients with del-19 received osimertinib after acquired resistance to

afatinib. Recently, the GioTag study showed that sequential afatinib and osimertinib conferred OS of about 3.5 years, and time to treatment failure of over 2 years, in NSCLC patients with *EGFR* mutation.[66] However, because of the limited patient numbers, it is uncertain whether osimertinib has favorable effectiveness for patients with complex *EGFR* mutations after acquired resistance to afatinib. Future study with more patients is warranted for this issue.

Although this study has the largest number of patients with complex *EGFR* mutations so far, it has some limitations. First, all included patients received first- and second-generation EGFR TKIs only. We have not evaluated the effectiveness of the third-generation EGFR TKI osimertinib, although the FDA has approved it as first-line treatment for metastatic NSCLC with classical *EGFR* mutations and T790M mutations. Second, the re-biopsy samples of patients after they acquired resistance to EGFR TKIs were limited. Obtaining tissue specimens for molecular analysis is a key point in current precision medicine. However, the patients' conditions may be too poor to be suitable for re-biopsy, especially when they experience disease progression after EGFR TKI treatment. Liquid biopsy to detect T790M mutations from circulating cancer cells or cell-free DNA from the blood may be another choice.[67]

In conclusion, this study enrolled the largest number of patients with complex *EGFR* mutations. Afatinib as the first-line therapy provided a better treatment efficacy and survival benefit in lung adenocarcinoma with complex *EGFR* mutations, especially with uncommon mutation pattern. Patients with classical mutation pattern had longer PFS and OS than those with uncommon patterns. Moreover, SCLC transformation remained one of the mechanisms for acquired resistance to EGFR TKIs, in addition to T790M mutations. Because of the treatment efficacy and survival benefit, afatinib is an effective first-line treatment for patients with complex *EGFR* mutations, especially those with uncommon mutation patterns.

Author contribution
SG Wu and JY Shih had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study design and patient specimen collection: SG Wu, JY Shih, CJ Yu and James CH Yang. Data collection, literature search, and drafting of the manuscript: SG Wu and JY Shih. Study supervision: CJ Yu, James CH Yang and JY Shih. All authors approved the final draft of the submitted manuscript.

Conflict of interest statement
SG Wu has received speaking honoraria from Roche, AstraZeneca and Pfizer. JY Shih has received personal fees for advisory boards from AstraZeneca, Roche, Boehringer Ingelheim, Eli Lilly, Merck Sharp & Dohme, Ono Pharmaceutical, Chugai Pharmaceutical, and Bristol-Myers Squibb; speaking honoraria from AstraZeneca, Roche, Boehringer Ingelheim, Eli Lilly, Pfizer, Novartis, Merck Sharp & Dohme, Ono Pharmaceutical, Chugai Pharmaceutical, and Bristol-Myers Squibb; and travel expenses from Roche, Pfizer, Merck Sharp & Dohme, Chugai Pharmaceutical, and Bristol-Myers Squibb. CJ Yu declares no conflict of interest. JCH Yang has received advisory board and speaker honoraria from Boehringer Ingelheim, AstraZeneca, Roche/Genentech, Pfizer, Novartis, MSD, Merck Serono, Clovis Oncology, and Bayer.

Ethics approval and consent to participate
The study was approved by the Institutional Review Board of the National Taiwan University Hospital Research Ethics Committee (REC No. 201103013RC and 201111039RIC). All patients provided written informed consent for the collection of demographic and clinical outcome data and for molecular analysis before the collection of tissue specimens.

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Supplemental material

Supplemental material for this article is available online.

References

1. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; 68: 7–30.

2. Shaw AT and Engelman JA. ALK in lung cancer: past, present, and future. *J Clin Oncol* 2013; 31: 1105–1111.

3. Recondo G, Facchinetti F, Olaussen KA, *et al*. Making the first move in EGFR-driven or ALK-driven NSCLC: first-generation or next-generation TKI? *Nat Rev Clin Oncol* 2018; 15: 694–708.

4. Shaw AT, Solomon BJ, Chiari R, *et al*. Lorlatinib in advanced ROS1-positive non-small-cell lung cancer: a multicentre, open-label, single-arm, phase 1–2 trial. *Lancet Oncol* 2019; 20: 1691–1701.

5. Shaw AT, Ou SH, Bang YJ, *et al*. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med* 2014; 371: 1963–1971.

6. Planchard D, Smit EF, Groen HJM, *et al*. Dabrafenib plus trametinib in patients with previously untreated BRAF(V600E)-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol* 2017; 18: 1307–1316.

7. Mok TS, Wu YL, Thongprasert S, *et al*. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; 361: 947–957.

8. Yatabe Y and Mitsudomi T. Epidermal growth factor receptor mutations in lung cancers. *Pathol Int* 2007; 57: 233–244.

9. Chiu CH, Yang CT, Shih JY, *et al*. Epidermal growth factor receptor tyrosine kinase inhibitor treatment response in advanced lung adenocarcinomas with G719X/L861Q/S768I mutations. *J Thorac Oncol* 2015; 10: 793–799.

10. Yang JC, Sequist LV, Geater SL, *et al*. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. *Lancet Oncol* 2015; 16: 830–838.

11. New indication approved for afatinib in NSCLC. *Oncology Times* 2018; 40: 18.

12. Hsieh MH, Fang YF, Chang WC, *et al*. 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.

13. Zhang B, Wang S, Qian J, *et al*. Complex epidermal growth factor receptor mutations and their responses to tyrosine kinase inhibitors in previously untreated advanced lung adenocarcinomas. *Cancer* 2018; 124: 2399–2406.

14. Chen Z, Feng J, Saldivar JS, *et al*. EGFR somatic doublets in lung cancer are frequent and generally arise from a pair of driver mutations uncommonly seen as singlet mutations: one-third of doublets occur at five pairs of amino acids. *Oncogene* 2008; 27: 4336–4343.

15. Shih JY, Gow CH and Yang PC. EGFR mutation conferring primary resistance to gefitinib in non-small-cell lung cancer. *N Engl J Med* 2005; 353: 207–208.

16. Wu SG, Chang YL, Hsu YC, *et al*. 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.

17. Wu JY, Wu SG, Yang CH, *et al*. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. *Clin Cancer Res* 2008; 14: 4877–4882.

18. Wu SG, Chang YL, Yu CJ, *et al*. Lung adenocarcinoma patients of young age have lower EGFR mutation rate and poorer efficacy of EGFR tyrosine kinase inhibitors. *ERJ Open Res* 2017; 3.

19. Liang SK, Ko JC, Yang JC, *et al*. Afatinib is effective in the treatment of lung adenocarcinoma with uncommon EGFR p.L747P and p.L747S mutations. *Lung Cancer* 2019; 133: 103–109.

20. Wu JY, Yu CJ, Chang YC, *et al*. 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.

21. Travis WD, Brambilla E, Noguchi M, *et al*. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol* 2011; 6: 244–285.

22. Goldstraw P, Crowley J, Chansky K, *et al*. The IASLC lung cancer staging project: proposals

for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. *J Thorac Oncol* 2007; 2: 706–714.

23. Centers for Disease Control and Prevention. Cigarette smoking among adults—United States, 2006. *MMWR Morb Mortal Wkly Rep* 2007; 56: 1157–1161.

24. Oken MM, Creech RH, Tormey DC, *et al.* Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982; 5: 649–655.

25. Eisenhauer EA, Therasse P, Bogaerts J, *et al.* New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; 45: 228–247.

26. Wu SG, Gow CH, Yu CJ, *et al.* Frequent epidermal growth factor receptor gene mutations in malignant pleural effusion of lung adenocarcinoma. *Eur Respir J* 2008; 32: 924–930.

27. Tsai TH, Su KY, Wu SG, *et al.* RNA is favourable for analysing EGFR mutations in malignant pleural effusion of lung cancer. *Eur Respir J* 2012; 39: 677–684.

28. Shih JY, Gow CH, Yu CJ, *et al.* Epidermal growth factor receptor mutations in needle biopsy/aspiration samples predict response to gefitinib therapy and survival of patients with advanced nonsmall cell lung cancer. *Int J Cancer* 2006; 118: 963–969.

29. Liu Y, Sun L, Xiong ZC, *et al.* Meta-analysis of the impact of de novo and acquired EGFR T790M mutations on the prognosis of patients with non-small cell lung cancer receiving EGFR-TKIs. *Onco Targets Ther* 2017; 10: 2267–2279.

30. Kobayashi S, Boggon TJ, Dayaram T, *et al.* EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005; 352: 786–792.

31. Hata A, Yoshioka H, Fujita S, *et al.* Complex mutations in the epidermal growth factor receptor gene in non-small cell lung cancer. *J Thorac Oncol* 2010; 5: 1524–1528.

32. Zhang T, Wan B, Zhao Y, *et al.* Treatment of uncommon EGFR mutations in non-small cell lung cancer: new evidence and treatment. *Transl Lung Cancer Res* 2019; 8: 302–316.

33. Zhang Y, Wang Z, Hao X, *et al.* Clinical characteristics and response to tyrosine kinase inhibitors of patients with non-small cell lung cancer harboring uncommon epidermal growth factor receptor mutations. *Chin J Cancer Res* 2017; 29: 18–24.

34. Otsuka T, Mori M, Yano Y, *et al.* Effectiveness of tyrosine kinase inhibitors in Japanese patients with non-small cell lung cancer harboring minor epidermal growth factor receptor mutations: results from a multicenter retrospective study (HANSHIN Oncology Group 0212). *Anticancer Res* 2015; 35: 3885–3891.

35. Russo A, Franchina T, Ricciardi G, *et al.* Heterogeneous responses to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in patients with uncommon EGFR mutations: new insights and future perspectives in this complex clinical scenario. *Int J Mol Sci* 2019; 20: 1431.

36. Kobayashi Y, Togashi Y, Yatabe Y, *et al.* EGFR exon 18 mutations in lung cancer: molecular predictors of augmented sensitivity to afatinib or neratinib as compared with first- or third-generation TKIs. *Clin Cancer Res* 2015; 21: 5305–5313.

37. Banno E, Togashi Y, Nakamura Y, *et al.* Sensitivities to various epidermal growth factor receptor-tyrosine kinase inhibitors of uncommon epidermal growth factor receptor mutations L861Q and S768I: what is the optimal epidermal growth factor receptor-tyrosine kinase inhibitor? *Cancer Sci* 2016; 107: 1134–1140.

38. Chiba M, Togashi Y, Bannno E, *et al.* Efficacy of irreversible EGFR-TKIs for the uncommon secondary resistant EGFR mutations L747S, D761Y, and T854A. *BMC Cancer* 2017; 17: 281.

39. Paz-Ares L, Tan EH, O'Byrne K, *et al.* Afatinib versus gefitinib in patients with EGFR mutation-positive advanced non-small-cell lung cancer: overall survival data from the phase IIb LUX-Lung 7 trial. *Ann Oncol* 2017; 28: 270–277.

40. Chen YR, Fu YN, Lin CH, *et al.* Distinctive activation patterns in constitutively active and gefitinib-sensitive EGFR mutants. *Oncogene* 2006; 25: 1205–1215.

41. Tam IY, Leung EL, Tin VP, *et al.* Double EGFR mutants containing rare EGFR mutant types show reduced in vitro response to gefitinib compared with common activating missense mutations. *Mol Cancer Ther* 2009; 8: 2142–2151.

42. Shi Y, Au JS, Thongprasert S, *et al.* A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol* 2014; 9: 154–162.

43. Ho HL, Chang FP, Ma HH, *et al.* Molecular diagnostic algorithm for epidermal growth factor receptor mutation detection in Asian lung adenocarcinomas: comprehensive analyses of 445

Taiwanese patients with immunohistochemistry, PCR-direct sequencing and Scorpion/ARMS methods. *Respirology* 2013; 18: 1261–1270.

44. Tseng JS, Wang CL, Yang TY, *et al*. Divergent epidermal growth factor receptor mutation patterns between smokers and non-smokers with lung adenocarcinoma. *Lung Cancer* 2015; 90: 472–476.

45. Riess JW, Gandara DR, Frampton GM, *et al*. Diverse EGFR exon 20 insertions and co-occurring molecular alterations identified by comprehensive genomic profiling of NSCLC. *J Thorac Oncol* 2018; 13: 1560–1568.

46. Yang JC, Schuler M, Popat S, *et al*. Afatinib for the treatment of NSCLC harboring uncommon EGFR mutations: a database of 693 cases. *J Thorac Oncol* 2020; 15: 803–815.

47. Maheswaran S, Sequist LV, Nagrath S, *et al*. Detection of mutations in EGFR in circulating lung-cancer cells. *N Engl J Med* 2008; 359: 366–377.

48. Wu SG and Shih JY. Management of acquired resistance to EGFR TKI-targeted therapy in advanced non-small cell lung cancer. *Mol Cancer* 2018; 17: 38.

49. Wu SG, Liu YN, Tsai MF, *et al*. The mechanism of acquired resistance to irreversible EGFR tyrosine kinase inhibitor-afatinib in lung adenocarcinoma patients. *Oncotarget* 2016; 7: 12404–12413.

50. Hanna N, Johnson D, Temin S, *et al*. Systemic therapy for stage IV non-small-cell lung cancer: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol* 2017; 35: 3484–3515.

51. Nakamura A, Inoue A, Morita S, *et al*. Phase III study comparing gefitinib monotherapy (G) to combination therapy with gefitinib, carboplatin, and pemetrexed (GCP) for untreated patients (pts) with advanced non-small cell lung cancer (NSCLC) with EGFR mutations (NEJ009). *J Clin Oncol* 2018; 36: 9005.

52. Byeon S, Kim Y, Lim SW, *et al*. Clinical outcomes of EGFR exon 20 insertion mutations in advanced non-small cell lung cancer in Korea. *Cancer Res Treat* 2019; 51: 623–631.

53. Tu HY, Ke EE, Yang JJ, *et al*. A comprehensive review of uncommon EGFR mutations in patients with non-small cell lung cancer. *Lung Cancer* 2017; 114: 96–102.

54. Yasuda H, Kobayashi S and Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. *Lancet Oncol* 2012; 13: e23–e31.

55. Fang W, Huang Y, Hong S, *et al*. EGFR exon 20 insertion mutations and response to osimertinib in non-small-cell lung cancer. *BMC Cancer* 2019; 19: 595.

56. Naidoo J, Sima CS, Rodriguez K, *et al*. Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: clinical outcomes and response to erlotinib. *Cancer* 2015; 121: 3212–3220.

57. Beau-Faller M, Prim N, Ruppert AM, *et al*. Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: a multicentre observational study by the French ERMETIC-IFCT network. *Ann Oncol* 2014; 25: 126–131.

58. Arcila ME, Nafa K, Chaft JE, *et al*. EGFR exon 20 insertion mutations in lung adenocarcinomas: prevalence, molecular heterogeneity, and clinicopathologic characteristics. *Mol Cancer Ther* 2013; 12: 220–229.

59. Oxnard GR, Lo PC, Nishino M, *et al*. Natural history and molecular characteristics of lung cancers harboring EGFR exon 20 insertions. *J Thorac Oncol* 2013; 8: 179–184.

60. Kuiper JL, Hashemi SM, Thunnissen E, *et al*. Non-classic EGFR mutations in a cohort of Dutch EGFR-mutated NSCLC patients and outcomes following EGFR-TKI treatment. *Br J Cancer* 2016; 115: 1504–1512.

61. Wu JY, Yu CJ and Shih JY. Effectiveness of treatments for advanced non-small-cell lung cancer with exon 20 insertion epidermal growth factor receptor mutations. *Clin Lung Cancer* 2019; 20: e620–e630.

62. Baek JH, Sun JM, Min YJ, *et al*. Efficacy of EGFR tyrosine kinase inhibitors in patients with EGFR-mutated non-small cell lung cancer except both exon 19 deletion and exon 21 L858R: a retrospective analysis in Korea. *Lung Cancer* 2015; 87: 148–154.

63. Cho JH, Lim SH, An HJ, *et al*. Osimertinib for patients with non-small-cell lung cancer harboring uncommon EGFR mutations: a multicenter, open-label, phase II trial (KCSG-LU15-09). *J Clin Oncol* 2020; 38: 488–495.

64. Yang M, Tong X, Xu X, *et al*. Case report: osimertinib achieved remarkable and sustained disease control in an advanced non-small-cell lung cancer harboring EGFR H773L/V774M mutation complex. *Lung Cancer* 2018; 121: 1–4.

65. Astaras C, Bettini A and Betticher DC. Response to osimertinib in a patient with non-small

cell lung cancer and two uncommon EGFR mutations. *healthbook TIMES Oncol Hematol* 2020; 3: 10–13.

66. Hochmair MJ, Morabito A, Hao D, *et al*. Sequential afatinib and osimertinib in patients with EGFR mutation-positive non-small-cell lung cancer: updated analysis of the observational GioTag study. *Future Oncol* 2019; 15: 2905–2914.

67. Sorber L, Zwaenepoel K, Deschoolmeester V, *et al*. Circulating cell-free nucleic acids and platelets as a liquid biopsy in the provision of personalized therapy for lung cancer patients. *Lung Cancer* 2017; 107: 100–107.