Published OnlineFirst April 29, 2011; DOI: 10.1158/1078-0432.CCR-10-3408

Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

Effectiveness of Tyrosine Kinase Inhibitors on "Uncommon" Epidermal Growth Factor Receptor Mutations of Unknown Clinical Significance in Non–Small Cell Lung Cancer

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Abstract

Purpose: Clinical features of epidermal growth factor receptor (EGFR) mutations, L858R, deletions in exon 19, T790M, and insertions in exon 20, in non–small cell lung cancer (NSCLC) are well known. The clinical significance of other uncommon EGFR mutations, such as their association with the effectiveness of EGFR tyrosine kinase inhibitors (TKI), is not well understood. This study aimed to improve the understanding of these uncommon EGFR mutations of unknown clinical significance.

Patients and Methods: Specimens from 1,261 patients were tested for EGFR mutations. We surveyed the clinical data and the effectiveness of gefitinib and erlotinib in NSCLC patients with uncommon EGFR mutations.

Results: Of the 1,261 patients, 627 (49.8%) had EGFR mutations. This included 258 patients with deletions in exon 19, 260 patients with L858R, 25 patients with insertions or duplications in exon 20, 6 patients with *de novo* T790M, and 78 (12.4%) patients with uncommon mutations. Of the 78 patients, 62 received either gefitinib or erlotinib treatment. The response rate of TKIs treatment was 48.4%, and the median progression-free survival (PFS) was 5.0 months. Mutations on G719 and L861 composed a major part (28 of 62) of uncommon mutations, and were associated with a favorable effectiveness of EGFR TKIs (response rate, 57.1%; median PFS, 6.0 months). Mutations other than G719 and L861 led to a worse response to EGFR TKIs (response rate, 20.0%; median PFS, 1.6 months).

Conclusions: Uncommon EGFR mutations constituted a distinct part of the whole group of EGFR mutations. Their composition was heterogeneous, and their associations with EGFR TKIs differed. *Clin Cancer Res; 17(11); 3812–21. ©2011 AACR.*

Introduction

Mutations of the epidermal growth factor receptor (EGFR) were found in 10% to 20% of Caucasian patients and in 30% to 60% of Asian patients with non–small cell lung cancer (NSCLC; refs. 1–5). In addition to their association with ethnicity, EGFR mutations occur more frequently in NSCLC of women, never smokers, and those with adenocarcinoma cell type (2, 5, 6). The 2 EGFR tyrosine kinase inhibitors (TKI), erlotinib and gefitinib, have been shown to possess favorable clinical efficacy for advanced NSCLC with EGFR mutations (1, 3, 5, 7, 8).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-10-3408

EGFR mutations exist in exons 18 to 21, which encompass most of the tyrosine kinase binding domain of EGFR (9, 10). As reported in the literature, 2 major EGFR mutations are deletions in exon 19 and L858R in exon 21. They constitute approximately 50% to 90% of total EGFR mutations (2–4, 11, 12). These 2 mutations are the most well-documented mutations in NSCLC patients who have a good response to gefitinib treatment (1, 4, 9, 12).

Besides deletions in exon 19 and L858R, 2 other categories of EGFR mutations, which are also well known, are T790M (13, 14) and insertions (or in-frame duplications) in exon 20 (15, 16). Insertions or in-frame duplications in exon 20 are primary somatic mutations. T790M can be a primary mutation (17, 18), or a secondary mutation acquired after treatment with EGFR TKIs (13, 14). Both these mutation types bring about resistance to EGFR TKIs.

The earlier-mentioned EGFR mutations, including deletions in exon 19, L858R, T790M, and insertions in exon 20, are well reported in the literature and have "known" clinical significance. However, there are still other EGFR mutations, such as amino acid substitutions in E709, G719, S768, L861, and others, which are also part of the spectrum of EGFR mutations (2, 12). The numbers of these mutations are small. They were scattered in separate study populations worldwide. Mutations such as G719 and L861

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Translational Relevance

Deletions in exon 19, L858R, T790M, and insertions in exon 20 are epidermal growth factor receptor (EGFR) mutations with well-known clinical significance in non–small cell lung cancer. However, there are a distinct part of EGFR mutations that are relatively rare and their associations to EGFR tyrosine kinase inhibitors (TKI) are not well clarified. They are uncommon EGFR mutations with unknown clinical significance. In this study, we included 78 patients with uncommon EGFR mutations, in which 62 patients received EGFR TKIs treatments. We found that these uncommon EGFR mutations of unknown clinical significance are heterogeneous and their association to EGFR TKIs differed from each other. Understanding individual EGFR mutational expression may help accomplish personalized treatment of advanced non–small cell lung cancer in the future.

were analyzed in many institutions, but were also reported in small case numbers and their influences on the effectiveness of EGFR TKIs have not been fully elucidated. These small populations of EGFR mutations are collectively named "uncommon mutations of unknown clinical significance" in this text, to distinguish them from mutations such as deletions in exon 19, L858R, T790M, and insertions in exon 20, which have well-described clinical significance. In this study, we investigated the clinical features of these uncommon EGFR mutations to increase comprehension of the entire EGFR mutation spectrum.

Materials and Methods

Patients

The study group included NSCLC patients diagnosed at the National Taiwan University Hospital between January 2000 and December 2009. All patients underwent complete cancer staging—including bronchoscopy; computed tomography (CT) of the head, chest, and abdomen; and whole-body bone scintigraphy—in the hospital. The patients' clinical data, including demographic information, performance status, smoking status, cancer cell type, and imaging studies, were reviewed. Never smokers were defined as those who had smoked less than 100 cigarettes in their lifetime. Date of diagnosis, treatments received, and responsiveness to treatments were recorded. Lung cancer histology was defined on the basis of the World Health Organization pathology classification (19). Tumor specimens obtained by either surgical or needle biopsy/aspiration procedures, from primary lung tumors, other distant metastases, and malignant effusion cell blocks, were sequenced for mutational analysis. Clinical staging was decided according to the sixth edition of tumor node metastasis classification of NSCLC. This study was approved by the hospital's Institutional Review Board. Written informed consent for use of tissue in molecular analysis was acquired from patients at the procurement of tumor specimens.

Use of EGFR TKIs and evaluation of effectiveness

Treatments of gefitinib or erlotinib for stage IIIb with malignant pleural effusion or stage IV NSCLC were identified from the records of the hospital's Department of Pharmacy. The timing of different EGFR TKIs depended on the physicians' discretion. Erlotinib was taken 150 mg daily, whereas gefitinib was taken 250 mg daily orally. Baseline assessments were generally carried out 2 weeks before treatment. Chest radiography was routinely carried out and assessed every 2 to 3 weeks to evaluate the response to treatment, whereas a chest CT scan (including liver and adrenal glands) was carried out every 2 to 3 months as per routine clinical practice and, as needed, to confirm response and disease progression.

Treatment responses were defined as complete response, partial response, stable disease, and progressive disease, according to the criteria of the RECIST (response evaluation criteria in solid tumors) group (20). Patients with partial or complete response were regarded as responders, and the rest were classed as nonresponders to antitumor therapy. Disease control status comprised complete response, partial response, and stable disease (21). The cutoff date for data collection was August 31, 2010. Overall survival was measured from the first day of erlotinib or gefitinib treatment to the day of death or cutoff date. Progression-free survival (PFS) with erlotinib or gefitinib was measured from the first day of erlotinib or gefitinib treatment until the first objective or clinical sign of disease progression or death.

Mutational analysis of EGFR

The EGFR mutation status of the lung cancer specimens was reviewed retrospectively. Tumor specimens, including paraffin blocks or frozen tissues of surgical specimens, fine needle biopsies, and pleural effusions, were obtained for mutational analysis. Formalin-fixed and paraffin-embedded blocks were procured from the hospital's pathology department. Acquisition of tumor specimens for testing for EGFR mutations were done before EGFR TKI treatments. Patients screened for EGFR mutations included: (i) those participating in clinical trial studies (22); (ii) those who underwent fine needle biopsy or thoracentesis for pleural effusions after July 2004, when consecutive recruitment for EGFR mutations was started at the hospital; (iii) whose resected tumors were retrospectively sequenced; and (iv) those who were recruited for retrospective NSCLC studies (6, 15, 23–26). Tissue sections were examined for adequacy by microscopy with hematoxylin and eosin staining; tissue samples that consisted of more than 80% tumor content, were selected for the study. Macrodissection was used.

The mutational analysis of *EGFR* genes was as previously reported (6, 23, 27). In summary, DNA was derived from tumors embedded in paraffin blocks by using a QIAmp DNA Mini Kit (Qiagen). The tyrosine kinase domain of the

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Table 1. Demographics of patients with *EGFR* mutations and patients with uncommon mutations of unknown clinical significance

	All patients with *EGFR* mutations	Uncommon mutations of unknown clinical significance	Deletion in exon 19 and L858R in exon 21	*P*[a]	Insertions in exon 20	Wild-type *EGFR*
No. of patient	627	78	518		25	634
Age, median (range); y	67 (29–91)	67 (35–91)	67 (29–91)	0.984	61 (35–84)	67 (25–96)
Gender				0.053		
Male	242	38	193		7	365
Female	385	40	325		18	269
Smoking				0.092		
Smoker	132	22	103		4	297
Never smoker	495	56	415		21	337
Histology				0.737		
Adenocarcinoma	605	76	501		22	466
Nonadenocarcinoma	22	2	17		3	168
EGFR mutation						
Deletion in exon 19	258		258			
L858R	260		260			
De novo T790M	6					
Insertion or duplication in exon 20	25				25	
Uncommon mutation	78	78				

[a]Comparison between uncommon mutations of unknown clinical significance and classical mutations (deletion in exon 19 and L858R in exon 21).

EGFR coding sequence, exons 18, 19, 20, and 21, was amplified, whereas independent PCR amplifications were purified and sequenced in an automatic ABI Prism 3100 or 3700 DNA Analyzer (Applied Biosystems).

Frozen lung cancer tissues were procured at surgery, immediately snap frozen in liquid nitrogen, and stored until use. Malignant pleural effusion fluid was centrifuged at 250 × *g* for 10 minutes, and the cell pellets were frozen in RNAlater (Qiagen).

Total mRNA was extracted from resected cancer tissue or cell pellets from pleural effusion by using an RNA extraction kit (RNeasy Mini Kit; Qiagen). The 4 exons (exons 18–21) that code for the TK domain of the *EGFR* gene were amplified by reverse transcription (RT)-PCR by using a Qiagen One-Step RT-PCR Kit (Qiagen). The primers and RT-PCR conditions were as previously reported (5, 23, 27). The cDNA amplicons were purified and sequenced.

All sequencing reactions were carried out in both forward and reverse directions by using tracings from at least 2 PCRs.

Statistical analyses

All categorical variables were analyzed with χ^2 tests, except where a small size (<5) necessitated the use of Fisher's exact test. A Student's *t* test was conducted for continuous variables for comparisons between the 2 groups. Overall survival and PFS after gefitinib or erlotinib treatment were estimated by the Kaplan–Meier method to assess the time to death or progression. A log-rank test was used to compare cumulative survival in different groups. All *P* values were 2-sided and $P < 0.05$ was considered statistically significant. All analyses were carried out by SPSS software (version 13.0; SPSS Inc.).

Results

Characteristics of lung cancer patients

Specimens from 1,261 NSCLC patients were examined for mutations of the EGFR tyrosine kinase domain. They included 464 surgical specimens, 396 fine-needle biopsies (echo-guided, CT-guided, or bronchoscopic), and 401 pleural effusions (377 pleural effusion cytology and 24 cell block preparations of pleural effusion). Surgical specimens were tested for EGFR mutations retrospectively because of recurrence of cancer after a period of time after initial curative treatment. There were 1,071 adenocarcinomas and 190 nonadenocarcinomas (93 squamous cell, 3 large cell, 4 adenosquamous cell, and 90 NSCLC not otherwise specified). Of the total 1,261 patients, 832 were smokers and 429 were never smokers; 627 (49.8%) patients had EGFR mutations, and 634 patients had wild-type EGFR (Table 1). The mutations were more frequent in never smokers than smokers (59.5% vs. 30.8%, $P < 0.001$), in adenocarcinomas than nonadenocarcinomas (56.5% vs. 11.6%, $P < 0.001$), and in women than in men (58.9% vs. 39.9%, $P < 0.001$).

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Of the 627 patients with EGFR mutations, 258 (41.1%) had deletions in exon 19, 260 (41.5%) had L858R in exon 21, 25 (4.0%) had insertions or duplications in exon 20, 6 had *de novo* T790M (1.0%, all 6 were T790M + L858R *de novo* complex mutation), and 78 (12.4%) had other single or complex uncommon mutations. The demographics of total patients with EGFR mutations and patients with uncommon mutations of unknown clinical significance are listed in Table 1. There were larger proportions of men (48.7% vs. 37.3%, $P = 0.053$) and smokers (28.2% vs. 19.9%, $P = 0.092$) in the group of patients with uncommon EGFR mutations of unknown clinical significance than in the group of patients with classical EGFR mutations.

EGFR TKI treatment in patients with uncommon EGFR mutations of unknown clinical significance

Medical records from all 78 patients with uncommon EGFR mutations of unknown clinical significance were reviewed in detail. Of the 78 patients, 62 had received EGFR TKI treatment (Table 2; Supplementary Table S1). Of the 62 patients, 1 (case 39) received radiotherapy with TKI treatments, and the other 61 patients received TKIs solely (without concurrent chemotherapy agents or radiotherapy for the primary lung tumor). Some of these patients had been reported in previously published studies (6, 15, 22–26, 28, 29). Gefitinib was administered to 51 patients and the other 11 received erlotinib. EGFR TKIs were used as first-line treatment for 38 patients, second line for 14, and third or later lines for 10. At the start of EGFR TKI treatment, 15 were stage IIIb patients with malignant pleural effusion, and the other 47 were stage IV patients.

The response rate to EGFR TKIs in patients with uncommon EGFR mutations of unknown clinical significance was 48.4% (30 of 62) and the disease control rate was 62.9% (39 of 62). The median PFS of EGFR TKIs was 5.0 months (range, 0.2–37.5). The median overall survival after start of EGFR TKIs was 15.0 months (range, 1.1–59.3 months).

To evaluate the effectiveness of EGFR TKI treatment in patients with uncommon EGFR mutations of unknown clinical significance, we included patients with classical EGFR mutations, L858R and deletions in exon 19, for comparison. In the 518 patients with classical mutations, clinical staging was follows: 69 stage I patients, 16 stage II patients, 16 stage IIIa patients, 61 stage IIIb patients, and 356 stage IV patients. Compared with the clinical stages of patients with uncommon mutations (3 stage Ib patients, 1 stage IIb patients, 1 stage IIIa patients, 7 stage IIIb patients, and 66 stage IV patients), the clinical staging was not significantly different between patients with classical mutations and those with uncommon mutations of unknown clinical significance ($P = 0.100$).

Two hundred seventy-eight patients with classical mutations received TKI treatment. In patients with uncommon mutations of unknown significance, we excluded case 39 (G719C + S768I), who received TKI and concomitant radiotherapy, from the survival comparison (Table 2). The response rate to EGFR TKIs was significantly higher in individuals with the classical EGFR mutations than in those with uncommon mutations of unknown clinical significance (74.1% vs. 47.5%, $P < 0.001$). The median PFS (8.5 vs. 5.0 months) and median overall survival after start of EGFR TKIs (19.6 vs. 15.0 months) were also greater in the classical EGFR mutations than in uncommon mutations of unknown clinical significance, but the difference did not reach statistical significance ($P = 0.101$ and 0.477; Figs. 1 and 2).

G719 and L861 mutations

Of the 62 patients who had uncommon EGFR mutations of unknown clinical significance and received EGFR TKI treatment, 30 had a single mutation and the other 32 had complex mutations (Table 3; Supplementary Table S1). Amino acid substitution mutations, G719 (G719A, G719C, G719D, and G719S), and mutations of L861 (L861Q and L861R) comprised the 2 largest groups. G719 mutations were noted in 15 patients (8 single and 7 complex mutations), and L861 mutations were also noted in 15 patients (7 single and 8 complex mutations). Two patients had complex mutations located in both G719 and L861 (cases 40 and 41).

The effectiveness of EGFR TKI treatment on mutations on G719 and L861 was also evaluated separately. The response rate in patients with mutations on G719 was 53.3% (8 of 15). The median PFS was 8.1 months and median overall survival was 16.4 months. The response rate in patients with mutations on L861 was 60% (9 of 15). The median PFS was 6.0 months and median overall survival was 15.2 months.

In all the patients with G719 or L861 mutations, the response rate to TKIs was 57.1% and the median PFS was 6.0 months. The effectiveness of TKI treatment was similar between the entire population of uncommon mutations of unknown clinical significance and the mutations on G719 or L861.

Mutations other than G719 and L861

Besides G719 and L861, 5 patients had mutations on E709 and 4 patients had mutations on S768. Unlike G719 and L861, mutations of E709 and S768 did not exist alone. They were all complex mutations, which occurred with other mutations such as G719 or L858R.

Out of the total of 62 patients, 15 had mutations without G719, L861, and not in combination with L858R or deletions in exon 19. This group is distinct from G719 and L861. They had a lower response rate to EGFR TKIs (20.0%; 3 of 15). Their median PFS (1.6 vs. 6.0 months, $P = 0.002$) and median overall survival (11.1 vs. 16.4 months, $P = 0.157$) were both lower than those in patients with mutations on G719 or L861 (Table 4).

Patients who did not receive EGFR TKI treatment

Of the 78 patients who had uncommon EGFR mutations of unknown clinical significance, 16 did not receive TKIs in their treatment course (Supplementary Table S2). Four patients (cases 67, 68, 74, and 77) had been diagnosed

Published OnlineFirst April 29, 2011; DOI: 10.1158/1078-0432.CCR-10-3408

Table 2. Comparison between uncommon mutations of unknown clinical significance and different mutations in EGFR TKI-treated NSCLC patients

	Uncommon mutations of unknown clinical significance	Deletion in exon 19 and L858R in exon 21	*P*[a]	Wild-type EGFR	*P*[b]	Insertions in exon 20	*P*[c]
No. of patients	61[d]	278		272		11	
Age, median (range); y	66 (39–92)	65 (33–91)	0.606	66 (25–94)	0.953	58 (44–84)	0.319
Gender			0.082		0.331		0.213
Male	29	99		148		3	
Female	32	179		124		8	
Smoking			0.095		0.147		0.733
Smoker	19	59		112		4	
Never smoker	42	219		160		7	
Histology			0.324		0.004		0.166
Adenocarcinoma	60	266		230		10	
Nonadenocarcinoma	1	12		42		1	
Stage			0.272		0.311		0.064
IIIb	5	37		35		3	
IV	56	241		237		8	
Performance status			0.228		0.811		0.244
0–1	44	178		138		6	
>1	17	100		134		5	
TKI			0.754		0.090		0.464
Gefitinib	50	223		194		10	
Erlotinib	11	55		78		1	
TKI line			0.391		0.538		0.939
First line	37	158		161		7	
Second line	14	52		60		2	
Third or later lines	10	68		51		2	
Response to TKI							
Responders	29 (47.5%)	206 (74.1%)	<0.001	45 (16.5%)	<0.001	0 (0%)	0.003
PFS (median month)	5.0	8.5	0.101	2.0	<0.001	1.4	<0.001
OS (median month)	15.0	19.6	0.477	10.4	0.030	4.8	0.242

Abbreviation: OS, overall survival.

[a]Comparison between uncommon mutations of unknown clinical significance and classical mutations (deletion in exon 19 and L858R in exon 21).

[b]Comparison between uncommon mutations of unknown clinical significance and wild-type EGFR.

[c]Comparison between uncommon mutations of unknown clinical significance and insertions in exon 20.

[d]One patient who received concurrent radiotherapy and gefitinib was excluded from the comparison.

in early stage, and received an operation without subsequent recurrence. One patient (case 70) was in stage IIIa when lung cancer was diagnosed. Seven patients received chemotherapy for their cancer (cases 63, 64, 66, 72, 75, 76, and 78). One patient received treatment in another hospital after a diagnosis of cancer (case 69). Three patients (case 65, 71, and 73) received supportive care after their diseases were diagnosed as being at an advanced stage.

EGFR TKI effectiveness with patients having insertions/duplications in exon 20, *de novo* T790M, and with wild-type EGFR

We compared the effectiveness of EGFR TKIs in patients having insertions/duplications in exon 20 and patients with uncommon EGFR mutations of unknown clinical significance. In the 25 patients having insertions/duplications in exon 20, 11 received EGFR TKI treatment. Both the response rate (0% vs. 47.5%, $P = 0.003$) and the median PFS (1.4 vs. 5.0months, $P < 0.001$) of EGFR TKI treatment were worse in patients having insertions/duplications in exon 20 (Table 2; Fig. 1). Median overall survival was also short in patients having insertions/duplications in exon 20 (4.8 vs. 15.0 months, $P = 0.242$; Fig. 2), but the difference was not significantly different, which might be due to small case number.

In the 6 patients having *de novo* T790M (all having *de novo* T790M and L858R at baseline), 1 had stage IIa disease and received curative surgery; 1 had stage IV disease and was

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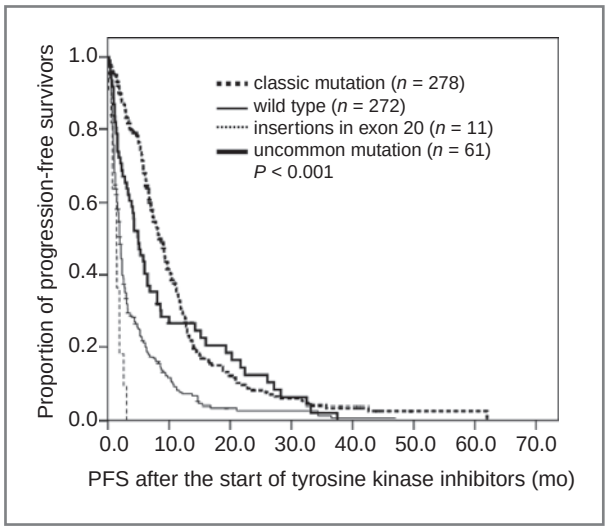

Figure 1. PFS after the start of EGFR TKIs in patients with classical EGFR mutations, wild-type EGFR, insertions in exon 20, or uncommon mutations of unknown clinical significance.

treated with chemotherapy without EGFR TKI; 4 patients received EGFR TKI treatment and all were not responsive to treatment (response rate, 0%, median PFS, 1.2 months).

We also compared the effectiveness of EGFR TKIs in patients with uncommon EGFR mutations of unknown clinical significance and patients with wild-type EGFR (Table 2). There were 272 patients with wild-type EGFR who received EGFR TKIs during this period. The response rate was higher in uncommon mutations than in the wild-type EGFR (47.5% vs. 16.5%, $P < 0.001$). The median PFS (5.0 vs. 2.0 months, $P < 0.001$) and median overall survival (15.0 vs. 10.4 months, $P = 0.030$) were also greater in uncommon mutations than in the wild-type EGFR (Figs. 1 and 2).

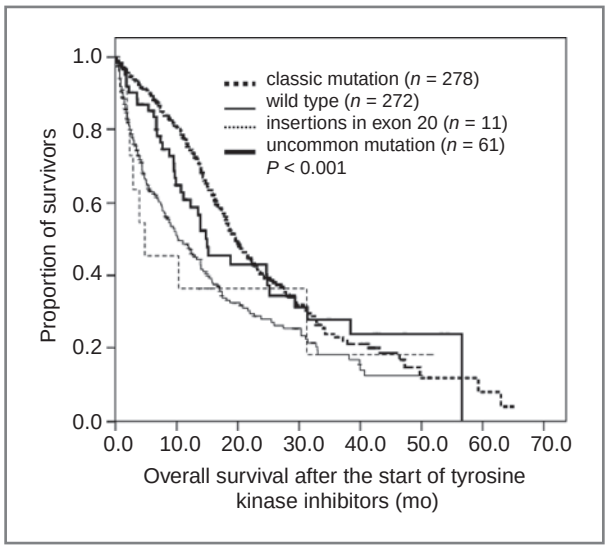

Figure 2. Overall survival after the start of EGFR TKIs in patients with classical EGFR mutations, wild-type EGFR, insertions in exon 20, or uncommon mutations of unknown clinical significance.

Discussion

This study included a large group of patients who underwent testing for EGFR mutations, and presented patients with uncommon EGFR mutations of unknown clinical significance. These relatively rare mutations comprised 12.4% of the entire EGFR mutations. Generally, patients with these uncommon mutations who received EGFR TKI treatment had shorter, though not statistically significant, PFS and overall survival compared with patients with L858R or deletions in exon 19. On the contrary, their outcomes were better than those with wild-type EGFR. Moreover, these uncommon mutations of unknown clinical significance were composed of heterogeneous groups, which had different responses to EGFR TKIs.

The mutations of amino acid substitutions at G719 and L861 were the 2 major groups in our population. These mutations were also noted in other study populations, and they were, in some studies, regarded as having well-known relevance to EGFR TKIs (12, 30). However, case numbers were small in previous studies and conclusions could not be made on the basis of these 2 EGFR mutation groups. In the large series of Shigematsu and colleagues (2), 130 mutations were detected in 617 tumor samples, with 3 G719 mutations (G719A, G719C, and G719S) and 1 L861Q. In the BR21 clinical trial, there were no G719 or L861 mutations in a total of 40 tumors with EGFR mutations (8). And in the series of Pallis and colleagues (12), which comprised 25 EGFR mutant tumors, 1 G719D and 1 L861P (combined with L858R) were found. In this study, 15 tumors contained L861 (L861Q and L861R) mutations and 15 tumors harbored G719 (G719A, G719C, G719D, and G719S) mutations. In a review by Mitsudomi (31), the response rate to TKIs of G719 was 55.6% (5 responders in 9 patients). In the study of Hata (32), 5 patients with complex mutation G719S + L858R received gefitinib and 2 (40.0%) had a response. In our study, the response rates were 53.3% for G719, 60.0% for L861, and 74.1% for classical mutations (deletions in exon 19 and L858R). We found that EGFR TKI treatment could lead to favorable responses in patients who had G719 or L861 mutations, though not as favorable as for patients with classical mutations. Our findings confirmed that G719 and L861 mutations were also sensitive mutations for EGFR TKIs (33).

This finding was also supported by a laboratory study conducted by Kancha and colleagues (34). Distinct biological features of different EGFR mutations were displayed. L858R and deletions in exon 19 were sensitive to both gefitinib and erlotinib, with very low inhibition concentration 50% (IC_{50}). G719S and L861Q required higher drug concentrations to inhibit the cancer cells than did L858R and deletions in exon 19. Moreover, S768I was more resistant to either gefitinib or erlotinib than were G719S and L861Q, and T790M had the highest IC_{50}.

Published OnlineFirst April 29, 2011; DOI: 10.1158/1078-0432.CCR-10-3408

Table 3. Types of uncommon *EGFR* mutations and response to gefitinib or erlotinib of 62 patients

No. of patients	*EGFR* mutation[a]	Mutation exon	Response to TKIs
2	DelE709-T710 insD	18	1 SD, 1 PD
6	G719A [23]	18	3 PR, 3 PD
2	G719D	18	1 PR, 1 SD
1	V742A [6]	19	1 SD
2	L747P [23]	19	2 PD
1	V774A	20	1 PR
1	V774M	20	1 PD
1	F784F	20	1 PD
1	K806E [15]	20	1 PD
1	N826Y	21	1 PD
1	V834I	21	1 PR
1	L838P [6], [25]	21	1 PR
1	N842S	21	1 PD
1	T847I	21	1 PD
1	V851I [25], [29]	21	1 PD
6	L861Q [23], [29]	21	4 PR, 1 SD, 1 PD
1	L861R	21	1 PR
1	Q701L + I706T + G719S	18	1 PR
1	E709A + L858R	18 + 21	1 SD
1	E709G + G719C	18	1 PD
2	E709G + L858R	18 + 21	1 PR, 1 PD
1	E709V + L858R	18 + 21	1 PR
1	G719A + S720F [23], [24]	18	1 PR
1	G719A + S768I [24]	18 + 20	1 PD
1	G719C + S768I [24]	18 + 20	1 PR
1	G719D + L861Q	18 + 21	1 PR
1	G719S + L861Q [24]	18 + 21	1 SD
1	E746G + L861Q	19 + 21	1 PR
1	L747S + L858R	19 + 21	1 PD
1	E758G + L858R [24]	19 + 21	1 PR
2	S768I + L858R [22], [24]	20 + 21	2 PR
1	V769M + Del in exon19	20 + 19	1 PD
1	R776G + L858R [15]	20 + 21	1 PD
1	R776H + L858R [24]	20 + 21	1 PR
1	R776H + L861Q [22], [24]	20 + 21	1 SD
1	G779S + L858R [24]	20 + 21	1 PR
1	E804K + Del in exon19 [24]	20 + 19	1 PR
1	R831C + L861R [28]	21	1 PR
1	R831H + L861Q [22], [24]	21	1 SD
1	V834L + L858R [24]	21	1 PR
2	H850D + L858R [22], [24]	21	1 SD, 1 PD
1	K860I + L858R [24]	21	1 PR
1	K860I + L861Q [24]	21	1 SD
1	L861F + L858R [24]	21	1 PR
1	A871E + L858R	21	1 PD
1	A871V + Del in exon19 [6]	21+19	1 PR

Abbreviations: Del, deletion; ins, insertion; PR, partial response; SD, stable disease; PD, progressive disease
[a]References mentioned are those in which the case was previously reported.

Chen and colleagues (35) showed that EGFR mutations are seldom singlets, but are actually almost doublets if the mutations occur at 1 of the 5 amino acids, E709, G719, S768, T790, and L861. Our findings are partly consistent with Chen's study. Mutations of either E709 or S768 were found in 9 patients, and their mutations were all doublets.

Published OnlineFirst April 29, 2011; DOI: 10.1158/1078-0432.CCR-10-3408

Table 4. Different categories of *EGFR* mutations and response to EGFR TKIs

No. of patients	*EGFR* mutation	RR (%)	PFS (mo)	OS (mo)
278	Single classical mutation (deletions in exon 19 or L858R)	74.1	8.5	19.6
272	Wild type	16.5	2.0	10.4
11	Insertions in exon 20	0	1.4	4.8
15	G719 (single or complex)	53.3	8.1	16.4
15	L861 (single or complex)	60.0	6.0	15.2
20	Uncommon mutations with combination with deletions in exon 19 or L858R	60.0	5.3	18.8
15	Uncommon mutations without combination with deletions in exon 19 or L858R or G719 or L861	20.0	1.6	11.1

Abbreviations: RR, response rate; OS, overall survival.

However, approximately half of G719 and L861 occur as single mutation.

Some other uncommon EGFR mutations, that is, those without G719, L861, or combinations of classical mutations, are associated with less effectiveness of TK inhibitors. With these EGFR mutations, this group of patients (15 patients in this study) had a lower response rate (20.0%) and a PFS of only 1.6 months. The clinical associations of uncommon EGFR mutations with effectiveness of EGFR TKIs are heterogeneous, as revealed in this study.

Identifications of very rare EGFR mutations may sometimes be due to PCR artifact with the use of formalin-embedded tissue (36, 37). Reports of the same rare EGFR mutations from different investigators help to decrease the possibility of identification of novel mutations that do not really exist. By searching the database (38), we found that some uncommon EGFR mutations in this study also could be found in other studies. V742A and T847I were also found in the phase III BR 21 study (8). Complex mutation V769M and deletion in exon 19 were noted in Huang's study (39). V851I was presented in Cappuzzo's study, in which a patient with mutation V851I did not respond to gefitinib treatment, as was the case for the patient in our study (40). Complex mutation R776H with L858R exists in a patient in Kosaka's study, and gefitinib was also effective to the patient, as in our study (41). Our findings of these uncommon mutations of unknown clinical significance and their association to EGFR TKIs, combined with those in other reports, help to guide the use of EGFR TKIs in patients with EGFR mutations (Supplementary Tables S3 and S4).

L747S EGFR mutation has been reported as an acquired resistant mutation after previous gefitinib use. In the study of Costa and colleagues (42), 1 patient, who had a tumor harboring L858R mutation, had a partial response after taking gefitinib. After a period of successful gefitinib treatment, he experienced failure of gefitinib with the new development of L747S mutation. The tumor carried L747S and L858R whereas resistance to gefitinib was noted, and still responded to erlotinib treatment. In our study, 1 patient also had the complex mutation L747S and L858R (case 43). This complex mutation existed before the administration of EGFR TKI, and so was a *de novo* mutation rather than an acquired mutation. Similarly, the patient who had L747S and L858R was not responsive to gefitinib in our study. Another point mutation in amino acid L747 (L747P) was noted in 2 patients in our population (cases 12 and 13). It was a single mutation in the 2 patients, and both patients did not respond to TK inhibitors (1 gefitinib and the 1 erlotinib). As for T790M and insertions in exon 20, mutations in point L747 can indicate poor responsiveness to EGFR TKIs. Besides L747, another 2 mutations, V769M and A871E, existed together with classical mutation deletion in exon 19 and L858R in 2 patients of our study group. These 2 mutations, V769M and A871E, also led to poor response to TKIs, despite the coexistence of sensitive classical mutations. Further clinical data are needed to clarify the nature of these 2 EGFR mutations.

Compared with the EGFR mutations of unknown clinical significance, another category of EGFR mutations, insertion or duplication in exon 20, makes up an even smaller proportion. In this study, insertions or duplications in exon 20 account for only 4.0% of all EGFR mutations. A part of the report was presented in our previous study (15). Their clinical relevance is clear despite their low number (15, 16). We did not include these mutations in the criteria of "uncommon mutations of unknown clinical significance." In this study, 25 patients in our population had insertions or duplications in exon 20. Of these 25 patients, 11 received tyrosine kinase, and none of them were responsive to the treatment. The finding was consistent with those of our previous report (15).

The major limitation of this report is its retrospective nature. In addition, although the number of patients with uncommon EGFR mutations in this study was relatively

Published OnlineFirst April 29, 2011; DOI: 10.1158/1078-0432.CCR-10-3408

large compared with other studies, more clinical experience in the treatment of patients with these EGFR mutations is needed. We anticipate more clinical studies on these uncommon mutations.

In conclusion, we reported on a large group of lung cancer patients with uncommon EGFR mutations of unknown clinical significance and their association with treatment of EGFR TKIs. These EGFR mutations comprised a distinct proportion of the total EGFR mutations populations, and are worthy of notice. G719 and L861 composed a major portion of these EGFR mutations, and were associated with favorable effectiveness of TKIs, which were a little worse than the well-known classical EGFR mutations, deletions in exon 19 and L858R in exon 21. On the contrary, others of these rare uncommon EGFR mutations failed to respond favorably to EGFR TKIs. Our report may help to guide choices for therapy in NSCLC patients.

Disclosure of Potential Conflicts of Interest

C.-H. Yang, C.-J. Yu, and J.-Y. Shih have received honoraria from AstraZeneca and Roche.

Acknowledgments

The authors thank the Department of Medical Research, National Taiwan University Hospital for providing support to this study.

Grant Support

This study was supported by grants 98-2314-B-002-117-MY3 and 98-2628-B-002-087-MY3 (National Science Council, Taiwan); DOH98-TD-G-111-031 (Department of Health, Executive Yuan, Taiwan); and 99C101-101 (National Taiwan University, Taiwan).

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Received December 23, 2010; revised February 11, 2011; accepted March 22, 2011; published OnlineFirst April 29, 2011.

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Published OnlineFirst April 29, 2011; DOI: 10.1158/1078-0432.CCR-10-3408

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Effectiveness of Tyrosine Kinase Inhibitors on "Uncommon" Epidermal Growth Factor Receptor Mutations of Unknown Clinical Significance in Non–Small Cell Lung Cancer

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Clin Cancer Res 2011;17:3812-3821. Published OnlineFirst April 29, 2011.