Good Response to Gefitinib in Lung Adenocarcinoma of Complex Epidermal Growth Factor Receptor (EGFR) Mutations with the Classical Mutation Pattern

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Key Words. EGFR mutation • Gefitinib • Lung cancer • EGFR TKI • Complex mutation pattern

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ABSTRACT

Background. Epidermal growth factor receptor (EGFR) mutations are usually detected in lung adenocarcinoma and are associated with a response to EGFR tyrosine kinase inhibitors (TKIs). However, not all EGFR mutations have similarly high clinical response rates. This study aimed to investigate the clinical characteristics and response to gefitinib in lung adenocarcinoma patients with complex EGFR mutations.

Materials and Methods. Three hundred thirty-nine specimens of lung adenocarcinoma from patients treated with gefitinib were collected for EGFR sequencing. Nineteen patients with complex EGFR mutations were enrolled for the study after excluding three patients with the EGFR T790M mutation, which confers resistance to gefitinib.

Results. Among the 19 patients, 12 had complex mutations with the classical mutation pattern (L858R or deletion in exon 19). When compared with those without the classical mutation pattern, patients with this mutation pattern had a higher response rate (83% versus 29%), longer progression-free survival duration (median, 12.7 months versus 4.9 months), and longer overall survival time (median, 24.7 months versus 12.3 months) after gefitinib treatment.

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Comparing patients harboring complex $\text{EGFR}$ mutations with a classical mutation pattern with those harboring single classical mutations, there were no statistical differences in the response rate (83% versus 73%), progression-free survival time (median, 12.7 months versus 8.1 months), or overall survival time (median, 24.7 months versus 16.4 months).

**Conclusion.** Patients with complex $\text{EGFR}$ mutations with the classical mutation pattern had the same response rate, progression-free survival duration, and overall survival time as those with single classical mutations. $\text{EGFR}$ TKIs may be the choice of treatment for this type of lung adenocarcinoma. *The Oncologist* 2008; 13:000–000

**INTRODUCTION**

Epidermal growth factor receptor (EGFR) is a member of the ErbB receptor family and is a transmembrane glycoprotein encoded by a gene located in the short arm of chromosome 7 [1]. Activation of $\text{EGFR}$ controls cell proliferation, antiapoptosis, angiogenesis, differentiation, and invasion [2], which are regulated via the phosphorylation of several tyrosine kinase residues after ligands bind to the extracellular domain of $\text{EGFR}$. The expression of $\text{EGFR}$ is correlated with poor prognosis and is seen in 50% of non-small cell lung cancer (NSCLC) cases on immunohistochemistry [3].

The $\text{EGFR}$ tyrosine kinase inhibitors (TKIs) gefitinib (Iressa®; AstraZeneca, Wilmington, DE) and erlotinib (Tarceva®; Genentech, South San Francisco, CA) are used to treat NSCLC. A higher response to $\text{EGFR}$ TKIs is noted in specific subgroups that include females, never smokers, patients with adenocarcinoma histology, and East Asians [4, 5]. Higher $\text{EGFR}$ mutation rates are also noted in these subgroups [6] and are also related to a better response to $\text{EGFR}$ TKIs and longer survival [7–9]. The in-frame deletion in exon 19 (del-19) accounts for 45% of mutations, while the point mutation L858R in exon 21 accounts for around 40%–45% of $\text{EGFR}$ mutations in lung cancer [3, 7, 8, 10–13].

Patients with both the del-19 and the L858R mutations have good responses to $\text{EGFR}$ TKIs and these are termed “classical mutations” [11]. Patients with these mutations exhibit objective response rates in the range of 75%–95% [7–9, 14–16]. In addition to the classical mutations, other $\text{EGFR}$ mutations in exons 18–21 have also been reported, although quite rarely. Patients with these nonclassical mutations have variable responses to $\text{EGFR}$ TKIs [12, 17, 18].

Most studies on the relationship between $\text{EGFR}$ mutations and response to $\text{EGFR}$ TKIs show a single site mutation. However, two or more concomitant sites of $\text{EGFR}$ mutations have also been detected despite the low patient number. Chen et al. [19] showed that the frequency of double $\text{EGFR}$ mutations is around 6% in all $\text{EGFR}$ mutations of lung cancer by an analysis of the literature. Concomitant $\text{EGFR}$ mutations have been termed “complex mutations” [20]. A second mutation may substantially alter the biological properties of the mutant $\text{EGFR}$ and sensitivity to TKIs [21]. For example, T790M is a noteworthy acquired $\text{EGFR}$ mutation after $\text{EGFR}$ TKI treatment in patients with L858R or del-19, which confers resistance to $\text{EGFR}$ TKI treatment [22, 23].

Although most studies have shown that patients with $\text{EGFR}$ mutations have a good response to gefitinib, it remains uncertain whether patients with complex $\text{EGFR}$ mutations have a similarly good response. This study investigated the clinical characteristics and response to gefitinib of patients with lung adenocarcinoma and complex $\text{EGFR}$ mutations. Because T790M is a well-known gefitinib resistance mutation, we excluded those patients with T790M from this analysis. A literature review of all published reports on complex $\text{EGFR}$ mutations and $\text{EGFR}$ TKI responsiveness is also provided.

**MATERIALS AND METHODS**

**Patients and Tissue Procurement**

The study group included lung adenocarcinoma patients diagnosed at the National Taiwan University Hospital between June 2004 and July 2007. Tumor specimens obtained by either surgical or needle biopsy/aspiration procedures, including primary lung tumors, malignant effusion cell blocks, and other distant metastases, were sequenced for mutational analysis. Written informed consent for use of the tissue in a molecular analysis was acquired from the patients at the procurement of the tumor specimens. This study was approved by the institutional review board of the National Taiwan University Hospital.

Lung cancer histology was defined according to the World Health Organization pathology classification [24]. A complete lung cancer staging workup was performed as routine practice, which included bronchoscopy, computed tomography (CT) of the head, chest, and abdomen, and whole-body bone scintigraphy. The date of diagnosis, all chemotherapy received, and responsiveness to the chemotherapy were recorded.

All patients with stage IIIb or IV NSCLC who received
gefitinib were identified from the records of the Department of Pharmacy in the hospital. Their clinical data, including demographic information, cancer cell type, smoking status, and imaging studies, were collected. Patients who had smoked <100 cigarettes in their lifetime were categorized as nonsmokers. Those who had smoked within 1 year of the diagnosis were categorized as current smokers. The rest were categorized as former smokers.

Response Evaluation of Lung Adenocarcinoma Patients

Gefitinib was taken orally at a dose of 250 mg daily. No concurrent chemotherapy or radiotherapy for the lung tumors was given during gefitinib therapy. Chest radiography every 2–4 weeks and a chest CT scan (including the liver and adrenal glands) every 2–3 months were performed as routine clinical practice, and as needed to confirm response and disease progression.

The unidimensional method was used according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines for measuring solid tumors [25]. Complete response (CR), partial response (PR), and stable disease (SD) were confirmed by a sustained four-week follow-up. Only patients with CR and PR were regarded as responders.

The progression-free survival duration was calculated from the date of initiation of gefitinib treatment to the date of disease progression, death, last follow-up, or the final follow-up day of the study. The overall survival duration after gefitinib was calculated from the date of initiation of gefitinib treatment to the date of death, last follow-up, or the final follow-up day of the study.

Sequencing of EGFR Exons 18–21

Tumor specimens, including frozen tissues or paraffin blocks, were collected. DNA was derived from tumors embedded in paraffin blocks using the QIAmp DNA Mini Kit (Qiagen, Valencia, CA) for EGFR mutation analysis as described previously [26]. The tyrosine kinase domain of the EGFR coding sequence, exons 18, 19, 20, and 21, was amplified by independent polymerase chain reaction (PCR) amplifications.

RNA was extracted from frozen tissue with a QIAamp RNA Mini Kit (Qiagen) according to the manufacturer’s protocol. Spectrophotometry was used to measure the amount of extracted RNA, according to the protocol of Mitsuomi et al. [9]. Exons 18–21 of the EGFR gene were amplified from cDNA with the forward primer 5′-AGCTTGTGGAGCCTCTTACACC-3′ and the reverse primer 5′-TAAAATTGATTCATGCCATCC-3′. A Qiagen OneStep reverse transcription (RT)-PCR kit (Qiagen) was used for the RT-PCR, as described previously [9]. PCR amplicons were sequenced with an ABI PRISM 3100 (Applied Biosystems, Foster City, CA) in both the sense and antisense directions. Specimens with EGFR mutations were confirmed twice. Only specimens with the same results identified in both rounds were recorded as mutation-positive. Mutations were also checked against the single nucleotide polymorphism database. The single-site EGFR mutations in-frame del-19 or point mutation L858R in exon 21 were defined as classical mutations. Other mutations were infrequently detected, and were defined as nonclassical EGFR mutations. When two or more concomitant different EGFR mutations were detected, the mutation types were defined as complex EGFR mutations. The term “classical mutation pattern” was used when the complex EGFR mutation had either an in-frame del-19 or a point mutation L858R in exon 21.

Statistical Analysis

All of the categorical variables were analyzed with Fisher’s exact test because of the small study size. A univariate analysis of the patient characteristics was used for the predictive factor of EGFR TKI response. The overall survival curve and progression-free survival curve were plotted using the Kaplan–Meier method and compared by a log-rank test. Two-sided p-values <.05 were considered significant. All analyses were performed using SPSS software (version 13.0 for Windows; SPSS Inc., Chicago, IL).

RESULTS

Clinical Characteristics and EGFR Mutations of Lung Adenocarcinoma Patients

A total of 725 NSCLC patients received gefitinib treatment between June 2004 and October 2007. Specimens from 339 lung adenocarcinoma patients who had measurable disease and received gefitinib therapy for stage IIIb or IV lung adenocarcinoma were collected for EGFR sequencing. Five patients’ tissue samples were inadequate for EGFR sequencing.

Finally, 334 gefitinib-treated patients (124 men and 210 women) provided EGFR mutation data. Among them, 211 patients had tumors harboring EGFR mutations (63.2%, 211 of 334). One hundred sixty-eight patients had tumors with classical mutations (83 with del-19 and 85 with L858R), and 43 patients had tumors with nonclassical EGFR mutations, including complex EGFR mutations. These patients have been reported in our previous studies [16, 17, 26–28]. In the present study, we update the follow-up data and focus on those patients who had complex mutations.
Clinical Characteristics and EGFR Mutations of Lung Adenocarcinoma Patients with Complex EGFR Mutation Patterns

Because patients with T790M mutations have been shown to have a high resistance to EGFR TKIs [23, 29, 30], three patients with T790M were excluded from this study. There was a total of 19 lung adenocarcinoma patients who had undergone gefitinib treatment and had complex EGFR mutations (Tables 1 and 2). The median age at which lung cancer was diagnosed was 62.8 years (range, 45.5–78.3 years), with four smokers and 15 nonsmokers. The distribution of lung cancer stages was one stage IIIA patient, one stage IIIB patient, and 17 stage IV patients. The patient with stage IIIA refused surgical treatment and took systemic chemotherapy according to patient preference. Twelve patients had the classical mutation pattern of L858R or del-19. No concurrent del-19 and L858R mutations were observed.

Response of Adenocarcinoma Patients with Complex EGFR Mutations Treated with Gefitinib

Among the 19 patients with complex EGFR mutations, 13 had gefitinib as first-line treatment. Two and four patients took gefitinib as second-line and third-line treatment, respectively. The maximal responses of the previous chemotherapies were one PR, two SD, two progressive disease (PD), and one unevaluated.

Among the 19 patients, 12 had the classical mutation pattern. There were no differences in gender, smoking status, age (≥65 or <65 years), lung cancer stage, and prior chemotherapy use between patients with complex EGFR mutations with the classical mutation pattern and those with complex EGFR mutations without the classical mutation pattern. Patients with the classical mutation pattern had a higher gefitinib response rate (83%, 10 of 12) than those without the classical mutation pattern (29%, 2 of 7) (p = .045) (Table 2).

PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL AFTER EGFR TKI TREATMENT

Patients with nonclassical EGFR mutations (n = 43) had a lower gefitinib response rate (44% [19 of 43] versus 73% [122 of 168]; p < .001), shorter progression-free survival time (median, 4.8 months versus 8.1 months; p = .009), and shorter overall survival (median, 13.5 months versus 16.4 months; p = .239) than patients with classical EGFR mutations (n = 168).

Among the 19 patients with complex EGFR mutations,

<p>| Table 1. Clinical features and response to gefitinib of 19 lung adenocarcinoma patients with complex EGFR mutations |
|---|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>n</th>
<th>Gender</th>
<th>Age</th>
<th>Smoker</th>
<th>Stage</th>
<th>Gefitinib response</th>
<th>Progression</th>
<th>PFS (mos)</th>
<th>Status</th>
<th>OS (mos)</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
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<td>F</td>
<td>53.5</td>
<td>N</td>
<td>IV</td>
<td>PR</td>
<td>+</td>
<td>14.3</td>
<td>Dead</td>
<td>14.4</td>
<td>Del-19 + E804K</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>65.4</td>
<td>N</td>
<td>IV</td>
<td>PR</td>
<td>+</td>
<td>8.1</td>
<td>Dead</td>
<td>24.7</td>
<td>Del-19 + A871V</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>73.6</td>
<td>Current</td>
<td>IV</td>
<td>PR</td>
<td>+</td>
<td>6.0</td>
<td>Dead</td>
<td>13.9</td>
<td>L858R + E758G</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>45.5</td>
<td>N</td>
<td>IV</td>
<td>PR</td>
<td>−</td>
<td>19.3</td>
<td>Alive</td>
<td>19.3</td>
<td>L858R + S768I</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>54.6</td>
<td>N</td>
<td>IV</td>
<td>PR</td>
<td>+</td>
<td>16.0</td>
<td>Alive</td>
<td>31.4</td>
<td>L858R + R776H</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>76.1</td>
<td>N</td>
<td>IV</td>
<td>PR</td>
<td>+</td>
<td>18.8</td>
<td>Dead</td>
<td>22.4</td>
<td>L858R + G779S</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>64.0</td>
<td>N</td>
<td>IV</td>
<td>PR</td>
<td>+</td>
<td>12.7</td>
<td>Alive</td>
<td>23.1</td>
<td>L858R + G779S</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>59.3</td>
<td>N</td>
<td>IV</td>
<td>PR</td>
<td>+</td>
<td>26.1</td>
<td>Alive</td>
<td>42.7</td>
<td>L858R + V834L</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>57.5</td>
<td>N</td>
<td>IIIB</td>
<td>PR</td>
<td>−</td>
<td>14.8</td>
<td>Alive</td>
<td>14.8</td>
<td>L858R + K860I</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>66.4</td>
<td>N</td>
<td>IV</td>
<td>PR</td>
<td>+</td>
<td>5.3</td>
<td>Dead</td>
<td>13.5</td>
<td>L858R + L861F</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>46.6</td>
<td>Former</td>
<td>IV</td>
<td>SD</td>
<td>+</td>
<td>3.5</td>
<td>Alive</td>
<td>21.6</td>
<td>L858R + H850D</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>50.0</td>
<td>N</td>
<td>IV</td>
<td>PD</td>
<td>+</td>
<td>1.6</td>
<td>Dead</td>
<td>9.9</td>
<td>L858R + H850D</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>62.8</td>
<td>N</td>
<td>IV</td>
<td>PR</td>
<td>+</td>
<td>8.1</td>
<td>Dead</td>
<td>24.8</td>
<td>G719A + S720F</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>78.3</td>
<td>Current</td>
<td>IV</td>
<td>PR</td>
<td>−</td>
<td>6.3</td>
<td>Alive</td>
<td>6.3</td>
<td>G719C + S768I</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>66.1</td>
<td>N</td>
<td>IV</td>
<td>PD</td>
<td>+</td>
<td>0.6</td>
<td>Dead</td>
<td>1.8</td>
<td>G719A + S768I</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>47.7</td>
<td>N</td>
<td>IIIA</td>
<td>SD</td>
<td>−</td>
<td>3.1</td>
<td>Dead</td>
<td>14.8</td>
<td>G719S + L861Q</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>65.9</td>
<td>N</td>
<td>IV</td>
<td>SD</td>
<td>+</td>
<td>1.8</td>
<td>Dead</td>
<td>6.7</td>
<td>R776H + L861Q</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>63.2</td>
<td>N</td>
<td>IV</td>
<td>SD</td>
<td>+</td>
<td>4.9</td>
<td>Dead</td>
<td>6.7</td>
<td>R831H + L861Q</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>58.1</td>
<td>Current</td>
<td>IV</td>
<td>SD</td>
<td>+</td>
<td>4.2</td>
<td>Dead</td>
<td>12.3</td>
<td>K860I + L861Q</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR, epidermal growth factor receptor; N, nonsmoker; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.
the median progression-free survival and overall survival times after gefitinib treatment were 8.1 months (95% confidence interval [CI], 4.2–12.0 months) and 22.4 months (95% CI, 8.4–36.4 months), respectively.

Patients with complex mutations with the classical mutation pattern had a longer progression-free survival time than those without the classical mutation pattern (median, 12.7 months; 95% CI, 3.4–22.0 months versus 4.9 months; 95% CI, 1.5–8.3 months; p = .048, by the log-rank test) (Fig. 1A). Patients with complex mutations with the classical mutation pattern also had a longer overall survival time after gefitinib treatment than those without the classical mutation pattern (median, 24.7 months; 95% CI, 13.0–36.4 months versus 12.3 months; 95% CI, 4.5–20.1 months; p = .027, by the log-rank test) (Fig. 1B).

Comparing patients with complex EGFR mutations with the classical mutation pattern (n = 12) with those harboring single classical mutations (n = 168), there were no statistical differences in the response rate (83%, 10 of 12 versus 73%, 122 of 168; p = .52), progression-free survival (median, 12.7 months versus 8.1 months; p = .39), or overall survival (median, 24.7 months versus 16.4 months; p = .170).

**DISCUSSION**

Prior studies on EGFR mutations in NSCLC patients have mostly shown single EGFR mutations and very few complex EGFR mutations. The responses to EGFR TKIs in these patients were not clear. Here, we demonstrate the characteristics of 19 lung adenocarcinoma patients with

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**Table 2.** Clinical characteristics of gefitinib-treated patients with complex EGFR mutations with or without the classical mutation pattern

<table>
<thead>
<tr>
<th>Variable</th>
<th>Complex EGFR mutations</th>
<th>Classical mutation pattern, n (%)</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total n</td>
<td>19</td>
<td>12 (63)</td>
<td></td>
</tr>
<tr>
<td>Gefitinib treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responder</td>
<td>12</td>
<td>10 (83)</td>
<td>.045</td>
</tr>
<tr>
<td>Nonresponder</td>
<td>7</td>
<td>2 (29)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>4 (57)</td>
<td>1.000</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>8 (67)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥65</td>
<td>7</td>
<td>4 (57)</td>
<td>1.000</td>
</tr>
<tr>
<td>&lt;65</td>
<td>12</td>
<td>8 (67)</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>15</td>
<td>10 (67)</td>
<td>.603</td>
</tr>
<tr>
<td>Former or current smoker</td>
<td>4</td>
<td>2 (50)</td>
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<tr>
<td>Stage</td>
<td></td>
<td></td>
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<tr>
<td>III</td>
<td>2</td>
<td>1 (50)</td>
<td>1.000</td>
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<tr>
<td>IV</td>
<td>17</td>
<td>11 (65)</td>
<td></td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13</td>
<td>8 (62)</td>
<td>1.000</td>
</tr>
<tr>
<td>1–2</td>
<td>6</td>
<td>4 (67)</td>
<td></td>
</tr>
</tbody>
</table>

aAnalyzed with Fisher’s exact test.

Abbreviation: EGFR, epidermal growth factor receptor.
complex EGFR mutations and their responses to gefitinib. Patients with complex EGFR mutations with the classical mutation pattern had the same response rate, progression-free survival time, and overall survival time as those with single classical mutations. Patients with complex EGFR mutations with the classical mutation pattern had a better response to gefitinib than those without the classical mutation pattern. Longer progression-free survival and overall survival times after gefitinib therapy were also noted in patients with the classical mutation pattern.

The present study is the first to determine and assert that patients with complex EGFR mutations with the classical mutation pattern (excluding those with T790M) have the same response rate, progression-free survival time, and overall survival time as patients with single classical mutations (L858R or del-19) after gefitinib treatment. The response rate to gefitinib, 63% (12 of 19), in the patients with complex mutations is also similar to that in patients with single EGFR mutations in other studies [31, 32]. Among the patients with single classical mutations (L858R or del-19), Takano et al. [32] and Riely et al. [33] also showed a progression-free survival duration of 12 months and a median survival time of 20 months. Their survival data were similar to those from patients with complex mutations with the classical mutation pattern in our study. Therefore, the addition of a second mutation, other than T790M, to an exon 19 deletion or L858R substitution does not alter the sensitivity to EGFR TKIs, and it is reasonable to suggest that patients with complex EGFR mutations with the classical mutation pattern can still benefit from gefitinib.

Complex EGFR mutations can appear before treatment [7, 8, 13] or be induced by TKI sequentially [21–23]. For example, a novel high resistance mutation, T790M, is an acquired mutation after EGFR TKI treatment [22, 23]. However, a primary somatic mutation of T790M has also been found [30]. Complex EGFR mutations change not

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**Table 3. Comparison of lung adenocarcinoma of complex EGFR mutation studies related to EGFR TKI responsiveness**

<table>
<thead>
<tr>
<th>Study</th>
<th>Responder</th>
<th>Complex mutation</th>
<th>Study</th>
<th>Nonresponder</th>
<th>Complex mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsieh et al. [20]</td>
<td>PR</td>
<td>Del-19 + L858R</td>
<td>Han et al. [42]</td>
<td>SD</td>
<td>L858R + V819A</td>
</tr>
<tr>
<td></td>
<td>PR × 3b</td>
<td>F712S + D855G + E868G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>V765M + L798H + K806E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ L814P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>Del-19 + L861Q</td>
<td></td>
<td>Asahina et al. [44]</td>
<td>PD</td>
<td>S768I + V769L</td>
</tr>
<tr>
<td>Han et al. [43]</td>
<td>PR</td>
<td>G719A + S768I</td>
<td>Ichihara et al. [45]</td>
<td>NC</td>
<td>L858R + D761Y</td>
</tr>
<tr>
<td>Taron et al. [46]</td>
<td>PR</td>
<td>Del-19 + L861Q</td>
<td>Jackman et al. [38]</td>
<td>SDa</td>
<td>Del-19 + L861Q</td>
</tr>
<tr>
<td>Takano et al. [32]</td>
<td>PR</td>
<td>L858R + S768I</td>
<td>Tokumo et al. [47]</td>
<td>PD</td>
<td>L858R + D761Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G719C + S768I</td>
<td>Chou et al. [48]</td>
<td>NPD</td>
<td>Del-19 + R803W</td>
</tr>
<tr>
<td>van Zandwijk et al. [49]</td>
<td>PR</td>
<td>E709A + G719A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Han et al. [31]</td>
<td>PR</td>
<td>E709K + G719A</td>
<td>Pallis et al. [50]</td>
<td>PD</td>
<td>T847A + G863S</td>
</tr>
<tr>
<td>Kosaka et al. [51]</td>
<td>PR</td>
<td>L858R + L833V</td>
<td></td>
<td>SD</td>
<td>L858R + V843I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PR L858R + R776H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choong et al. [52]</td>
<td>PR</td>
<td>L858R + E884K (exon 22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pugh et al. [53]</td>
<td>PR</td>
<td>Del-19 + V774L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PR S768I + L815L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kimura et al. [54]</td>
<td>PR</td>
<td>L858R + V689L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oshita et al. [55]</td>
<td>PR</td>
<td>Del-19 + F856L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Treated with erlotinib.
* Abbreviations: CR, complete remission; EGFR, epidermal growth factor receptor; NC, no change; NPD, nonprogressive disease; PD, progressive disease; PR, partial response; SD, stable disease; SR, serum response; TKI, tyrosine kinase inhibitor.
* Three patients.
only the amino acid sequence but also the molecular con-
formation. The characteristics of the biological properties
related to complex EGFR mutations have not yet been clar-
ified.

Chen et al. [34] used the H1299 cell line stably trans-
fected with various EGFR mutant constructs. They showed
that different complex EGFR mutations had different ge-
fitinib responsiveness in engineered cell line studies, but in
vitro biochemical and growth inhibition cannot be extrapo-
lated to in vivo tumor response in patients.

In a review of the English medical literature up to 2007,
there was a total of 216 papers on “gefitinib” and “EGFR
mutation” and 146 papers on “erlotinib” and “EGFR muta-
tion.” We collected data on lung adenocarcinoma patients
with complex EGFR mutations from these published re-
ports. Moreover, the patients had records on their response
to gefitinib or erlotinib. Consistently, the enrolled patient
number was relatively small in each report (Table 3). A to-
total of 34 lung adenocarcinoma patients could be included
from the published data, and two of the 34 were treated with
erlotinib. Different responses to EGFR TKIs were noted
from the published data, and two of the 34 were treated with
gefitinib. Seven patients who had a PR to EGFR TKIs, one with a CR, and one with
a serum response (SR). There were seven patients who had
PD despite EGFR TKIs.

The response rate of patients harboring complex EGFR
mutations with the classical mutation pattern (12 of 20,
60%) is the same as those without the classical mutation
pattern (9 of 14, 64%) (Table 3). The data pooled from the
literature are quite different from our study data. The dis-
crepancy might be caused by several reasons. First, the cri-
eteria of tumor response to EGFR TKIs were different in
the different studies. Seven studies adopted the RECIST [25],
five studies used the World Health Organization criteria
[35], and two studies used the Southwest Oncology Group
standard response criteria [36]. Some authors used other re-
sponse criteria, such as SR, no change (NC), or nonprogres-
sive disease NPD). SR was defined as a >50% decrease in
serum carcinoembryonic antigen levels. Some studies did
not define their criteria. We classified patients with both
NC and NPD as nonresponders, including the three patients
harboring the classical mutation pattern. Second, of the en-
rolled patients from the other reference papers, two patients
received erlotinib, not gefitinib. The drug effect for lung
cancer may be different between erlotinib and gefitinib
[37]. Only a small number of patients was reported in each of
the reviewed reports. No conclusion could therefore be
made from the pooled data of the literature review.

Our study had the largest group of patients with com-
plex EGFR mutations. After combining all 34 patients with
complex EGFR mutations from the other studies and the
present study, there was a total of 53 lung adenocarcinoma
patients with complex EGFR mutations. There were some
common complex mutations among the 53 patients, such as
three with V765M, L798H, K806E, and L814P; three
with del-19 and L861Q; two with del-19 and L858R; two with
L858R and R776H; two with L858R and S768I; two with
L858R and G779S; and two with G719A and S768I (Table
4). Patients with all the above complex EGFR mutations
had good responses (CR, PR, or SR) to EGFR TKIs. Among
the three patients with del-19 plus L861Q, two had a
PR to gefitinib and one had SD with erlotinib [38]. Pa-
patients with the other common complex mutations, including
two with L858R plus D761Y and two with L858R plus
H850D, had SD or PD with EGFR TKIs. D761Y has been
reported as a secondary resistance mutation, like T790M, to
EGFR TKIs [39]. It would be interesting to verify whether
patients with H850D also have a high resistance to EGFR
TKIs. Two patients with G719A plus S768I had different
maximum responses to EGFR TKIs. One patient had a PR,
whereas the other had PD. A similar condition was also
noted in two patients with G719S plus L861Q.

Although the patients with complex EGFR mutations
with the classical mutation pattern had a better response to
gefitinib, for those without the classical EGFR mutation
pattern or with the T790M mutation, irreversible EGFR
TKIs might have some effect. In preclinical cell culture
studies, irreversible EGFR inhibitors can potentially pro-
vide a solution to the T790M TKI resistance problem [40].

Table 4. Lung adenocarcinoma patients with the same complex EGFR mutations and response to EGFR TKIs

<table>
<thead>
<tr>
<th>n of</th>
<th>Mutation type</th>
<th>Response to EGFR TKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>V765M + L798H + K806E + L814P</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>Del-19 + L861Q</td>
<td>1 PR, 1 SR, 1 SD*</td>
</tr>
<tr>
<td>2</td>
<td>Del-19 + L858R</td>
<td>1 CR, 1 PR</td>
</tr>
<tr>
<td>2</td>
<td>L858R + R776H</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>L858R + S768I</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>L858R + G779S</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>G719C + S768I</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>G719A + S768I</td>
<td>1 PR, 1 PD</td>
</tr>
<tr>
<td>2</td>
<td>G719S + L861Q</td>
<td>1 PR, 1 PD</td>
</tr>
<tr>
<td>2</td>
<td>L858R + D761Y</td>
<td>1 NC, 1 PD</td>
</tr>
<tr>
<td>2</td>
<td>L858R + H850D</td>
<td>1 SD, 1 PD</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete remission; EGFR, epidermal growth factor receptor; NC, no change; PD, progressive disease; PR, partial response; SD, stable disease; SR, serum response; TKI, tyrosine kinase inhibitor.

*Treated with erlotinib.
For example, HKI-272, an irreversible dual inhibitor of both EGFR and human epidermal growth factor receptor 2, can suppress EGFR signaling in the context of an EGFR complex mutation with T790M [40, 41]. Another irreversible EGFR TKI, BIBW 2992, also shows the effect in erlotinib-resistant cell lines [42]. Clinical studies are necessary to prove the effect of irreversible EGFR TKIs for patients with complex mutations.

Although our study had the largest group of patients with complex EGFR mutations, it had limitations in that the sample size was still too small to draw any firm conclusions because we were comparing two groups of 12 and seven patients. Even after adding the data from the other studies, there are still limitations in the analysis because of the different tissue sampling methods and treatment response criteria.

CONCLUSION
We demonstrated a larger series of lung adenocarcinoma patients with complex EGFR mutations. Patients with complex EGFR mutations with the classical mutation pattern had a better response, longer survival time, and longer progression-free survival time after EGFR TKI therapy than those without the classical mutation pattern. EGFR TKIs may still be the best choice of treatment for these patients.

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Final approval of manuscript: Shang-Gin Wu, Yih-Leong Chang, Ya-Chieh Hsu, Jenn-Yu Wu, Chi-Hsin Yang, Chong-Jen Yu, Meng-Feng Tsai, Jin-Yuan Shih, Pan-Chyr Yang

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