Clinical Outcomes in Non–Small Cell Lung Cancers Harboring Different Exon 19 Deletions in EGFR

Kuei-Pin Chung¹, Shang-Gin Wu⁴, Jenn-Yu Wu⁴, James Chih-Hsin Yang³, Chong-Jen Yu², Pin-Fei Wei¹, Jin-Yuan Shih², and Pan-Chyr Yang²

Abstract

Purpose: Several deletions in exon 19 of epidermal growth factor receptor (EGFR) gene have been reported in non–small cell lung cancer (NSCLC). It is unknown if deletions occurring at different amino acid positions or of different sizes are associated with different clinical outcome to EGFR tyrosine kinase inhibitors (TKI).

Experimental Design: This study enrolled NSCLC patients with deletions in EGFR exon 19. Patients who had received EGFR TKIs for advanced NSCLC were further evaluated for response rate (RR), progression-free survival (PFS), and overall survival (OS).

Results: In 308 patients with deletions in EGFR exon 19, 298 had deletions encompassing the entire amino acid string from L747 through E749 (LRE deletions). EGFR TKIs were used in 204 patients with advanced NSCLC. Patients with non-LRE deletions had the least RR, compared with those with deletions from E746 or L747 (42.9%, 68.2%, and 79.6%, respectively; P = 0.022). Patients with non-LRE deletions had relatively short median PFS, though not significantly different from those with deletions from E746 or L747 (5.9, 9.8, and 10.5 months, respectively; P = 0.665). The OS was not different among patients with deletions occurring at different amino acid positions (P = 0.776). Deletions in exon 19 of different sizes were not associated with different RR, PFS, or OS.

Conclusions: Non-LRE deletions in exon 19 were associated with worse response to EGFR TKIs, compared with LRE deletions. Therefore, the expected clinical outcome under EGFR TKIs depends on not only the existence but also the types of deletions in exon 19. Clin Cancer Res; 18(12); 3470–7. ©2012 AACR.

Introduction

The activation of epidermal growth factor receptor (EGFR) and its downstream signaling pathways are important to the survival and proliferation of non–small cell lung cancer (NSCLC; ref. 1). To date, several EGFR mutations have been discovered in NSCLC (2), which are associated with the therapeutic response to EGFR tyrosine kinase inhibitors (TKI; refs. 3–5). The most frequent EGFR mutations are L858R and deletions in exon 19 (6–8). For advanced NSCLC, with sensitizing EGFR mutations, randomized controlled studies have shown that the progression-free survival (PFS) under first-line gefitinib or erlotinib treatment was longer than PFS under platinum-based chemotherapy (9–12). Thus, EGFR TKI has become an important therapeutic option for advanced NSCLC.

Several deletions in exon 19 have been found in NSCLC. Most of these deletions encompass the amino acids from codons L747 to E749 (LRE deletions; ref. 13), while other deletions do not involve any of the LRE fragment. According to the Somatic Mutations in EGFR Database (SM-EGFR-DB; ref. 14), the most frequent deletions in exon 19 of EGFR were delE746-A750 (66.1%), followed by delL747-P753insS (56.8%), delL747-A750insP (4.0%), and delL747-T751 (3.7%). In addition, the most common non-LRE deletion was delS752-I759. Structural analyses showed allosteric mechanism important for EGFR kinase domain activation (15). The deletions in EGFR exon 19, located between strand β3 and helix αC, could disrupt inactive conformation of EGFR kinase domain and enhance the effectiveness of EGFR TKIs (15–18). Although previous study has shown that the outcome of patients with NSCLCs under erlotinib treatment was not affected by the number of amino acids deleted (19), different response rates (RR) to EGFR TKIs, ranging from 70% to 100%, were observed in NSCLC, with deletions in exon 19 occurring at different amino acid positions, such as deletions from E746 or from L747, and deletions not encompassing the entire LRE...
Deletions in Exon 19 of EGFR in NSCLC

Translational Relevance

The most frequent mutations observed in the epidermal growth factor gene (EGFR) in non–small cell lung cancer (NSCLC) are L858R and deletions in exon 19, both of which are sensitive to EGFR tyrosine kinase inhibitor (TKI) treatment. However, it is unclear whether different deletions in exon 19 are associated with different therapeutic response and clinical outcomes under EGFR TKI treatment. We found that the therapeutic response associated with EGFR TKI treatment was different among NSCLC patients with deletions in exon 19 occurring at different amino acid positions, and patients with non-LRE deletions had the least response. NSCLC patients with non-LRE deletions also had relatively short PFS, though not significantly different to that in patients with LRE deletions. The results indicate that the expected clinical outcome under EGFR TKIs depends on not only the existence but also the types of deletions in exon 19.

EGFR mutation analysis

The process for mutation analysis of the EGFR gene was described previously (22–24). Briefly, DNA was extracted from tumors embedded in paraffin blocks by a QIAmp DNA Mini kit (QIAGEN). The tyrosine kinase domains of EGFR (exons 18–21) were amplified by PCR. RNA was extracted from frozen tumor specimens using RNeasy Mini Kit (QIAGEN). Reverse transcriptase PCR was used to amplify the 4 exons (exons 18–21) of the tyrosine kinase domain of the EGFR gene, with forward and reverse primers as 5’-GATCGGCCATCC-3’ and 5’-TAAAAATGATC-CATTGATC-3’, respectively (21). The amplicons were then sequenced on an automatic ABI PRISM 3700 DNA analyzer (Applied Biosystems). All sequencing reactions were carried out in both forward and reverse directions using tracings from at least 2 PCRs.

EGFR TKI treatment for NSCLC

Advanced NSCLC was defined as stage IV or postoperative recurrence. We identified advanced NSCLC patients who had received EGFR TKIs (including gefitinib and erlotinib) for treatment. Patients were excluded for further analysis if they received EGFR TKIs as maintenance therapy or in combination with chemotherapy. The final selected patients constituted the population for investigating the therapeutic response and clinical outcome in NSCLCs with deletions in exon 19 occurring at different amino acid positions or of different sizes. Chest radiographs were carried out every 2 to 3 weeks and chest CT scans every 2 to 3 months to evaluate treatment response and disease progression. The best overall response to EGFR TKI was determined according to the Response Evaluation Criteria in Solid Tumors Group (RECIST, version 1.0; ref. 25). PFS was defined as the length of time from the first day of TKI treatment until disease progression or death. Overall survival (OS) was defined as the length of time from the first day of cancer treatment until death or the last follow-up on May 31, 2011.

Statistical analysis

To investigate whether deletions in exon 19 occurring at different amino acid positions were associated with different therapeutic responses and clinical outcomes during EGFR TKI treatment, patients were categorized according to exon 19 deletions: encompassing the entire LRE fragment (LRE group) or not encompassing the entire LRE fragment (non-LRE group). In the LRE group, patients were further divided according to the deletion starting codon: from E746 (E746 group) or from L747 (L747 group). On the other hand, we also evaluated whether different number of nucleotides or amino acids deleted in exon 19 was associated with different clinical outcomes in NSCLC under EGFR TKI treatment, as the study by Taron and colleagues (19). Categorical variables were compared between 2 groups by the Pearson χ² or Fisher exact test, as appropriate. The logistic regression model was used (with adjustments for potential confounding factors) to compare the response to EGFR TKI treatment between NSCLCs with different

Materials and Methods

Study population

In this retrospective study, consecutive patients who were diagnosed with NSCLC between January 2004 and June 2010 in the National Taiwan University Hospital were identified. Tumor specimens were obtained by surgical or needle biopsy and included primary lung tumors, malignant pleural effusion cell blocks, and distant metastases. The lung cancer histology was classified according to the World Health Organization (Geneva, Switzerland) pathology classification system (20). The extent of disease was evaluated by computed tomography (CT) scans of the chest (from neck to adrenal glands) and brain, and bone scans. Clinical stages were determined according to the seventh edition of the American Joint Committee on Cancer (AJCC; ref. 21). The clinical characteristics of enrolled patients were recorded, including age, gender, smoking history, weight loss (≥5%) on presentation, and Eastern Cooperative Oncology Group (ECOG) performance status. Informed consent was obtained from each subject, and the study protocol was approved by the International Review Board of the National Taiwan University Hospital (IRB number: 201107010RC).
deletions in exon 19. OS and PFS between different stratified patient groups were evaluated by the Kaplan–Meier curve with log-rank tests. Multivariate analyses for OS and PFS were conducted using the Cox proportional hazard model. In addition to deletions in exon 19, other covariates included in the models were age (≥70 vs. <70 years), gender, history of smoking, ECOG performance status (≥2 vs. <2), weight loss (≥5% on presentation), disease stage (stage IV vs. postoperative recurrence), extent of disease (number of metastases ≥2 vs. <2), cancer histology (adenocarcinoma vs. nonadenocarcinoma), and the clinical setting of EGFR TKI treatment (first line, second line, third line, or subsequent line). Backward variable selection was carried out with significance for entry and stay set at 0.10. A 2-sided $P < 0.05$ was considered statistically significant. All statistical analyses were conducted with SPSS software (version 17.0 for Windows).

**Results**

**Deletions in EGFR exon 19 occurring at different amino acid positions**

From January 2004 to June 2010, 1,607 NSCLC patients were examined for EGFR mutations. Deletions in exon 19 of EGFR were detected in 308 patients, who constituted the study population (Table 1). The entire LRE fragment was involved in 298 deletions (96.7%). The most frequent

<table>
<thead>
<tr>
<th>deletion</th>
<th>Insertion</th>
<th>Number, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>E746-A750</td>
<td>203 (65.9)</td>
<td></td>
</tr>
<tr>
<td>T S P K A N K E I</td>
<td>E746-A750</td>
<td></td>
</tr>
<tr>
<td>Q P T S P K A N K E I</td>
<td>E746-A750</td>
<td></td>
</tr>
<tr>
<td>A P T S P K A N K E I</td>
<td>E746-A750</td>
<td></td>
</tr>
<tr>
<td>V P S P K A N K E I</td>
<td>E746-T751</td>
<td></td>
</tr>
<tr>
<td>V A S P K A N K E I</td>
<td>E746-T751</td>
<td></td>
</tr>
<tr>
<td>A S P K A N K E I</td>
<td>E746-T751</td>
<td></td>
</tr>
<tr>
<td>V P K A N K E I</td>
<td>E746-S752</td>
<td></td>
</tr>
<tr>
<td>V S K A N K E I</td>
<td>E746-P753</td>
<td></td>
</tr>
<tr>
<td>L S K A N K E I</td>
<td>E746-P753</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1. Deletions in exon 19 of EGFR (N = 308)**

<table>
<thead>
<tr>
<th>Amino acid sequencea</th>
<th>Deletion</th>
<th>Insertion</th>
<th>Number, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>746</td>
<td>759</td>
<td>Deletions starting at E746 (AE746)</td>
<td></td>
</tr>
<tr>
<td>E L R E A T S P K A N K E I</td>
<td>219 (71.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q P T S P K A N K E I</td>
<td>E746-A750 QA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A P T S P K A N K E I</td>
<td>E746-A750 AP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V P S P K A N K E I</td>
<td>E746-T751 VP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V A S P K A N K E I</td>
<td>E746-T751 VA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A S P K A N K E I</td>
<td>E746-T751 A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V P K A N K E I</td>
<td>E746-S752 V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V S K A N K E I</td>
<td>E746-P753 VS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L S K A N K E I</td>
<td>E746-P753 LS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Deletions starting at L747 (AL747) |
| E L R E A T S P K A N K E I | 79 (25.6) |
| E A T S P K A N K E I | L747-E749 A |
| E S T S P K A N K E I | L747-A750 S |
| E P T S P K A N K E I | L747-A750 P |
| E S P K A N K E I | L747-T751 S |
| E P K A N K E I | L747-S752 P |
| E Q P K A N K E I | L747-S752 Q |
| E K A N K E I | L747-P753 A |
| S K A N K E I | L747-P753 S |
| S D K A N K E I | L747-P753 SD |
| A A A K E I | L747-K754 A |
| S R E A N K E I | L747-K754 SRE |
| S P H N K E I | L747-A755 SPH |

**Non-LRE deletions |
| E L R E A T S P K A N K E I | 10 (3.2) |
| E L R E A T S P K A N K E I | R748-S752 A |
| E L R E A T S P K A N K E I | R748-P753 A |
| E L R E A T S P K A N K E I | T751-E758 A |
| E L R E A T S P K A N K E I | T751-I759 D |
| E L R E A T S P K A N K E I | T751-I759 N |
| E L R E A T S P K A N K E I | S752-I759 T |
| E L R E A T S P K A N K E I | S752-I759 N |

aThe exon 19 of wild-type EGFR spans from G729 to D761, and deletions of exon 19 alter amino acid sequences from E746 to I759. The altered amino acid sequences are presented in this table.
Deletions in Exon 19 of EGFR in NSCLC

Table 2. Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total (N = 308)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
</tr>
<tr>
<td>&lt;70</td>
<td>220 (71.4)</td>
</tr>
<tr>
<td>≥70</td>
<td>88 (28.6)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>132 (42.9)</td>
</tr>
<tr>
<td>Female</td>
<td>176 (57.1)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Never-smoker</td>
<td>234 (76.0)</td>
</tr>
<tr>
<td>Current or former smoker</td>
<td>74 (24.0)</td>
</tr>
<tr>
<td>Weight loss</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>247 (80.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>61 (19.8)</td>
</tr>
<tr>
<td>ECOG</td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>270 (87.7)</td>
</tr>
<tr>
<td>≥2</td>
<td>38 (12.3)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>I/II/III, without recurrence</td>
<td>41 (13.3)</td>
</tr>
<tr>
<td>IV</td>
<td>213 (69.2)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>54 (17.5)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>291 (94.5)</td>
</tr>
<tr>
<td>Nonadenocarcinoma*</td>
<td>8 (0.26)</td>
</tr>
<tr>
<td>NSCLC-NOS</td>
<td>9 (0.29)</td>
</tr>
</tbody>
</table>

Abbreviation: NSCLC-NOS, NSCLC-not otherwise specified.

*Including squamous cell carcinoma (n = 6), adenosquamous cell carcinoma (n = 1), and lymphoepithelioma-like carcinoma (n = 1).

deletions in exon 19 were delE746-A750 (65.9%), followed by delL747-P753insS (8.1%) and L747-I751 (5.8%). The clinical characteristics of the study population are presented in Table 2. The mean patient age was 61.4 years (range, 27–95). Most of the patients were female (57.1%) and never-smokers (76%). Adenocarcinoma was the principal histology observed (94.5%). Ten patients (3.2%) constituted the non-LRE group (Table 3). The most common non-LRE deletion was delS752-I759 (40% of the non-LRE group). The mean age of these patients was 61.4 years (range, 43–95 years), and most patients were female (60%) and never-smokers (70%). All 10 non-LRE patients had adenocarcinoma.

Within the study population, 210 patients had received EGFR TKIs for advanced NSCLC. Six patients received EGFR TKIs as maintenance therapy or in combination with chemotherapy and were excluded from further analyses. The remaining 204 patients constituted the patient group for investigating therapeutic response and clinical outcomes in NSCLC patients who had deletions in exon 19 occurring at different amino acid positions and who were treated with EGFR TKIs. The clinical characteristics of these patients are summarized in Supplementary Table SA2. EGFR TKIs were used as first-line treatment in 114 patients (55.9%). Gefitinib was the principal EGFR TKI used by the patients (66.2%). The overall RR to EGFR TKIs was 70.1%.

Of the LRE patients (n = 197), 148 were in the ΔE746 group and 49 were in the ΔL747 group. More patients in the ΔE746 group had adenocarcinoma compared with patients in the ΔL747 group (97.3% vs. 87.8%, P = 0.026). Gefitinib was used in 63.5% of the ΔE746 patients and in 69.4% of the ΔL747 patients (P = 0.455). Other clinical characteristics did not differ significantly between the 2 groups. Seven patients were in the non-LRE group (Table 3). The RRs of NSCLCs with delL747-P753insS, delL747-T751, and delL747-S752 were 76.9%, 70.0%, and 88.9%, respectively. Compared with patients in the ΔL747 group, the ΔE746 patients displayed a lower RR to EGFR TKI (adjusted OR, 0.265; P = 0.011). Patients in the non-LRE

Table 3. Clinical profiles of patients in the non-LRE group (N = 10)

<table>
<thead>
<tr>
<th>Deletion</th>
<th>Insertion</th>
<th>Age</th>
<th>Gender</th>
<th>Histology</th>
<th>Stage*</th>
<th>Smoking</th>
<th>Weight loss</th>
<th>ECOG TKI</th>
<th>Response</th>
<th>PFS months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 T751-I759</td>
<td>N</td>
<td>69</td>
<td>Female</td>
<td>Adenocarcinoma IB</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6.6</td>
</tr>
<tr>
<td>2 T751-E758</td>
<td>57 Female</td>
<td>67</td>
<td>Female</td>
<td>Adenocarcinoma IB</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>20.8</td>
</tr>
<tr>
<td>3 S752-I759</td>
<td>95 Female</td>
<td>67</td>
<td>Female</td>
<td>Adenocarcinoma IV</td>
<td>–</td>
<td>+</td>
<td>1</td>
<td>Gefitinib (second line) PR</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>4 R748-P753</td>
<td>57 Female</td>
<td>67</td>
<td>Female</td>
<td>Adenocarcinoma R</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>Gefitinib (third line) PR</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>5 R748-S752</td>
<td>69 Male</td>
<td>67</td>
<td>Male</td>
<td>Adenocarcinoma R</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>Gefitinib (second line) SD</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>6 S752-I759</td>
<td>59 Female</td>
<td>67</td>
<td>Female</td>
<td>Adenocarcinoma IV</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>Gefitinib (first line) SD</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>7 S752-I759</td>
<td>51 Male</td>
<td>67</td>
<td>Male</td>
<td>Adenocarcinoma IV</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>Gefitinib (first line) SD</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>8 S752-I759</td>
<td>58 Male</td>
<td>67</td>
<td>Male</td>
<td>Adenocarcinoma IV</td>
<td>–</td>
<td>+</td>
<td>2</td>
<td>Gefitinib (second line) PR</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>9 T751-I759</td>
<td>43 Female</td>
<td>67</td>
<td>Female</td>
<td>Adenocarcinoma IV</td>
<td>–</td>
<td>+</td>
<td>1</td>
<td>Gefitinib (first line) SD</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>10 T751-I759</td>
<td>56 Male</td>
<td>67</td>
<td>Male</td>
<td>Adenocarcinoma IV</td>
<td>+</td>
<td>–</td>
<td>0</td>
<td>Gefitinib (first line) SD</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>

*R, postoperative recurrence.
Patients with different median OS (Table 4). The clinical characteristics were not significantly different between patients with deletion of 15 nucleotides and those with deletion of less or more than 15 nucleotides (Supplementary Table S3A). The RR, PFS, and OS between 2 patient groups were also not statistically different (Supplementary Fig. S2A and S2B). On the other hand, patients who received EGFR TKI treatment were categorized into 4 groups according to the numbers of amino acid deleted in exon 19: 5 amino acids in 154 patients (75.5%), 6 amino acids in 14 patients (6.9%), 7 amino acids in 20 patients (10.7%), as the results from COSMIC data-base (2). The therapeutic response associated with EGFR TKIs were not significantly different between the 4 groups, ranging from 62.5% to 85% (P = 0.370, by univariate logistic regression analysis). The PFS (Supplementary Fig. S3A) and OS under EGFR TKIs (Supplementary Fig. S3B) were not significantly different between 4 groups (P = 0.794 and 0.946 by log-rank test, respectively).

Discussion

In this study, we observed that deletions in exon 19 of EGFR not encompassing the entire LRE fragment were uncommon. We found the most frequent deletions were delE746-A750 (65.5%), delL747-P753insS (8.1%), and delL747-T751 (5.8%), as the results from COSMIC data-base (2). The therapeutic response associated with EGFR

Table 4. Best overall response to EGFR TKIs in NSCLC patients with deletions in exon 19 of EGFR occurring at different amino acid positions

<table>
<thead>
<tr>
<th>Response</th>
<th>N</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
<th>RR (%)</th>
<th>Adjusted OR* (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>204</td>
<td>143</td>
<td>37</td>
<td>24</td>
<td>70.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Patient groups categorized by deletions in exon 19 of EGFR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔL747</td>
<td>49</td>
<td>39</td>
<td>3</td>
<td>7</td>
<td>79.6</td>
<td>1</td>
<td>0.019</td>
</tr>
<tr>
<td>delL747-P753insS</td>
<td>13</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>76.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>delL747-T751</td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>70.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>delL747-S752</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>88.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ΔE746</td>
<td>148</td>
<td>101</td>
<td>30</td>
<td>17</td>
<td>68.2</td>
<td>0.265 (0.095–0.736)</td>
<td>0.011</td>
</tr>
<tr>
<td>delE746-A750</td>
<td>137</td>
<td>93</td>
<td>29</td>
<td>15</td>
<td>67.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Non-LRE</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>42.9</td>
<td>0.120 (0.020–0.737)</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease.

*Logistic regression was used to compare the RRs between different patient groups. The model covariates included history of smoking, ECOG performance status, disease stage, extent of disease, cancer histology, and the clinical setting of EGFR TKI treatment (first line, second line, third line, or more). Backward variable selection was carried out with entry and stay deemed significant at 0.10. The covariates in the final model included age, history of smoking, cancer histology, and deletions in exon 19 of EGFR occurring at different amino acid positions.

Deletions in EGFR exon 19 of different sizes

In the 308 patients with deletions in exon 19 of EGFR, the number of nucleotides deleted in exon 19 ranged from 9 to 27 and the most frequent number was 15 (233 patients, 75.6%). The numbers of amino acid deleted in exon 19 ranged from 3 to 9, and the most frequent number was 5 (230 patients, 74.7%). The response and survival to EGFR TKI treatment was evaluated in advanced NSCLC patients with deletions in EGFR exon 19 of different nucleotide or amino acid sizes. In the 204 patients who received EGFR TKIs, 156 patients (76.5%) had 15 nucleotides deleted in exon 19 of EGFR. The clinical characteristics were not significantly different between patients with deletion of 15 nucleotides and those with deletion of less or more than 15 nucleotides (Supplementary Table S3A). The RR, PFS, and OS between 2 patient groups were also not statistically different (P = 0.229, 0.949, and 0.758, respectively; Supplementary Fig. S2A and S2B). On the other hand, patients who received EGFR TKI treatment were categorized into 4 groups according to the numbers of amino acid deleted in exon 19: 5 amino acids in 154 patients (75.5%), 6 amino acids in 14 patients (6.9%), 7 amino acids in 20 patients (9.8%), and other numbers of amino acid in 16 patients (7.8%) (Supplementary Table S4A). The RRs to EGFR TKIs were not significantly different between the 4 groups, ranging from 62.5% to 85% (P = 0.370, by univariate logistic regression analysis). The PFS (Supplementary Fig. S3A) and OS under EGFR TKIs (Supplementary Fig. S3B) were not significantly different between 4 groups (P = 0.794 and 0.946 by log-rank test, respectively).
also showed that a new class of EGFR mutation, exon 19
the EGFR kinase domain (15). In addition, one recent study
conformation enhances the compatibility of erlotinib with
L861 on the activating loop, and disruption of the inactive
between strand
[76x165]lated by complex allosteric mechanism (15–18). The inac-
[76x176]deletions. The activation of EGFR kinase domain is regu-
[76x187]ciated with lower RR under EGFR TKIs, compared with LRE
[76x209]D
[76x231]ferent amino acid positions.
[76x242]patients with deletions in
[76x253]PFS and OS, were not statistically different in NSCLC
[76x264]LRE deletions. However, the clinical outcomes, including
[76x297]deletions in exon 19 occurring at different amino acid
[76x308]TKI treatment was different among NSCLC patients with
[76x376]Kaplan
[76x367]OS and (B) PFS were plotted with strati-
[76x332]median survival time.
[76x340]exon 19 of
[111x340]EGFR
[111x340]occurring at different amino acid positions. MST,
[129x209]L747 group, as the results from SM-EGFR-DB
[142x143]–
[142x143]Meier curves of OS and PFS. Kaplan
[157x526]Log-rank test
[157x533]Non-LRE
[157x539]L747
[157x546]Δ
[157x549]E746
[157x709]N
[157x709]= 7
[157x702]= 49
[157x702]N
[157x708]= 148 MST 33.1 (95% CI, 26.7–39.6)
[157x702]Δ
[157x702]E746
[157x708](95% CI, 21.2–46.3)
[157x702]L747
[157x702]Δ
[157x708](95% CI, 19.7–46.0)
[157x689]= 0.665
[157x702]= 0.665
[157x526]P
[157x526]= 0.776
[157x533]MST 33.7
[157x539]MST 32.8
[157x702]MST 10.5
[185x696](95% CI, 3.9–7.8)
[187x689]= 0.665
[185x702]= 0.665
[237x533](95% CI, 21.2–46.3)
[237x539](95% CI, 19.7–46.0)
[238x696](95% CI, 3.9–7.8)
[238x702](95% CI, 7.7–13.3)
[246x376]Meier curves of (A)
[278x106]Figure 1. Kaplan–Meier curves of OS and PFS. Kaplan–Meier curves of (A)
OS and (B) PFS were plotted with stratification for NSCLC with deletions
in exon 19 of EGFR occurring at different amino acid positions.
The OS and PFS rates did not differ between patients with deletions in
exon 19 of EGFR occurring at different amino acid positions.
TKI treatment was different among NSCLC patients with
deletions in exon 19 occurring at different amino acid
positions. Patients with non-LRE deletions in exon 19
showed a worse response to EGFR TKIs than those with
LRE deletions. However, the clinical outcomes, including
PFS and OS, were not statistically different in NSCLC
patients with deletions in EGFR exon 19 occurring at dif-
ferent amino acid positions.

We found the average RR in ΔE746 group would be lower
than that in ΔL747 group, as the results from SM-EGFR-DB
(14). Furthermore, we also found non-LRE deletions asso-
ciated with lower RR under EGFR TKIs, compared with LRE
deletions. The activation of EGFR kinase domain is regu-
lated by complex allosteric mechanism (15–18). The inac-
tive conformation of EGFR kinase domain needs the loop
between strand β3 and helix αC packing against L858 and
L861 on the activating loop, and disruption of the inactive
conformation enhances the compatibility of erlotinib with
the EGFR kinase domain (15). In addition, one recent study
also showed that a new class of EGFR mutation, exon 19
insertions, is associated with sensitivity to EGFR TKI (26).
All the identified exon 19 insertions result in substitution
for L747 with a proline residue. The authors did structural
analysis and found L747 was a key hydrophobic core
stabilizing the inactive form of EGFR, and the substitution
was predicted to disrupt the hydrophobic core and favored
the active conformation of EGFR. Therefore, our results are
consistent with previous studies and suggest the biological
importance of the LRE residues in sensitivity to EGFR TKI in
NSCLC. Further crystallographic studies would be needed
to evaluate whether deletions in exon 19 occurring at
different amino acid positions cause different conformation
changes in EGFR kinase domain and are associated with
different sensitivity to EGFR TKI.

The RR associated with gefitinib or erlotinib in NSCLC
with sensitizing EGFR mutations ranges from 54.5% to 83%
and the median PFS ranges from 9.2 months to 13.1 months
(9–12). Different RR to EGFR TKIs in these studies might be
due to different frequencies of activating EGFR mutations in
the study populations. Previous studies showed L858R
mutation was associated with relatively lower RR than
deletion in exon 19 (10, 27, 28). In addition, our results
also showed deletions in exon 19 occurring at different
amino acid positions were associated with different RR. In
patients with advanced NSCLC under EGFR TKIs, the RR
to EGFR TKIs correlate with the length of median PFS (29).
However, at least 14% increase of RR was needed to prolong
median PFS by 1 month. Consequently, the population size
needed to detect differences in median PFS would be larger
than that to detect differences of RR. This could be one
reason why several studies did not show different median
PFS under EGFR TKIs between NSCLC with L858R
mutation and that with deletion in exon 19 (9–11). Further-
more, although non-LRE deletions were associated with shorter
median PFS than LRE deletions in this study, the population
size of non-LRE group is small and would not have adequate
power for the differences in median PFS to be detected.
Further studies would be required to evaluate whether
NSCLC patients with non-LRE deletions in exon 19 have
shorter median PFS than those with LRE deletions.

Several techniques were developed to screen for EGFR
mutations in NSCLC patients (30). With some techniques,
including direct sequencing and fragment length analysis,
detection of all deletions in exon 19 is possible. Other
techniques, such as smart amplification process, detect only
specific deletions in exon 19 and may miss uncommon non-
LRE deletions (30–32). Our results show NSCLC patients
with non-LRE deletions in exon 19 have shorter median PFS
than those with LRE deletions.
sensitivity to EGFR TKIs in NSCLC patients with K745. Additional studies are required to determine the clinical outcomes associated with EGFR TKI treatment in Therefore, our results do not clarify the response and EGFR had deletions starting at codon I744 or K745. none of our patients with LRE deletions in exon 19 of positions were independent of cancer histology. Finally, patients with deletions occurring at different amino acid RR. As a result, the differences in response among NSCLC patients with non-LRE deletions to EGFR TKI treatment may be different among NSCLC patients. We found that the response associated with EGFR distribution of deletions in exon 19 of D

to EGFR TKIs. Furthermore, crystal structural analysis of the different exon 19 deletions may also disclose the different structural effects on the EGFR activation and response to EGFR TKIs. Second, this is a retrospective study and thus, could contain selection bias. We found that more patients in the D

in NSCLC compared with the ΔL747 group. However, this confounding factor was adjusted during analysis for the RR. As a result, the differences in response among NSCLC patients with deletions occurring at different amino acid positions were independent of cancer histology. Finally, none of our patients with LRE deletions in exon 19 of EGF had deletions starting at codon I744 or K745. Therefore, our results do not clarify the response and clinical outcomes associated with EGFR TKI treatment in NSCLC patients with deletions starting at codon I744 or K745. Additional studies are required to determine the sensitivity to EGFR TKIs in NSCLC patients with ΔI744 or ΔK745 deletions.

In conclusion, this study highlights the frequency and distribution of deletions in exon 19 of EGFR in NSCLC patients. We found that the response associated with EGFR TKI treatment may be different among NSCLC patients with deletions occurring at different amino acid positions and patients with non-LRE deletions had the least response. NSCLC patients with non-LRE deletions also had relatively short PFS, though not significantly different to that in patients with LRE deletions. Therefore, this study showed that, in advanced NSCLC patients, the expected clinical outcome under EGFR TKIs depends not only the existence but also the types of deletions in exon 19.

Table 5. Multivariate analyses for OS and PFS in advanced NSCLC patients who received EGFR TKI treatment

<table>
<thead>
<tr>
<th>Covariate</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (≥70/&lt;70)</td>
<td>0.717 (0.501–1.027)</td>
<td>0.069</td>
</tr>
<tr>
<td>Stage (recurrence/IV)</td>
<td>0.630 (0.405–0.981)</td>
<td>0.041</td>
</tr>
<tr>
<td>Metastases (≥2 sites/&lt;2 sites)*</td>
<td>1.596 (1.156–2.203)</td>
<td>0.005</td>
</tr>
<tr>
<td>Histology (adenocarcinoma/nonadenocarcinoma and NSCLC-NOS)</td>
<td>0.373 (0.172–0.808)</td>
<td>0.012</td>
</tr>
<tr>
<td>Weight loss (≥5%/&lt;5%)</td>
<td>1.506 (0.968–2.343)</td>
<td>0.070</td>
</tr>
<tr>
<td>ECOG performance status (≥2/&lt;2)</td>
<td>2.479 (1.404–4.376)</td>
<td>0.002</td>
</tr>
<tr>
<td>Metastases (≥2 sites/&lt;2 sites)*</td>
<td>1.685 (1.119–2.538)</td>
<td>0.013</td>
</tr>
<tr>
<td>Histology (adenocarcinoma/nonadenocarcinoma and NSCLC-NOS)</td>
<td>0.288 (0.130–0.636)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Abbreviation: NSCLC-NOS, NSCLC-not otherwise specified.

*Number of metastases more than 1; the sites of metastasis included malignant pleural effusion, malignant pericardial effusion, the lungs, bone, liver, adrenal gland, soft tissue, brain, peritoneum, spleen, and distant lymph node.

TKI in exon 19 deletions occurring at different amino acid positions. In vitro analyses, using EGFR TKIs to treat transfected cell lines harboring EGFR exon 19 deletions occurring at different amino acid positions, will be valuable to understand how these deletions affect sensitivity to EGFR TKIs. Furthermore, crystal structural analysis of the different exon 19 deletions may also disclose the different structural effects on the EGFR activation and response to EGFR TKIs. Therefore, our results do not clarify the response and clinical outcomes associated with EGFR TKI treatment in NSCLC patients with deletions starting at codon I744 or K745.

In conclusion, this study highlights the frequency and distribution of deletions in exon 19 of EGFR in NSCLC patients. We found that the response associated with EGFR TKI treatment may be different among NSCLC patients with deletions occurring at different amino acid positions and patients with non-LRE deletions had the least response. NSCLC patients with non-LRE deletions also had relatively short PFS, though not significantly different to that in patients with LRE deletions. Therefore, this study showed that, in advanced NSCLC patients, the expected clinical outcome under EGFR TKIs depends not only the existence but also the types of deletions in exon 19.

Disclosure of Potential Conflicts of Interest

J.-Y. Shih and C.-J. Yu received honoraria from AstraZeneca and Roche for giving a speech. J.C.-H. Yang received honoraria from AstraZeneca and Roche for giving a speech and serving on an ad hoc advisory committee. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

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Analysis and interpretation of data: K.-P. Chung, J.C.-H. Yang, J.-Y. Shih, P.-C. Yang

Writing, review, and/or revision of the manuscript: K.-P. Chung, J.C.-H. Yang, C.-J. Yu, J.Y. Shih, P.-C. Yang

Administrative, technical, or material support: S.-G. Wu, P.-C. Yang

Study supervision: J.C.-H. Yang, P.-C. Yang

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