Prior EGFR tyrosine-kinase inhibitor therapy did not influence the efficacy of subsequent pemetrexed plus platinum in advanced chemonaïve patients with EGFR-mutant lung adenocarcinoma

Background: Tumor cells before and after epidermal growth-factor receptor (EGFR) tyrosine-kinase inhibitor (TKI) therapy might display different characteristics. The aim of this study was to evaluate the influence of prior EGFR TKI therapy on the efficacy of subsequent pemetrexed plus platinum (PP) in advanced chemonaïve patients with EGFR-mutant lung adenocarcinoma.

Materials and methods: Advanced chemonaïve patients with EGFR-mutant lung adenocarcinoma receiving PP as first-line chemotherapy were enrolled retrospectively in two medical centers of Taiwan. The objective of this study was to compare objective response rate (ORR), disease-control rates (DCR), progression-free survival (PFS), and overall survival (OS) of PP in patients with and without prior EGFR TKI therapy.

Results: In total, 105 patients were analyzed. Sixty-one patients (58.1%) had prior EGFR TKI therapy and used PP as second-line treatment. The other 44 patients (41.9%) received PP as first-line therapy. ORRs of PP in patients with and without prior EGFR TKI therapy were 24.6% and 38.6%, respectively (P=0.138). DCRs of the two groups were 62.3% and 65.9%, respectively (P=0.837). The median PFS (6.1 versus 6.1 months, P=0.639) and OS (34.4 versus 32.3 months, P=0.394) were comparable between the groups with and without prior EGFR TKI therapy. In a subgroup analysis of patients with prior EGFR TKI therapy, there was no significant association between the efficacy of first-line EGFR TKI and the outcome of subsequent PP therapy.

Conclusion: Our results suggested that prior EGFR TKI therapy would not influence the efficacy of subsequent PP therapy in advanced chemonaïve patients with EGFR-mutant lung adenocarcinoma.

Keywords: non-small-cell lung cancer, epidermal growth-factor receptor mutation, epidermal growth-factor receptor tyrosine-kinase inhibitor, pemetrexed

Introduction
In the last decade, many studies focused on defining the individualization of non-small-cell lung cancer (NSCLC) therapy. Both histologic and molecular subtyping have been recognized as important predictive factors in NSCLC treatment.1-2 Several Phase III studies disclosed the significantly higher response rate and longer progression-free survival (PFS) of epidermal growth-factor receptor (EGFR) tyrosine-kinase inhibitor (TKI) therapy compared with chemotherapy as first-line treatment in patients with EGFR-mutant NSCLC.3-6

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However, studies on EGFR TKI in the first-line setting did not show significant overall survival (OS) benefit.\(^7\)

Pemetrexed plus platinum (PP) is one of the standard frontline therapies for chemonaïve patients with advanced lung adenocarcinoma. In 2008, Scagliotti et al compared first-line pemetrexed plus cisplatin to gemcitabine plus cisplatin in chemonaïve patients with advanced NSCLC, and demonstrated a survival benefit of pemetrexed/cisplatin in patients with nonsquamous histology.\(^8\) The efficacy of PP for patients with EGFR-mutant lung adenocarcinoma was even better than nonselected patients, as shown in a recent Phase III study.\(^9\) However, all the reported efficacy of PP was confined to the treatment-naïve cohort, and whether the results can be applied to patients with prior EGFR TKI therapy is still unclear.

Many recent studies suggest EGFR TKI as first-line therapy for patients with EGFR-mutant lung adenocarcinoma, and PP may be reserved for patients experiencing progression of first line EGFR TKI.\(^10\) However, the mechanism of EGFR TKI resistance is complex,\(^11\) and the post-EGFR TKI-treatment cancer cells may display different characteristics compared with the treatment-naïve cells. It is unclear if clinical resistance to EGFR TKI might also confer resistance to subsequent PP therapy. We conducted this study to evaluate whether prior EGFR TKI use influences the efficacy of subsequent PP in advanced chemonaïve patients with EGFR-mutant lung adenocarcinoma.

**Materials and methods**

**Patients**

This was a retrospective study consisting of patients with advanced lung adenocarcinoma harboring EGFR mutations and treated with PP as first-line chemotherapy regimen at two medical centers of Taiwan (Taichung Veterans General Hospital and National Taiwan University Hospital) from May 2007 to June 2013. We included lung cancer patients with histologically or cytologically confirmed and inoperable lung adenocarcinoma, known EGFR mutations, and clinically measurable disease. Patients were excluded if they had only evaluable lesions, other active malignancy, prior history of other chemotherapy agents, incomplete data records, or received other treatments, such as radiotherapy, concurrently. Tumor, node and metastases (TNM) staging was done according to the seventh edition of *AJCC Cancer Staging Handbook*.\(^12\) This study was approved by the institutional review board of each institute.

**Data records and response evaluation**

Clinical data for analysis included patients’ age, sex, Eastern Cooperative Oncology Group performance status (ECOG PS), tumor stage, smoking status, EGFR-mutation status, prior EGFR TKI treatment, and PP-treatment history. Chest computed tomographies, which were followed up with an interval of 8–12 weeks and included the liver and adrenal glands, and other required image studies for response evaluation were reviewed by two chest physicians. Unidimensional measurements as defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 were used in this study.\(^13\) The objective of the study was to compare the objective response rate (ORR), disease-control rate (DCR), PFS, and OS of PP treatment in patients with and without prior EGFR TKI therapy. OS was determined from the date of the start of treatment (EGFR TKI in the group with prior EGFR TKI therapy, and PP in the group without prior EGFR TKI therapy) until the date of death, irrespective of cause.

**EGFR-mutation tests**

EGFR-mutation analyses using either direct sequencing or protein nucleic acid-locked nucleic acid polymerase chain reaction (PNA-LNA PCR) clamp methods were performed in patients with adequate specimens. Different methods were used in this study, because we set up a direct-sequencing method for *EGFR*-mutation analysis starting in 2007 and then shifted to the PNA-LNA PCR clamp in late 2009. Informed consent was obtained from all subjects.

Tumor specimens were procured for *EGFR*-mutation analysis as previously described.\(^14\) Briefly, deoxyribonucleic acid (DNA) was extracted from the tumors using a QIAmp DNA minikit (Qiagen, Venlo, the Netherlands) following the manufacturer’s protocols. For direct sequencing, the tyrosine-kinase domain of the *EGFR*-coding sequence – exons 18, 19, 20, and 21 – was amplified by PCR and sequenced bidirectionally with an ABI Prism® 3730 DNA analyzer (Life Technologies, Carlsbad, CA, USA) following standard protocol. For the PNA-LNA PCR clamp, real-time amplification monitoring was done using a SmartCycler® (Cepheid, Sunnyvale, CA, USA) to detect mutations on the tyrosine-kinase domain of the *EGFR*-coding sequence: exons 18, 19, 20, and 21.

**Statistical methods**

Patients were divided into without-prior EGFR TKI and with-prior EGFR TKI groups. Student’s *t*-test was used to compare ages between the two groups. Other univariate analysis comparing clinical characteristics and history of prior EGFR TKI therapy on ORR and DCR was performed by exact test. Multivariate analyses using the logistic regression model with stepwise selection method were performed for ORR and DCR. The Kaplan–Meier method was used to
estimate PFS and OS. Differences in survival time regarding prior EGFR TKI therapy were analyzed using the log-rank test. Multivariate analyses using the Cox proportional hazard model with stepwise selection method were performed for PFS and OS. All statistical tests were done with SAS version 9.1 software (SAS Institute, Cary, NC, USA). Two-tailed tests and P-values <0.05 for significance were used.

Results

Patient characteristics

There was a total of 351 patients with advanced lung adenocarcinoma and history of PP treatment from May 2007 to June 2013. A total of 163 patients were excluded, including 85 with prior history of other chemotherapies, 62 without adequate tissue for EGFR-mutation analysis, 12 with incomplete chart records or without measurable lesions, and four who were receiving concurrent treatments (two radiotherapy, one bevacizumab, and one erlotinib). Of the remaining 188 patients, 105 patients had detectable EGFR mutations, and 83 did not. These 105 cases were included for analysis of PP efficacy.

The baseline characteristics are shown in Table 1. Patients without prior EGFR TKI therapy were older than those with prior EGFR TKI therapy (61.6 versus 57.2 years), but this was not statistically significant (P=0.054). Otherwise, there were no significant differences in baseline characteristics between the two groups.

Efficacy of pemetrexed plus platinum chemotherapy

The results of best tumor response and PFS are shown in Table 2. Of the overall 105 patients, 32 patients achieved a partial response, and 35 patients had stable disease. No patients achieved a complete response. The ORR and DCR of the overall population were 30.5% and 63.8%, respectively. Survival data were followed up until the end of November 2013. At the end of data cutoff, five patients (two partial response and three stable disease, total 7.1%) had not experienced progression, and 61 patients were still alive at the last observation, and their data were therefore censored. The median PFS was 6.1 (95% confidence interval [CI] 5.2–7.0) months. The median OS was 32.3 (95% CI 26.7–37.8) months.

ORRs of patients with and without prior EGFR TKI therapy were 24.6% and 38.6%, respectively (P=0.138). DCRs in the two groups were 62.3% and 65.9%,
respectively ($P=0.837$). Univariate analysis for best response regarding patient characteristics and choice of platinum revealed that no factor correlated significantly with ORR or DCR (data not shown), and no covariate reached the significance level to enter the multivariate logistic regression model.

The results of survival analysis are shown in Table 2 and Figure 1. The median PFS (6.1 versus 6.1 months, $P=0.639$) and OS (34.4 versus 32.3 months, $P=0.394$) were comparable between the groups with and without prior EGFR TKI therapy. Univariate analysis for survival regarding patient characteristics and choice of platinum revealed that no factor correlated significantly with PFS or OS (data not shown), and no covariates reached the significance level to enter the multivariate Cox proportional hazard model.

### Association between efficacies of prior EGFR TKI therapy and subsequent pemetrexed-plus-platinum chemotherapy

In the EGFR-mutation analysis, 55 patients (52.4%) had exon 19 deletion, and 40 patients (38.1%) had L858R. The EGFR statuses of the remaining ten patients were heterogeneous, including G719A/S, L861Q, T790M, and other complex mutations. In 55 patients harboring exon 19 deletion, seven had complex mutations, including four with T790M. In 40 patients harboring L858R, five revealed complex mutations, including two with T790M. In 61 patients with EGFR TKI as...
first-line therapy, 46 received gefitinib, 13 received erlotinib, and two received afatinib. The ORR and DCR of EGFR TKI were 67.2% (47 of 61) and 90.2% (55 of 61), respectively. The median PFS of EGFR TKI was 8.0 (95% CI 5.6–10.4) months. EGFR TKI therapy was stopped in 58 patients (95.1%) due to disease progression. Another three patients withdrew from EGFR TKI therapy because of hepatotoxicity (n=2) and financial considerations (n=1), respectively.

Results of univariate analysis for ORR and DCR in subgroups of patients with prior EGFR TKI therapy are shown in Table 3. Neither responses nor PFS of prior EGFR TKI therapy significantly influenced the efficacy of subsequent PP therapy.

Subsequent treatments after pemetrexed-plus-platinum chemotherapy

In the group treated with PP initially (n=44), two patients did not receive further treatment, one patient received other chemotherapy and 41 patients received EGFR TKI as second-line therapy. Eight of the 41 patients were still undergoing EGFR TKI therapy without PD, and 27 of the remaining 33 patients (81.8%) received at least one subsequent therapy after progression to second-line EGFR TKI. In the group with prior EGFR TKI therapy (n=61), five patients were still undergoing PP therapy without PD, and 53 of the remaining 56 patients (94.6%) received at least one subsequent therapy after progression to PP.

Table 3 Univariate analysis of response rate and disease-control rate in subgroup patients with prior EGFR TKI therapy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>ORR (%)</th>
<th>P-value</th>
<th>DCR (%)</th>
<th>P-value</th>
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<tr>
<td>Sex</td>
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<td></td>
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<tr>
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<td>±65</td>
<td>48</td>
<td>22.9</td>
<td>0.156</td>
<td>58.3</td>
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<tr>
<td>&gt;65</td>
<td>13</td>
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<td>0.676</td>
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<td>0–1</td>
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<td>0.511</td>
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<td>19Del or L858R</td>
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<td>25.5</td>
<td>1.000</td>
<td>58.2</td>
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<td>&gt;9 months</td>
<td>18</td>
<td>22.2</td>
<td></td>
<td>55.6</td>
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</table>

Notes: By Fisher’s exact test; aresponders – patients with partial response, nonresponders – patients with stable or progressive disease.

Abbreviations: ORR, objective response rate; DCR, disease-control rate; ECOG PS, Eastern Cooperative Oncology Group performance status; NS, nonsmoker; C/FS, current/former smoker; EGFR, epidermal growth-factor receptor; TKI, tyrosine-kinase inhibitor; PFS, progression-free survival.
of each other, and drugs used in frontline therapy, whether EGFR TKI or chemotherapy, may destroy more tumor cells. In 2006, Chang et al showed that chemonaïve patients had a higher response rate to gefitinib than chemotherapy-treated patients, and hypothesized that tumor cells will evolve into a more heterogeneous and resistant phenotype with a longer time after diagnosis.\(^1\) Recently, Bai et al also showed that chemotherapy may reduce EGFR-mutation frequency in both plasma and tumor tissue, and suspected a reduction of overall clinical benefit of subsequent EGFR TKI after chemotherapy.\(^2\) However, in the present study, we showed that neither responses nor survival time of PP therapy were significantly different between patients with and without prior EGFR TKI therapy. As PFS represents the duration of tumor control of an investigational therapy, PFS together with ORR and DCR are important indicators to demonstrate the effectiveness of the study regimen irrespective of subsequent treatment,\(^3\) and all these outcome parameters were similar in each group of the present study. By contrast, OS represents the greatest clinical benefit but could be skewed by the effects of subsequent therapies. In the group with PP as first-line therapy, 41 of 44 (93.2%) received EGFR TKI in subsequent therapies, which might explain the similar OS of both groups. The ORR of second-line EGFR TKI among 35 patients with measurable disease was 62.9%, which was similar to that of patients receiving EGFR TKI as first-line therapy (\(P=0.663\)).

In 2008, Deng et al used the lung adenocarcinoma cell lines PC9 and PC9/G with acquired resistance to gefitinib to explore the influence of acquired resistance of EGFR TKI on the sensitivity of tumor cells to chemotherapeutic drugs, and showed that no significant difference of sensitivity to pemetrexed was found between these two cell lines.\(^4\) Furthermore, a study by Maemondo et al that compared the efficacy of gefitinib and carboplatin plus paclitaxel as first-line treatment for patients with advanced NSCLC harboring EGFR mutations also showed a similar response rate of carboplatin plus paclitaxel either in the first-line setting or as subsequent therapy after progression to first-line gefitinib (30.7% versus 28.8%, respectively).\(^5\) In the gefitinib group, 67.5% of patients received carboplatin plus paclitaxel as second-line therapy. We suggest that there might be no clinically meaningful interference between EGFR TKI and chemotherapy.

In a study by Sun et al, there was no significant association between the efficacy of prior EGFR TKI and subsequent pemetrexed therapy.\(^6\) However, the results might have been limited by the diversity of previous treatments and the lack of EGFR-mutation analysis. The present study may provide a more solid result, because all patients with prior EGFR TKI therapy received PP therapy as second-line therapy, and all of them harbored EGFR mutations. Our results suggested that neither efficacy of prior EGFR TKI therapy nor the EGFR-mutation type would influence the outcome of subsequent PP therapy.

There are three limitations of this study. First, this was a retrospective study. Although data were collected retrospectively, we tried to ensure the validity of patients’ characteristics, and excluded patients with confounding factors that could lead to incorrect response evaluation. A prospective trial is needed to evaluate the extent of the impact of interaction between EGFR TKI and PP in advanced EGFR-mutant lung adenocarcinoma patients. Second, the EGFR status in this study was not assessed in all patients. Third, there was no biological analysis in the present study, such as thymidylate synthase-expression level between the treatment-naïve and post-EGFR TKI tumor tissue, which has been recognized to be associated with pemetrexed efficacy.\(^7\)

**Conclusion**

In conclusion, our results suggested that the efficacy of PP therapy in chemonaïve patients with EGFR-mutant adenocarcinoma was comparable between patients with and without prior EGFR TKI therapy. Furthermore, in patients with prior EGFR TKI therapy, there was no significant association between the efficacy of first-line EGFR TKI and the outcome of subsequent PP therapy.

**Acknowledgments**

We would like to thank the Comprehensive Cancer Center and Clinical Informatics Research and Development Center of Taichung Veterans General Hospital for the assistance of data collection and management, and acknowledge the technical services provided by the Pharmacogenomics Lab of the National Research Program for Biopharmaceuticals (NRPB) (TR6-3) and Integrated Core Facility for Functional Genomics of the National Core Facility Program for Biotechnology (NCFPB) (C5), which were supported by the National Science Council (NSC102-2325-B-002-078 and NSC102-2319-B-002).

**Disclosure**

The authors have no conflicts of interest in this work.
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