Comparison of therapeutic effects of EGFR-tyrosine kinase inhibitors on 19Del and L858R mutations in advanced lung adenocarcinoma and effect on cellular immune function

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Keywords
Efficacy; EGFR mutation; EGFR-TKI; lung adenocarcinoma.

Abstract

Background: We compared the therapeutic effect of EGFR-tyrosine kinase inhibitors (TKIs) on 19Del and L858R mutations in advanced lung adenocarcinoma on cellular immune function and explored the factors influencing the curative effect and prognosis.

Methods: Clinical efficacy in the selected 71 patients with lung adenocarcinoma, including 52 patients with 19Del and L858R mutations and 19 wild type patients treated with EGFR-TKIs was retrospectively analyzed. The response rate (RR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS), and cellular immune function were analyzed.

Results: The RR, DCR, PFS, and OS of the 19Del group were higher than those of the L858R group; however, there were no statistically significant differences between the groups. χ² test results revealed that gender, smoking, and EGFR mutations were associated with DCR. Log-rank analytical results showed that EGFR mutation type was correlated to PFS and OS. Multivariate analysis implied that disease control and mutation type of EGFR were independent prognostic factors of OS. Following TKI treatment, the number of CD3+, CD4+, and NK cells and the CD4+/CD8+ ratio increased in both mutation groups; however the results were not statistically significant. There was also no significant difference in the upregulation of immunological function observed, with 46.43% in the 19Del mutation and 45.83% in the L858R mutation group.

Conclusion: EGFR 19Del and L858R mutations are good biomarkers for predicting the clinical response of EGFR-TKIs. 19Del mutations may have a better clinical outcome.

Introduction

To date, chemotherapy is the primary treatment method for non-small cell lung cancer (NSCLC). Although combination therapy of cisplatin with other medicine is the standard first-line treatment for NSCLC, the efficacy of this regimen has reached a peak, with an efficacy rate of only 20%–35% and 10–12 months median survival.¹ Individualized molecular target therapy according to NSCLC molecular phenotype has drawn attention in recent years. Statistical analysis has demonstrated that EGFR gene mutation in in Asian patients with advanced adenocarcinoma is as high as 51.4%.² However, in the latest NSCLC cases, the number of patients with adenocarcinoma has dramatically increased to 60%.³ The development of EGFR-tyrosine kinase inhibitors (TKI), applied as the first-line treatment for NSCLC, has gradually shown advantages in efficacy and safety to treat sensitive mutant advanced NSCLC patients, especially patients with adenocarcinoma.

At present, EGFR mutation represents the optimal biomarker for predicting EGFR-TKI sensitivity, suggesting that future therapy will be guided by EGFR sensitive mutations. In-frame deletion of exon 19 (19Del) and missense mutation of exon 21 (L858R) are the most common EGFR mutations.
mutations in NSCLC, and have become the common biomarkers for predicting a clinical response to EGFR-TKI. Nevertheless, there is no definitive conclusion regarding the differences in EGFR-TKI efficacy for the treatment of both mutations. Further clinical data accumulation is necessary to explore the impact of EGFR-TKI in NSCLC patients in order to clinically screen the optimal patients for EGFR-TKI treatment.

In this study, advanced adenocarcinoma patients with 19Del and L858R were selected from the Jiangsu Province Hospital of Traditional Chinese Medicine between May 2014 and May 2017. All patients were defined without surgical indicators. The clinical efficacy of EGFR-TKI treatment for both types of EGFR mutation was analyzed.

**Methods**

**Clinical data**

A total of 145 adenocarcinoma patients diagnosed by pathology or cytology, at stages IIIb and IV according to 7th American Joint Committee on Cancer staging system, were tested using the amplification-refractory mutation system to predict EGFR mutation. Adenocarcinoma staging was based on American Joint Committee on Cancer and Union for International Cancer Control tumor node metastasis (TNM) classifications. All testing specimens were taken from paraffin sections or fresh tumor tissues. Amplification-refractory mutation system testing showed that 62 patients (men 22, women 40) had EGFR gene mutations, including 30 cases of 19Del mutation (48.8%), 28 cases of L858R mutation (45.2%) and 4 cases of G719X mutation. All mutations were single site mutations.

The selected 71 patients (mutation type 52, wild-type 19) were treated with EGFR-TKIs. Patient age ranged from 38 to 84 years, and there were 18 smokers and 53 non-smokers. In the 52 mutation cases (men 19, women 33), there were 28 19Del and 24 L858R mutations, with 11 cases of stage IIIb and 41 of stage IV. In the 19 wild-type patients (men 9, women 10), there were 3 cases of stage IIIb and 16 of stage IV. As computed tomography scanning revealed that 71 patients were ineligible for surgery, the efficacy estimation was conducted on these selected patients only.

**Drug administration**

Gefitinib (250 mg/d) or erlotinib (150 mg/d) were administered orally to patients until disease progression or death.

**Indicators and treatment evaluations**

Evaluation of the efficacy of EGFR-TKIs commenced one month after the initiation of drug administration, and was then evaluated every two months. Timely re-examination was conducted after disease progression. The final follow-up visit occurred in April 2017. The median follow-up duration was 15.8 months (range: 0.5–30.1).

Immunological function testing: 4 ml of peripheral blood obtained from each patient was tested by flow cytometry to detect the number of T cell subsets before and after treatment to evaluate immune cell activity. X-ray and computed tomography diagnosis results were compared before and after treatment.

Response rates (RRs), disease control rates (DCRs), progression-free survival (PFS), and overall survival (OS) were analyzed in the 19Del, L858R, and wild-type groups. Complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) were defined according to the Response Evaluation Criteria in Solid Tumors. PR and DCR were calculated using the following formulas: \( RR = \frac{(CR+PR)}{\text{total cases}} \times 100\% \) and \( DCR = \frac{(CR+PR+SD)}{\text{total cases}} \times 100\% \). PFS was defined as the duration from initial drug administration to PD or death from any cause. If PD did not occur or the patient was still alive at the final follow-up, the date of the last disease evaluation was recorded.

**Statistical analysis**

SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the results. A chi-square test was applied to analyze efficacy, while Kaplan–Meier curves and log-rank tests were used to analyze survival. Multivariate analysis of factors influencing prognosis was performed by Cox proportional hazards model. \( P < 0.05 \) was defined as statistically significant.

**Results**

**Efficacy**

Complete remission was not observed in either the mutation or wild-type groups, while 40 cases of PR, 12 cases of SD, and 19 cases of PD were observed among the selected 71 patients. In the 19Del mutation group, the RR and DCR were 71.4% and 85.7%, respectively. In the L858R mutation group, the RR and DCR were 62.5% and 79.2%, respectively. The median PFS was 13 months (95% confidence interval [CI] 9.4–19.6) and the median OS was 30 months (95% CI 29.19–30.80) in the 19Del mutation group. In the L858R mutation group, the median PFS was 12 months (95% CI 9.8–14.2) and the median OS was 28 months.
The impacts factors of efficacy

The χ² test revealed that gender, smoking, and EGFR mutation were associated with short-term treatment efficacy (P < 0.05), while age, TNM staging classification, Eastern Cooperative Oncology Group performance status, and TKI drug selection were not impact factors (P > 0.05). Log-rank analysis determined that EGFR mutation type was correlated to PFS and OS (P < 0.05), while gender, age, smoking, TNM staging classification, Eastern Cooperative Oncology Group performance status, and TKI drug selection were not associated with PFS or OS (P > 0.05) (Table 2). Multivariate analysis indicated that disease control and EGFR mutation type were independent prognostic factors of OS (P < 0.05) and that EGFR mutation status was closely associated with PFS (P = 0.05) (Table 3).

### Changes in T lymphocyte subsets and NK cell activity

Following TKI treatment for 19Del and L858R mutations, CD3⁺, CD4⁺, and NK cell activity and the CD4⁺/CD8⁺ ratio increased in both groups; however, the results were not statistically significant (P > 0.05). In the wild-type group, CD3⁺, CD4⁺, and NK cell activity and the CD4⁺/CD8⁺ ratio decreased after TKI treatment; however, these results were also not statistically significant (P > 0.05). Increases in T and NK cell activity in both the 19Del and L858R mutation groups were not statistically significant (P > 0.05). Upregulation of immunological function was observed in both mutation groups, at 46.43% (13/28) in the 19Del mutation and 45.83% (11/24) in the L858R mutation group. Immune

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**Table 1 Clinical efficacy of the three groups (n = 71)**

| Groups     | RR   | DCR   | PFS (month, [95%CI]) | OS   |
|------------|------|-------|----------------------|------|
| 19Del      | 20 (71.4) | 24 (85.7) | 13 (9–20) 30 (29–31) |      |
| L858R      | 15 (62.5) | 19 (79.2) | 12 (10–14) 28 (22–34) |      |
| Wild-type  | 5 (26.3)† | 9 (47.4)† | 7 (6–11) 15 (13–17)† |      |

†Compared to 19Del and L858R groups (P < 0.05). CI, confidence interval; DCR, disease control rate; OS, overall survival; PFS, progression-free survival; RR, relative risk.

(95% CI 22.5–33.5). The RR and DCR were higher and the PFS and OS were longer in the 19Del mutation group compared to the L858R mutation group. However, there was no statistically significant difference in RR, DCR, PFS, and OS between the two mutation groups (P = 0.494, 0.534, 0.55, and 0.21, respectively). The RR and DCR in both mutation groups was significantly higher than in the wild-type EGFR group, while the PFS and OS in the mutation groups was dramatically prolonged compared to the wild-type group, with statistical significance (Table 1).

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**Table 2 Efficacy analysis of 71 patients with advanced lung adenocarcinoma**

| Characteristics | Case | mPFS | mOS | P       | DCR (%)  | P         |
|-----------------|------|------|-----|---------|----------|-----------|
| Gender          |      |      |     |         |          |           |
| Male            | 28   | 11   | 25  | 0.25    | 0.24     | 0.01      |
| Female          | 43   | 13   | 30  | 0.64    | 0.9      | 0.68      |
| Age (year)      |      |      |     |         |          |           |
| ≤ 65            | 42   | 13   | 27  | 0.28    | 0.18     | 0.40      |
| > 66            | 29   | 11   | 30  | 0.88    | 0.98     | 0.049     |
| TNM stage       |      |      |     |         |          |           |
| IIIb            | 14   | 13   | 35  | 0.91    | 0.1      | 0.26      |
| IV              | 57   | 11   | 28  | 0.30    | 0.24     | 0.90      |
| Smoking         |      |      |     |         |          |           |
| Non-smoker      | 18   | 12   | 29  | 0.88    | 0.98     | 0.049     |
| Smoker          | 53   | 11   | 27  | 0.91    | 0.1      | 0.26      |
| ECOG PS         |      |      |     |         |          |           |
| 0-1             | 62   | 11   | 27  | 0.30    | 0.24     | 0.90      |
| 2               | 9    | 12   | 33  | 0.30    | 0.24     | 0.90      |
| TKI drug        |      |      |     |         |          |           |
| Gefitinib       | 37   | 11   | 26  | 0.91    | 0.1      | 0.26      |
| Erlotinib       | 34   | 12   | 30  | 0.30    | 0.24     | 0.90      |
| Mutation type   |      |      |     |         |          |           |
| 19del           | 28   | 13   | 30  | < 0.01  | < 0.01   | < 0.01    |
| L858R           | 24   | 12   | 28  | 0.91    | 0.1      | 0.26      |
| Wild-type       | 19   | 7    | 15  | 0.88    | 0.98     | 0.049     |

All confidence intervals are 95%. DCR, disease control rate; ECOG PS, Eastern Cooperative Oncology Group performance status; mOS, median overall survival; mPFS, median progression-free survival; TKI, tyrosine kinase inhibitor; TNM, tumor node metastasis.
upregulation referred to increases in the number of CD4+ and NK cells observed. Although the differences between the two mutation groups were not statistically significant ($\chi^2 = 0.3095; P = 0.578$), the increase in both groups was significantly higher than in the wild-type group at 15.79% (95% CI = 9.8–21.7) and 30.80%, respectively. In the 19Del group, the median PFS and OS was 12 months (95% CI = 9.8–14.2) and 30 months (95% CI = 27.0–33.4), respectively. In the L858R group, the median PFS and OS was 15 months (95% CI = 12.3–18.2) and 30 months (95% CI = 27.0–33.4), respectively. Significant favorable ORR, DCR, PFS, and OS were observed in the mutation groups compared to the wild-type group ($P < 0.05$). These results showed that TKIs could be effectively used to treat EGFR-sensitive mutated disease. Further comparison between the two mutation groups revealed a statistically significant difference in OS rate ($\chi^2 = 6.838, P = 0.0089$) (Table 4).

### Discussion

Exploration of EGFR mutations and evaluation of the efficacy and safety of molecular target drugs in clinical use has proven that EGFR-TKIs have significant efficacy and safety for treating EGFR-sensitive advanced adenocarcinoma. EGFR-TKI treatment has demonstrated great benefit to patients with lung cancer and has increased the survival rate. The IPASS study was the first to demonstrate a significant correlation between EGFR-sensitive mutations and gefitinib treatment efficacy.4 Following the IPASS study, clinical results of phase III trials OPTIMAL, NEJ002, EUR-TAC, and WJT03405 showed that EGFR-TKI as first-line therapy could greatly improve quality of life and prolong PFS in lung cancer patients with EGFR mutations compared to chemotherapy.

The most common EGFR mutations detected in our study were 19Del (45%) and L858R (40–45%). These two mutations account for 85–90% of EGFR mutations.5–7 Previous studies have reported that an EGFR-TKI probe was more effective for treating patients with 19Del mutations than patients with L858R mutations, with more significant clinical outcomes and favorable median PFS and OS rates in the 19Del mutation group.5–9 However, inconsistent outcomes have also been reported in some phase III clinical trials. These phase III trial results suggested that there was no significant difference in PFS in patients with 19Del and L858R mutations treated with TKIs.10 This result requires confirmation by more evidence-based and basic research.

This retrospective analysis of patients with EGFR mutations treated with EGFR-TKIs yielded RRs of 71.4% and 62.5%, and DCRs of 85.7% and 79.2% in the 19Del and L858R groups, respectively. In the 19Del group, the median PFS and median OS rates were 13 months (95% CI 9.4–19.6) and 30 months (95% CI 29.19–30.80), respectively. In the L858R group, the median PFS and OS was 12 months (95% CI 9.8–14.2) and 28 months (95% CI 22.5–33.5), respectively. Significant favorable ORR, DCR, PFS, and OS were observed in the mutation groups compared to the wild-type group ($P < 0.05$). These results showed that TKIs could be effectively used to treat EGFR-sensitive mutated disease. Further comparison between the two mutation groups revealed a statistically significant difference in OS rate ($\chi^2 = 6.838, P = 0.0089$) (Table 4).

### Table 3 Cox multivariate analysis of EGFR expression status with PFS and OS

| Characteristics | PFS | | | OS | | |
|----------------|-----|-----|-----|-----|-----|-----|
|                | HR  | 95% CI | $P$  | HR  | 95% CI | $P$  |
| Gender         | 1.283 | 0.579–2.759 | 0.523 | 1.294 | 0.549–3.051 | 0.555 |
| Age            | 0.842 | 0.453–1.564 | 0.586 | 0.769 | 0.401–1.475 | 0.429 |
| Smoking        | 0.839 | 0.363–1.934 | 0.468 | 0.763 | 0.317–1.838 | 0.254 |
| TNM stage      | 1.485 | 0.563–3.916 | 0.424 | 1.781 | 0.660–4.802 | 0.546 |
| ECOG PS        | 0.917 | 0.586–1.435 | 0.704 | 0.592 | 0.341–1.027 | 0.062 |
| Efficacy       | 0.490 | 0.219–1.097 | 0.083 | 0.342 | 0.193–0.768 | 0.009 |
| EGFR type      | 0.610 | 0.368–1.012 | 0.055 | 0.207 | 0.096–0.446 | 0.000 |

CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; TNM, tumor node metastasis.

### Table 4 Changes in T lymphocyte subsets and NK cell activity in patients before and after therapy

| T lymphocyte subsets | Phase | 19Del | L858R | Wild-type |
|----------------------|-------|-------|-------|-----------|
|                      | ([X± S] %) | ([X± S] %) | ([X± S] %) | ([X± S] %) |
| CD3+                 | 73.60±1.33 | 70.41±1.09 | 73.89±1.08 |
|                      | 74.17±1.27 | 72.44±1.04 | 72.52±1.30 |
| CD4+                 | 31.83±1.70 | 31.05±1.64 | 30.18±1.99 |
|                      | 34.23±1.96 | 31.95±1.71 | 27.39±1.72 |
| CD4+/CD8+            | 1.00±0.12 | 1.02±0.12 | 0.88±0.12 |
|                      | 1.14±0.12 | 1.04±0.98 | 0.69±0.07 |
| NK cell              | 16.39±1.28 | 15.56±1.26 | 14.58±1.35 |
|                      | 16.90±1.09 | 16.00±1.18 | 13.85±1.13 |
groups revealed higher RR and DCR in the 19Del group compared to the L858R group. In addition, longer median PFS and OS rates were observed in the 19Del group. Although there were no statistically significant differences in RR, DCR, PFS, and OS between the mutation groups (P = 0.494, 0.534, 0.55, and 0.21, respectively), the results indicated that EGFR-TKI treatment was more effective in patients with 19Del mutations. The small size of the patient sample may explain why no statistically significant differences were observed between the two mutation groups.

Further correlation analysis regarding clinical characteristics and efficacy implied that gender and smoking were associated with DCR. A short-term TKI treatment advantage was observed in non-smoking female patients. The association between DCR, PFS, OS, and EGFR mutation type suggested the clinical efficacy of using TKIs to treat EGFR-sensitive mutations. The data showed that the use of different kinds of TKI drugs had no significant impact on DCR, PFS, or OS, indicating that the selection of TKI drugs has no dramatic influence on prognosis after treatment.

The results of Cox analysis suggested that the disease control profile and EGFR mutation status (P < 0.05) were independent prognostic factors of OS. EGFR mutation status was closely associated with PFS (P = 0.055), consistent with the results of previous studies. However, this result requires confirmation by studies using larger samples.

Downregulation and immune system dysfunction, along with the hypofunction of T and NK cells, was observed in advanced lung cancer patients during tumor progression. Cellular immune function is suppressed in cancer patients and as such, cannot activate an immune response. However, the survival duration in lung cancer patients was prolonged after immunotherapy with mixed T and NK effector cells. Studies have indicated that TKI drugs stimulate important immunoregulatory activity by participating in the development and activation of immune cells, leading to the activation of T and NK cells. Therefore, elevating the sensitivity of tumor cells to immune regulation enhances the immunologic function of immune cells. It is accepted that the CD4+/CD8+ ratio and NK cell activity and the CD4+/CD8+ ratio was improved after TKI treatment in both 19Del and L858R mutation groups; however, these results were not statistically significant (P > 0.05). Decreased activity of CD3+, CD4+, and NK cell activity and the CD4+/CD8+ ratio was observed in the wild-type group after TKI treatment; however, these results were also not statistically significant (P > 0.05). There was no statistically significant difference in increases in the degree of viability of these immune cells before and after TKI treatment in either mutation group (P > 0.05). After TKI treatment, upregulation of immunologic function was observed in 46.43% of 19Del and 45.83% of L858R mutation patients (χ² = 0.3095; P = 0.578). Although there was no statistically significant difference, the number of patients with immunological upregulation in both mutation groups was higher compared to the wild-type group. These results indicate that TKI treatment was effective for improving immunological function in advanced lung cancer patients with EGFR mutations.

In conclusion, EGFR mutation is the main impact factor of the clinical efficacy of TKI treatment for advanced adenocarcinoma. Compared to the L858R mutation group, higher RR and DCR and longer median PFS and OS rates were observed in the 19Del mutation group, demonstrating that TKI treatment was more effective for patients with 19Del mutations. As no statistically significant differences between the mutation groups were observed, further prospective randomized controlled studies of TKI treatment efficacy for patients with EGFR mutations should be conducted with large patient samples. Moreover, mechanisms behind the biological behavior of different EGFR mutant subtypes should also be explored.

Disclosure
No authors report any conflict of interest.

References
1. Schiller JH, Harrington D, Belani CP et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346: 92–8.
2. Shi Y, Au JS, Thongprasert S et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). J Thorac Oncol 2014; 9: 154–62.
3. Sakurai H, Asamura H, Goya T et al. Survival differences by gender for resected non-small cell lung cancer: A retrospective analysis of 12,509 cases in a Japanese Lung Cancer Registry study. J Thorac Oncol 2010; 5: 1594–601.
4. Fukuoka M, Wu YL, Thongprasert S et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011; 29: 2866–74.
5. Pao W, Miller VA. Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: Current knowledge and future directions. J Clin Oncol 2005; 23: 2556–68.
6. Zhang Y, Sheng J, Kang S et al. Patients with exon 19 deletion were associated with longer progression-free survival compared to those with L835R mutation after first
line EGFR-TKIs for advanced non-small cell lung cancer: A meta-analysis. *PLoS One* 2014; 9: e107161.

7 Riely GJ, Pao W, Pham D *et al*. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. *Clin Cancer Res* 2006; 12: 839–44.

8 Rosell R, Moran T, Queralt C *et al*. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* 2009; 361: 958–67.

9 Li JJ, Qu LL, Wei X *et al*. [Clinical observation of EGFR-TKI as a first-line therapy on advanced non-small cell lung cancer.]. *Chinese J Lung Cancer* 2012; 15: 299–304 (In Chinese.)

10 Maemondo M, Inoue A, Kobayashi K *et al*. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010; 362: 2380–8.

11 Zhou C, Wu YL, Chen G *et al*. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* 2011; 12: 735–42.

12 Cappuzzo F, Ciuleanu T, Stelmakh L *et al*. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: A multicentre, randomized, placebo-controlled phase 3 study. *Lancet Oncol* 2010; 11: 521–9.

13 Zhang G, Zhao H, Wu J *et al*. Adoptive immunotherapy for non-small cell lung cancer by NK and cytotoxic T lymphocytes mixed effector cells: Retrospective clinical observation. *Int Immunopharmacol* 2014; 21: 396–405.

14 Farsaci B, Higgins JP, Hodge JW. Consequence of dose scheduling of sunitinib on host immune response elements and vaccine combination therapy. *Int J Cancer* 2012; 130: 1948–59.

15 Tu C, Zhu Y, Jiang B, He W, Jin C. Correlation between circulating tumor cells EGFR expression and T cell subsets in advanced non-small cell lung cancer patients after tyrosine kinase inhibitor treatment. *Neoplasma* 2017; 64: 619–25.

16 Mustjoki S, Ekblom M, Arstila TP *et al*. Clonal expansion of T/NK-cells during tyrosine kinase inhibitor dasatinib therapy. *Leukemia* 2009; 23: 1398–405.