

Afatinib treatment response in advanced lung adenocarcinomas harboring uncommon mutations

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Abstract

Background: Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have improved the prognosis of mutant lung cancer; however, the clinical application value of TKIs for nonclassical EGFR mutation is unclear, especially for patients with rare uncommon mutations.

Methods: A retrospective study based on electronic medical records was conducted to collect data on the effectiveness of afatinib in patients with stage IIIB/IV lung adenocarcinoma (LUAD) bearing uncommon mutations between January 2017 and January 2021.

Results: Forty-two patients with uncommon mutations treated with afatinib were enrolled. The objective response rate (ORR) was 50.0% (10 of 20 patients). The median time to treatment failure (TTF) was 11.7 months (95% confidence interval = 8.5–18.3 months). Of the 42 patients, the median TTF was 15.0, 11.7, and 16.6 months in patients with Gly719Xaa (G719X), Ser768Ile (S768I), and Leu861Gln (L861Q) mutations, respectively. In patients with the rare uncommon mutation, the median TTF was 10.0 months, and the ORR was 50.0%. Afatinib demonstrated clinical activity across a set type of specific rare uncommon mutations, including EGFR L747P, A767_V769dup, and L833V/H835L, with a case having a TTF of more than 1 year. Molecular profiling reports of 16 afatinib-resistant biopsy samples were available, and the secondary T790M mutation was detected in one patient with L833V/H835L mutation and one harboring S768I/L858R mutation.

Conclusions: Our findings suggested that afatinib is effective in patients with uncommon mutations. Mechanisms of afatinib resistance vary and need further investigation.

KEYWORDS

afatinib, non-small cell lung cancer, uncommon mutation

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common malignancies worldwide.¹ With the deepening understanding of the molecular pathogenesis of NSCLC, using molecularly targeted anticancer drugs for optimal treatment is possible.² Recent studies have shown that epidermal growth factor receptor (EGFR) mutations are one of the most common occurrences in lung adenocarcinoma (LUAD) and occur most frequently in tumors from never-

smokers, women, and Asian populations.^{3,4} The identification of EGFR mutations and the discovery of their sensitivity to targetable tyrosine kinase inhibitors (TKIs) dramatically changed how LUAD is managed.⁵

Patients with EGFR mutations represent a heterogeneous population.⁶ The two most common EGFR mutations (i.e., EGFR19del and L858R mutations) account for 85% of all mutation-positive NSCLC cases and are sensitive to EGFR TKIs and called “common mutations”.^{7–9} Moreover, uncommon mutations are being paid increasing attention. In the Catalogue of Somatic Mutations in Cancer database, ~594 types of EGFR mutations were reported. Among them,

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~93% are present in the first four exons (18–21) of the gene encoding tyrosine kinase domain, including exon 18 Gly719Xaa (G719X) (~3%), exon 21 Leu861Gln (L861Q) (~1%), and exon 20 Ser768Ile (S768I) mutations (~1%), as well as other insertions in exon 19 or 20 (ins19; 0.6%, and ins20; ~6%).¹⁰ Although at low frequency, approximately tens of thousands of new cases worldwide are reported each year because of the large population base of NSCLC.¹¹ Meanwhile, with the rapid development of the next-generation sequencing (NGS) technology, it has been indicated that uncommon EGFR mutations are much more prevalent than previously realized.¹² Therefore, more attention is needed for developing effective therapeutic approaches to improve patient prognosis and survival rate.

Afatinib is an irreversible ErbB family inhibitor approved for treating patients with NSCLC harboring EGFR nonresistant mutations and has been currently approved in more than 80 countries worldwide.¹³ With such a drug mechanism, patients with uncommon mutations were eligible to enroll in registered clinical trials of afatinib. In a pooled analysis of clinical trials of lux-lung 2, lux-lung 3, and lux-lung 6, 75 (12%) patients harboring uncommon EGFR mutations were enrolled. The progression-free survival (PFS) was 13.8, 8.2, and 14.7 months, respectively, for patients with G719X, L861Q, and S768I mutations treated with afatinib.¹⁴ Concomitant to this, several retrospective studies in Asia have proven the efficacy of afatinib in patients with uncommon mutations with an objective response rate (ORR) of ~50%–70%.¹⁵ However, compared with major uncommon mutations (i.e., G719X, L861Q, and S768I), only a few case reports on some specific uncommon mutations, such as EGFR-KDD, are available.^{16,17} Furthermore, the outcomes and resistance mechanisms of specific rare uncommon mutations have not yet been reported.¹⁸ Therefore, there is a large unmet need for further detailed empirical evidence to focus on afatinib activity in uncommon mutations, especially for the “rare” group.¹⁹

Therefore, this study was designed to evaluate the responses to afatinib treatment of patients with G719X, L861Q, S768I, and other rare uncommon mutations, as well as to determine the clinical demographics and underlying mechanisms of resistance. A detailed report for rare uncommon mutations was listed, which can provide more references for clinical practice.

METHODS

Study design and participants

We retrospectively analyzed patients with advanced NSCLC harboring uncommon mutations who received afatinib between January 2017 and January 2021. The inclusion criteria were as follows: (1) adult patients; (2) those diagnosed with advanced NSCLC, harboring uncommon mutations; and (3) those that started with afatinib monotherapy within regular clinical practice. The exclusion criteria were

patients with acquired T790M mutations before afatinib treatment, those without follow-up visits, and those treated with combination therapy as the initial treatment.

Clinical data collection and efficacy evaluations

Baseline characteristics were collected, including sex, age, smoking history, history of treatment, site of metastasis, and types of mutations. The key endpoint was time to treatment failure (TTF), which was defined as the time from the start of afatinib monotherapy to the end of afatinib monotherapy treatment, the initiation of combination therapy, or death by any cause. The secondary objective was the ORR. The ORR will be evaluated only if the imaging date is available at least twice since the start of the afatinib treatment. G719X, S768I, and L861Q mutations were defined as major uncommon mutations. Other mutations, such as L747P and H835L, were defined as rare uncommon mutations.

Statistical analysis

TTF was analyzed using the Kaplan–Meier method and is expressed as median value and corresponding 95% confidence interval (CI). Survival curves were compared using the log-rank (Mantel–Cox) test. *p* values of <0.05 were used to denote statistical significance. All statistical analyses were exploratory, and there was no formal statistical analysis plan. We used Statistical Package for the Social Sciences (version 24; IBM) and R version 3.6.2 for all statistical analyses.

RESULT

Demographics

In the analysis, 42 patients with recurrent or metastatic NSCLC were enrolled from January 2017 to January 2021. Among them, 14 patients were included in our previous study, and the ORR was reevaluated, and the TTF was reported for the first time.²⁰

The most common uncommon EGFR mutation was G719X (*n* = 20; 48%), followed by S768I (*n* = 12; 29%) and L861Q (*n* = 3; 7%). Fifteen patients had mutations other than G719X, L861Q, and S768I; seven patients had concomitant common EGFR mutations. Patient characteristics are listed in Table 1. Among these patients, 62% were women, and 64% were never smokers. The median age was 61 years (range, 40–83 years), and 41 (98%) patients had adenocarcinoma. Of the 42 patients, 36 (86%) received afatinib treatment as the first-line therapy, and six (14%) received afatinib treatment as the second-line therapy or above. Moreover, 17 patients (40%) underwent surgery. At the start of afatinib treatment, 12 patients (29%) had central nervous system (CNS) metastasis, and 10 patients (24%) had liver metastasis.

Outcomes in patients treated with afatinib

At the time of data cut-off (January 31, 2021), following afatinib treatment, the median TTF of all patients was

TABLE 1 Baseline characteristics of the participants

| Characteristic | Patients (<i>n</i> = 42) |
|-------------------------|---------------------------|
| Median age (range), y | 61 (40–83) |
| Sex | |
| Men | 16 (38%) |
| Women | 26 (62%) |
| Smoking status | |
| Never smoked | 27 (64%) |
| Current or ex-smoker | 11 (26%) |
| Unknown | 4 (10%) |
| Histology | |
| Adenocarcinoma | 41 (98%) |
| Others | 1 (2%) |
| Line of therapy | |
| First line | 36 (86%) |
| Second line and above | 6 (14%) |
| Site of metastasis | |
| CNS | 12 (29%) |
| Liver | 10 (24%) |
| Both unknown | 4 (10%) |
| Surgical history | |
| Yes | 17 (40%) |
| No | 25 (60%) |
| Uncommon EGFR mutation* | |
| G719X | 20 (48%) |
| L861Q | 3 (7%) |
| S768I | 12 (29%) |
| Others | 15 (36%) |

Abbreviations: CNS, central nervous system; EGFR, epidermal growth factor receptor.
*Uncommon mutation categories overlap with patients with compound mutations, so each patient may belong to multiple categories.

11.7 months (95% CI = 8.5–18.3 months) (Figure 1(a)). The best tumor response was reported in 20 patients. In total, 10 of the 20 patients had partial remission (ORR = 50.0%), including G719X only or combined (*n* = 4), R776H/L858R (*n* = 2), and others (Figure 1(b)).

We next analyzed the TTF according to the number of treatment lines. In the first-line therapy (*n* = 36), the ORR was 47.1% (8 of 17 patients). The median TTF was 13.2 months (95% CI = 8.5–23.1 months). Of note, two patients with uncommon mutations (one with R776H/L858R and one harboring G719X) remained on treatment for more than 3 years. In patients pretreated before the afatinib treatment (*n* = 6), afatinib also demonstrated inhibitory activity against uncommon mutations (median TTF, 8.1 months; 95% CI = 6.6–NA; ORR = 66.7%). However, no statistically significant difference in the TTF was observed between the different therapy lines (1st vs. others: hazard ratio [HR], 0.7; 95% CI = 0.5–3.6; *p* = 0.5) (Figure 1(a)).

Studies have demonstrated that the CNS and liver metastases in patients with NSCLC might be associated with poor clinical outcomes. We further analyzed the data of those with metastases. The median TTF was 11.6 and 9.1 months in patients with CNS and liver metastases, respectively. In the subgroup analysis, the TTF of the no baseline liver metastases group and that of the baseline liver metastases group were not statistically significant (HR = 0.4; 95% CI = 0.2–1.0; *p* = 0.05) (Figure 2(a)). Similarly, no statistically significant difference in the TTF was found between the no baseline CNS metastases and with baseline CNS metastases groups (HR = 0.7; 95% CI = 0.3–1.8; *p* = 0.4) (Figure 2(b)).

Activity of afatinib against G719X/L861Q/S768I mutations

We further performed a subset analysis of the ORR and TTF according to the type of uncommon EGFR mutations, including G719X, L861Q, and S768I mutations, which are

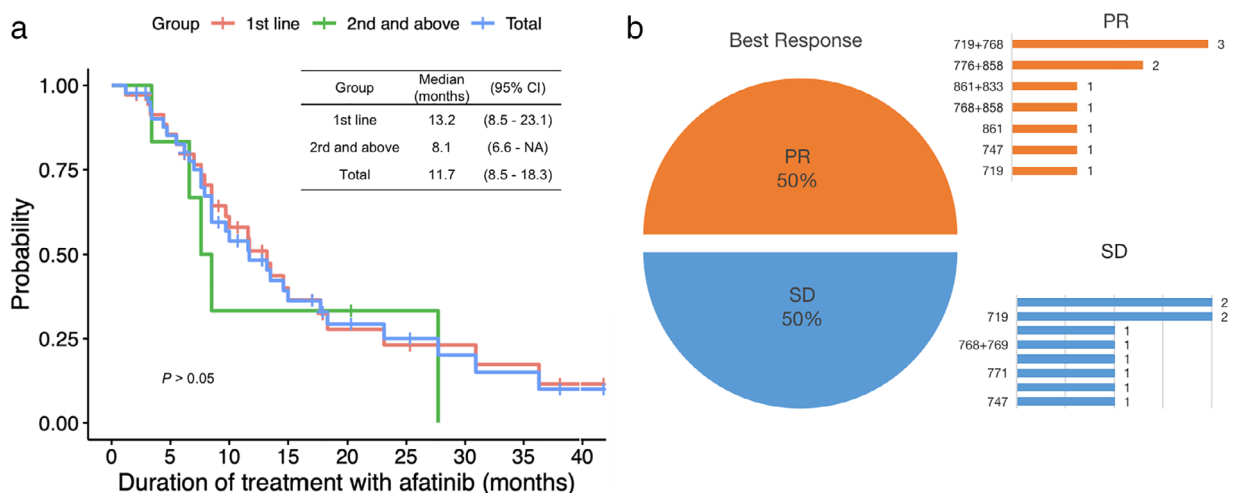


FIGURE 1 The TTF (a) and response (b) of afatinib in patients with NSCLC harboring uncommon mutations

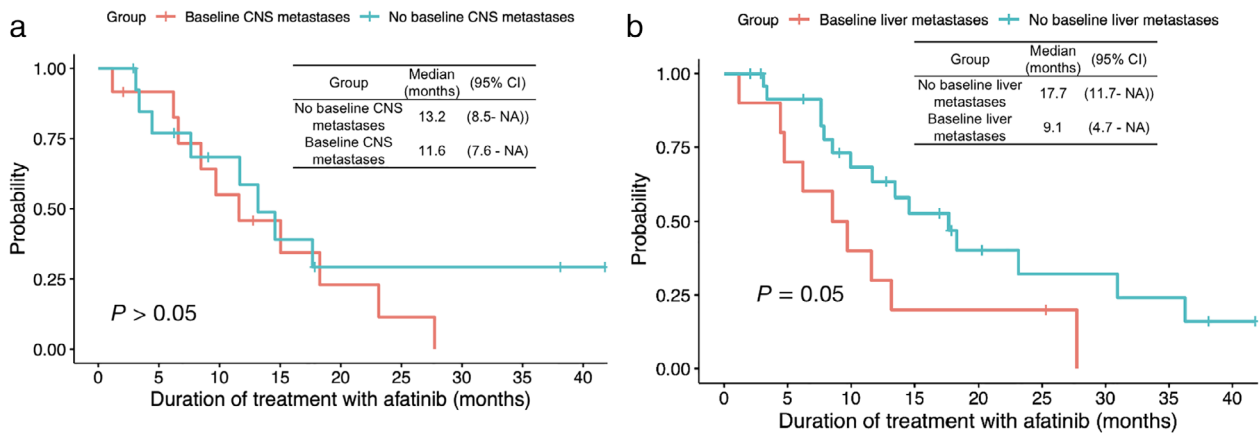


FIGURE 2 Subgroup analysis of the TTF in patients with NSCLC harboring uncommon mutations with central nervous system (CNS) (a) and liver (b) metastases

the three most frequently detected types of uncommon EGFR mutations (Table 2). Objective responses were noted in 50.0%, 50.0%, and 50.0% of patients with G719X, S768I, and L861Q mutations. Accordingly, the TTF of patients with G719X, S768I, and L861Q mutation was 15.0 months (range, 7.9–NA), 11.7 months (range, 7.0–NA), and 16.6 months (range, 5.5–NA), respectively (Figure 3(a)).

We analyzed the difference between single major mutations and compound mutations in first-line therapy. Considering that previous studies have suggested that the rare uncommon mutation group has a lower TTF, here, we analyzed only single major mutations and major mutations compound with major/common mutations, and patients with the rare uncommon mutation were excluded. Among 18 patients, nine harbored compound EGFR mutations: two patients had compound mutations with common EGFR mutations, and seven had compound G719X mutation with S768I mutation. In the subgroup analysis, the TTF of the compound mutation group (TTF, 30.9 months) and that of the single mutation group (15.0 months) were not statistically significant (HR = 0.6; 95% CI = 0.5–5.7; $p = 0.5$) (Figure 3(b)).

Activity of afatinib in specific rare uncommon mutation

Table 3 shows the specific uncommon mutation types and outcomes of patients with each mutation type. Twenty patients with tumors harboring rare uncommon mutations received afatinib. This was a highly heterogeneous category, including mutations across EGFR exon 18 ($n = 5$), exon 19 ($n = 3$), exon 20 ($n = 7$), exon 21 ($n = 3$), and others ($n = 2$). Afatinib demonstrated clinical activity across a broad set of uncommon mutations, including EGFR E709A/L858R, S720F/G719A, L747P, A767_V769dup (patient 1), R776H/L858R, and L833V/H835L with a TTF of more than 1 year. Subgroup analysis showed that the TTF of patients with rare uncommon mutation compound with or without major/common mutations also exhibited insignificant

TABLE 2 Activity of afatinib in major uncommon mutations

| Mutation | Objective response | | Median time to treatment failure, months (95% CI) |
|----------------------------|--------------------|-------|---|
| | No. | (%) | |
| G719X ($n = 20$) | 50.0% | (4/8) | 15.0 (7.9–NA) |
| G719X ($n = 8$) | | | |
| G719X + S768I ($n = 8$) | | | |
| G719X + others ($n = 4$) | | | |
| L861Q ($n = 3$) | 50.0% | (1/2) | 16.6 (5.5–NA) |
| L861Q ($n = 2$) | | | |
| L861Q + others ($n = 1$) | | | |
| S768I ($n = 12$) | 50.0% | (4/8) | 11.7 (7.0–NA) |
| S768I ($n = 1$) | | | |
| S768I + G719X ($n = 8$) | | | |
| S768I + others ($n = 3$) | | | |

Note: Uncommon mutation categories overlap with patients with compound mutations, so each patient may belong to multiple categories.

differences (median TTF: 10.0 vs. 11.6 months; HR = 0.8; 95% CI = 0.5–3.4; $p = 0.6$) (Figure 3(c)).

Considering the lack of reports on the treatment of EGFR exon 20 A767_V769dup mutation with afatinib, we further described the treatment details of patient 1. Patient 1 was a 54-year-old female and never-smoker and relapsed after the operation. On August 9, 2013, the patient underwent resection of the left lower lobe in our hospital and was diagnosed with EGFR exon20 A767_V769dup mutation stage IB (pT2N0M0) LUAD. Recurrence of the left pulmonary nodule was observed 4 years after the operation (August 2017). Meanwhile, genetic analysis of the peripheral blood at that time also showed a mutation of EGFR exon 20 as previously detected. Subsequently, the patient was treated with afatinib (30 mg, oral, daily) from September 9, 2017. On her first restaging scan at 2 months, the patient had no complete response/partial response and continued

on treatment. After 36 months of treatment (September 2020), the patient's CT image disclosed that some nodules of the left lung are larger than previously observed. At this visit, we adjusted her treatment strategy to afatinib combined with bevacizumab. As of article submission, the patient remained on treatment with afatinib combined with bevacizumab without disease progression.

Potential mechanism of resistance of afatinib against uncommon mutations

The NGS results of 16 patients after treatment failure were available. Among them, six patients had NGS reports at both baseline and progression (Table 4). Of all 16 patients, liquid biopsies were performed in 14 patients, and tissue biopsies

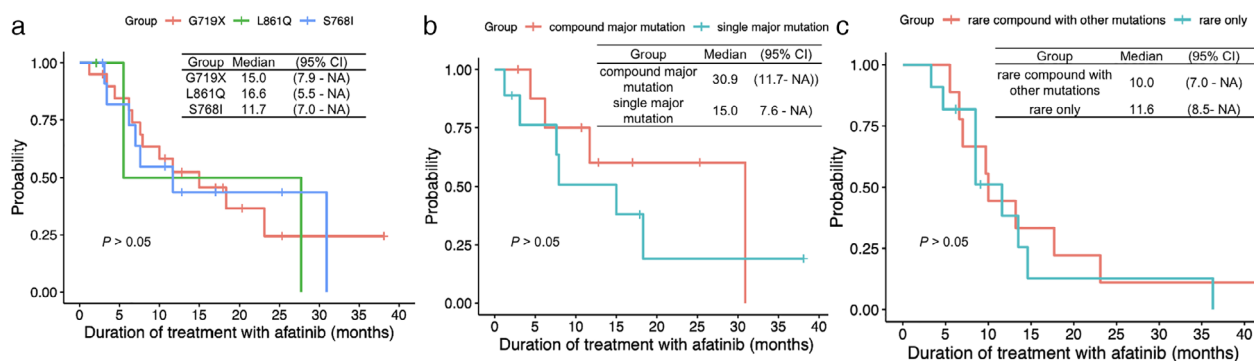


FIGURE 3 Subgroup analysis of the TTF in patients with NSCLC harboring uncommon mutations. (a) Patients with major EGFR uncommon mutations (i.e., S768I, G719X, and L861Q). (b) TKI-naïve patients with major uncommon mutations and major uncommon mutations compound with major/common mutations. (c) Patients with rare uncommon mutations and rare uncommon mutations compound with major/common mutations

| Uncommon mutation | Coexisting EGFR mutation | Other treatments failed before? | Best response | Time on treatment (months) |
|---------------------------------|--------------------------|---------------------------------|---------------|----------------------------|
| Exon 18 | | | | |
| E709A | L858R | No | NA | 17.7 |
| E709K | G719A | No | NA | 10.0 |
| L718V | L858R | No | NA | 9.7 |
| S720F | G719A | No | Non-CR/Non-PD | 23.1 |
| A722G | No | No | NA | 3.3 |
| Exon 19 | | | | |
| L747P | No | No | SD | 14.6 |
| L747P | No | Yes | PR | 8.5 |
| L747P | No | No | NA | 8.5 |
| Exon 20 | | | | |
| A763_Y764insFQEA | No | No | Non-CR/Non-PD | 6.2+ |
| A767_V769dup | No | No | Non-CR/Non-PD | 36.3 |
| N771 > GD | No | No | SD | 4.7 |
| V769L | S768I | No | SD | 7.0 |
| V769L | L861Q | No | NA | 5.5 |
| R776H | L858R | No | PR | 13.2 |
| R776H | L858R | No | PR | 41.8+ |
| Exon 21 | | | | |
| L833V | H835L | No | SD | 13.5 |
| L833V | G719S | Yes | NA | 6.6 |
| L833F | L861R | No | PR | 11.6 |
| Others | | | | |
| HER2 exon20 G778_P780dup | No | No | Non-CR/Non-PD | 8.5 |
| HER2 exon20 A775_G776insYVMA | No | No | Non-CR/Non-PD | 9.1+ |

TABLE 3 Activity of afatinib in specific rare uncommon mutations

Abbreviations: CR, complete response; EGFR, epidermal growth factor receptor; NA, not available; PD, progressive disease; PR, partial response; SD, stable disease.

TABLE 4 NGS reports at both baseline and progression of six patients

| No. | Baseline sample | Baseline NGS result | Line | Best response | TTF (months) | Secondary sample | Secondary NGS result |
|-----|------------------|--|-------------|---------------|--------------|------------------|--|
| 1 | Pleural fluid | EGFR p.L833V EGFR p.H835L EGFR cn_amp RET c.625 + 9C > T TSC2 p.N1564S TP53 p.P36fs | 1 | SD | 13.5 | Peripheral blood | EGFR p.T790M EGFR p.L833V EGFR p.H835L |
| 2 | Tissue | EGFR p.L861Q EGFR p.V769L TP53 p.YE220* | 1 | NA | 5.5 | Peripheral blood | EGFR p.V769L EGFR p.L861Q TP53 p.R273C TP53 p.R248W TP53 p.Y220* |
| 3 | Peripheral blood | EGFR p.L747P TP53 p.C242fs | 1 | SD | 14.6 | Peripheral blood | POLE p.N1638S |
| 4 | Tissue | EGFR p.G719A TP53 p.Y220C | 1 | PR | 7.6 | Peripheral blood | (-) |
| 5 | Tissue | EGFR p.L747P SMAD2 p.N148Kfs*7 TP53 p.E224D | 2 and above | SD | 8.5 | Peripheral blood | EGFR p.L747S |
| 6 | Tissue | EGFR p.G719C EGFR p.S768I KDR p.P328H TP53 p.D184fs CCND1 cn_amp EGFR cn_amp FGF19 cn_amp FGF3 cn_amp | 2 and above | SD | 3.4 | Peripheral blood | EGFR p.G719C EGFR p.S768I PMS2 p.G207* |

Abbreviations: NA, not available; NGS, next-generation sequencing; PR, partial response; SD, stable disease; TTF, time to treatment failure.

*Termination codon (Ter).

were performed in two patients. Five of the 14 patients who underwent liquid biopsy had negative EGFR mutation results. Of the remaining cases, only two cases were positive for EGFR T790M mutations. Of the two patients with T790M mutation, they both received afatinib as the first-line treatment. Among them, one harbored EGFR exon 20 S768I and exon 21 L8585R mutations at baseline, which was expected, and the other one harbored EGFR exon 21 L833V/H835L mutation (patient 2).

Patient 2 was a 75-year-old male and never-smoker diagnosed with EGFR exon 21 L833V/H835L mutation stage IV LUAD. In addition to the EGFR mutation, the tumor harbored RET c.625 + 9C > T, TSC2 p.N1564S and TP53 p.P36fs mutations and EGFR amplification, as determined by the pleural fluid-based NGS platform. From November 20, 2019, the patient received treatment with afatinib (30 mg, oral, daily). After 12 months (November 25, 2020), chest CT revealed a significant increase in tumor size, and brain MRI demonstrated multiple parenchymal brain metastases. According to the response evaluation criteria in solid tumors (version 1.1), the patient had progressive disease and was resistant to afatinib. On December 17, 2020, NGS of plasma detected resistant EGFR T790M and two other uncommon mutations (L833V and H835L). The treatment was switched to osimertinib. On January 31, 2021, the first evaluation of the effectiveness had not yet been assessed.

DISCUSSION

This study was one of the largest retrospective studies demonstrating the efficacy of afatinib on uncommon mutations. The ORR was 50.0%, and the median TTF was 11.7 months among 42 patients. The activity of afatinib was observed in G719X, L861Q, and S768I mutations and some rare uncommon mutations.

In the literature, several reports support our results. According to the combined post hoc analysis of patients with Lux2, Lux3, Lux6, and Lux27 (71.1%) mutations with G719X, L861Q, or S781I mutation treated with afatinib had objective responses. The median PFS was 10.7 months, and the median OS was 19.4 months. Additionally, this pooled analysis suggested that objective responses were most common in patients with S768I mutations (100%), followed by those with G719X (77.8%) and L861Q (56.3%) mutations.¹⁴ Our results are consistent with that and suggested that afatinib, a second-generation TKI, had a compromised efficacy in both TKI-naïve and pretreated patients harboring uncommon NSCLC mutations with a TTF of ~1 year. However, the ORR in this study is lower than that reported in the literature. Objective responses were noted in 50.0%, 50.0%, and 50.0% of patients with G719X, S768I, and L861Q mutations in this study. We considered the possibility that more treatment lines and the inaccessibility of some patients' efficacy evaluation data may explain the discrepancy in results.

Although there are many EGFR TKIs available for treating EGFR mutation-positive NSCLC, afatinib still has an advantage in treating uncommon mutations over first- or third-generation TKIs. In relation to this issue, preclinical studies have indicated that second-generation EGFR TKIs have relatively broader activity than others.²¹ The IC₅₀ values of afatinib were ~1–2 nM, which was much lower than that for gefitinib/osimertinib in cell lines with both common and uncommon EGFR mutations.¹⁰ Consistent with this, although there is no direct head-to-head comparison, afatinib still has the highest level of evidence and clinical reports in nonclassical mutations. For first-generation TKIs, the response rate of gefitinib/erlotinib in treating uncommon mutations was 40%–50% in some retrospective data.¹⁵ However, in the prospective NEJ002 study, the ORR of uncommon mutations treated with gefitinib was only 20%, which was significantly lower than that of common mutations, such as L858R.²² For third-generation TKIs, a phase II clinical trial (NCT03424759) has also shown that osimertinib is effective against uncommon mutations with an ORR of 50% and an mPFS of 8.2 months.²³ However, it is still much lower than that of classical mutations (i.e., 19del and L858R) and requires more study data. Obviously, the efficacy of first- or third-generation TKIs cannot meet the clinical needs, and therefore, afatinib has gained prominence in clinical practice. Similar to small-sample retrospective studies, our data confirmed the efficacy of afatinib on uncommon mutations with an ORR of 50.0% and a TTF of 11.7 months. In addition to this study, a larger study assessing the activity of afatinib in 693 patients with tumors harboring uncommon EGFR mutations treated in randomized clinical trials and real-world data was conducted in 2020. In that study, afatinib had clinical activity in major uncommon mutations (i.e., G719X, L861Q, and S768I) with a TTF of 10.8 months and a DoR of 17.1 months.²⁴ Based on the results of our study and other studies, we recommend afatinib as a first-line treatment for patients with uncommon EGFR mutations.

Studies have suggested that co-mutation affects the prognosis of patients. Some studies can give some insight and some clues on this issue. In a multicenter retrospective study, patients with uncommon compound EGFR mutations (i.e., G719X + L861Q and G719X + S768I) had a significantly longer PFS than those with a single mutation (median, 11.9 vs. 6.5 months; $p = 0.01$).²⁵ Similarly, in a database on afatinib uncommon mutations, afatinib is active across compound major mutations with a TTF of 14.7 months; however, the TTF of the single major mutation group was only 10.8 months.²⁴ Consistent with them, in our real clinical practice, the mTTF of the compound mutation group was 30.9 months, and that of the single group was 15.0 months in first-line therapy. Unfortunately, the TTF exhibited insignificant differences ($p = 0.5$). The small number of participants may explain why the result is not significant.

Tumor metastasis has always been an important aspect of cancer mortality and prognosis. Studies have shown that the improvement in survival observed following afatinib

administration in patients with brain metastases was similar to that seen in patients without brain metastases.²⁶ Similar to this, we also found that afatinib can provide therapeutic efficacy in patients with CNS metastases (11.6 months). Liver metastases were also reported to be one of the virtual prognostic factors in patients with NSCLC, which may be related to a “cold” tumor microenvironment in liver metastases from primary tumors.²⁷ Recently, it was shown that liver metastasis with TKI monotherapy was suboptimal.²⁸ In our cohort, the TTF was only 9.1 months in the group with liver metastasis. Given the small sample size, liver metastasis did not have a statistically significant impact on the TTF. In the future, it needs to be further confirmed by expanding the sample size and exploring better treatment strategies for patients with liver metastasis.

Currently, limited clinical data were available for the efficacy of EGFR TKIs in patients with NSCLC harboring rare uncommon mutations, and further observations are necessary. Some case reports or case series reports have shown the efficacy of afatinib in some rare mutations; however, the sample size was limited.^{29–31} In a database on afatinib uncommon mutation, researchers have provided the largest investigation of afatinib activity against other uncommon mutations. That study has shown that afatinib demonstrated activity against other uncommon mutations (4.5 months; 95% CI = 2.9–9.7 months).²⁴ In this study, the median TTF of afatinib for the rare uncommon mutations (10.0 months) seemed to be longer than previously reported (4.5 months). Although we do not have a definitive explanation for this result, patients with combined mutations that were not excluded from our subgroup analysis may in part contribute to the longer TTF. Moreover, we further reported the treatment details of one patient with EGFR exon20 A767_V769dup mutation treated with afatinib. To our knowledge, only osimertinib had been reported to be valid for this type of mutation and was recommended.³² In our case report, afatinib also showed good efficacy to EGFR exon20 A767_V769dup mutation with a TTF of approximately 36 months and can be a potential therapeutic option for this mutation. Patients with EGFR E709A/L858R, S720F/G719A, L747P, R776H/L858R, and L833V/H835L mutations also showed a TTF of more than 1 year, which was similar to the previous recommendations and increased our confidence to apply afatinib to these patients.^{29,30,33}

Presently, the mechanism of drug resistance of TKIs has also been widely concerned. For exon 19 deletions and exon 21 L858R mutations, the acquired EGFR T790M mutation is the primary resistance mechanism to first- and second-generation EGFR TKIs, followed by the acquisition of the second drive mutation; the emergence of new clones and the replacement of previously predominant clones; and the activation of some downstream pathways and histological transformations.³⁴ However, the mechanism of acquired resistance for uncommon mutations remains unclear.^{35,36} A multicenter study has shown that acquired T790M mutation was common in patients with NSCLC with icotinib-resistant

EGFR uncommon mutations. Other several resistance mechanisms to icotinib in NSCLC harboring uncommon mutations include EGFR extracellular domain mutation, BCL2L1 loss, MET amplification, ERBB2 amplification, MYC amplification, PTEN mutation, and PIK3CA mutation.^{18,37} In this study, apart from one patient with L858R mutation, one patient with L833V/H835L was detected with a secondary T790M mutation. This secondary drug resistance mechanism is consistent with a previous case report.³⁸ Unfortunately, we did not find the mechanism of drug resistance in some patients. We considered the following possible reasons: the sample size was small for both baseline and progressive NGS tests, and the detection rate of potential mutation by liquid biopsy was relatively lower. With the more application of secondary biopsy and NGS detection, more mechanisms of drug resistance will be discovered.

This study has some limitations to consider. First, the diversity of times of initial TKI treatment may have influenced the survival analysis after administration. Second, we used local testing to detect EGFR mutations. The mechanism of TKI resistance after initial TKI treatment is not yet investigated. Third, given the limited number of each subtype of uncommon EGFR mutation, additional studies with a large number of patients are warranted.

In summary, our findings suggest that afatinib is effective in patients with uncommon EGFR mutations. Detailed reports for specific rare uncommon mutations provide reliable evidence for clinical practice. Further studies are necessary to explore the resistance mechanism of uncommon mutations.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AVAILABILITY OF DATA AND MATERIAL

The data are not publicly available because of privacy and ethical restrictions.

CONSENT TO PARTICIPATE

This is a retrospective study. All private data on the included patients were erased, and the requirement for written informed consent was waived by the Institutional Review Board.

CONSENT FOR PUBLICATION

All authors agree with the content of the publication.

PREVIOUS PRESENTATION

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