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Efficacy analysis of tyrosine kinase inhibitors on rare non-small cell lung cancer patients harboring complex EGFR mutations

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The efficacy of epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) in patients with non-small cell lung cancer (NSCLC) is related to *EGFR* mutations. Although the p.L858R point mutation in exon 21 and the in-frame deletion mutation in exon 19 are well known, efficacy of EGFR-TKI in patients with more than one *EGFR* mutation is not well understood. 799 NSCLC patients were screened for *EGFR* mutations. Of the 799 patients, 443 (55.4%) had mutations, out of which 22 (2.75%) had multiple complex mutations. Most multiple mutations (20/22) harbored common mutations such as the p.L858R point mutation in exon 21 and the in-frame deletion mutation in exon 19. 11 out of 22 patients who had multiple *EGFR* mutations underwent TKI therapy and primary end-points of progression free and overall survival were determined. Our analysis revealed that cases with multiple mutations had similar end-point outcomes as single mutation to TKI therapy. Report of these cases will be helpful in decision making for treatment of NSCLC patients harboring multiple *EGFR* mutations.

Lung cancer has the highest incidence among malignant tumors, mostly refractory to surgical resection because of the advanced stage of the disease. The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI), gefitinib and erlotinib, are among the first targeting drugs used in treatment of advanced lung cancer patients in China. Clinical studies revealed that advanced non-small cell lung cancer (NSCLC) patients with *EGFR* mutations gained a significant advantage of efficacy and survival after using TKI[1–3].

The most common *EGFR* mutation is exon 19 deletion and p.L858R mutation in exon 21[3–4]. In a number of clinical studies on EGFR-TKI, the subgroup analyzes were collected in both mutant types. In the IPASS study[3], mutations subgroup efficacy analysis showed that after first-line treatment with TKI, the patients with exon 19 deletions and the p.L858R mutation in exon 21 had no significant difference in progression free survival (PFS) time (Hazards Ratio (HR), 0.78; 95% class interval (CI), 0.51–1.19). However, in the overall response rate (ORR), exon 19 deletions group was 84.8%, while the p.L858R mutation group was 60.9%, suggesting that the drug had better efficacy in the exon 19 deletion group; however, statistical analysis did not reveal significant difference. In a separate retrospective study involving 87 patients[4], PFS of the exon 19 deletion patients was 9.3 months, overall survival (OS) was 17.7 months, and response rate (RR) was 64%. In comparison, PFS of the L858R mutation patients was 6.9 months, OS was 20.5 months, and RR was 62%.

Yet another mutation characterized in exon 20 (p.T790M) is now attributed to drug resistance; however, whether p.T790M mutation is associated with poor prognosis is still debatable[5–6]. Other *EGFR* mutations have been characterized, inclusive of the p.L861Q, p.S768L, G719X, exon20 insertions[3,7], but their exact role in refractory behavior of patients harboring those mutations to TKI has not yet been elucidated. Cases of complex *EGFR* mutations have been reported; however, the relation between complex *EGFR* mutations and resistance to therapy with TKI has not been completely elucidated[8,9]. Hence, the goal of the current study was to retrospective analyze lung cancer patients with complex *EGFR* mutations and their correlation to treatment outcome with TKI in order to provide clinical reference for the treatment of lung cancer patients harboring complex *EGFR* mutations.

Results

Frequency of EGFR Mutations. There were 799 cases of lung cancer patients in the study time frame who underwent *EGFR* mutation detection, inclusive of 686 cases of non-squamous carcinoma (bronchioloalveolar and adenocarcinoma) and 113 cases of squamous and adenosquamous carcinoma. Of the 799 cases of lung cancer, there were 443 *EGFR* mutations detected, a single mutation being detected in 421 cases, accounting for 95.03% of all mutations. Among the single mutation cases, exon 18, 19, 20 and 21 mutations were detected in 10 (2.37%), 162 (38.48%), 114 (27.08%), and 135 (32.07%) cases, respectively. On the other hand, complex *EGFR* mutations were detected in 22 (4.97%) cases.

EGFR Complex Mutations and TKI Therapy. General condition, specimen source and mutation detection results of all patients of complex mutations are summarized in Table 1. Of the 22 cases of patients with complex *EGFR* mutations, 20 patients had at least one common mutation, 10 cases harbored missense mutations in exon 18, 7 cases harbored exon 19 deletion mutations, 9 cases harbored 20 missense mutations, and 16 cases harbored 21 missense mutations (Table 1). Of the 22 cases with complex *EGFR* mutations, 10 cases were Stage I (T1N0M0) – out of which 8 post-operative cases were not subjected to adjuvant chemo or radiotherapy – and did not exhibit any disease recurrence following surgical resection and did not undergo TKI therapy. Of the remaining 12 cases with advanced disease stage, one was lost and the remaining 11 underwent EGFR-TKI therapy (Table 2).

Response to EGFR-TKI in Patients with EGFR Complex Mutations. Efficacy of EGFR-TKI treatment in 11 lung cancer (out of 22 cases harboring more than one *EGFR* mutation) cases with advanced disease stage and complex *EGFR* mutations (more than one *EGFR* mutations) are summarized in Table 2. Serious adverse effect was observed in only 1 of the 11 patients. Complete and partial responses were observed in one patient each, whereas the remaining 8 patients had stable disease. Response to EGFR-TKI did not have any correlation to prior smoking history.

Discussion

In the present study, we retrospectively analyzed 799 lung cancer patients who were screened for *EGFR* mutations in People's Liberation Army General Hospital, and subsequently focused on the outcome of EGFR-TKI therapy on patients with complex *EGFR* mutations.

The incidence of *EGFR* mutations is dictated by ethnic groups. In the IPASS study[3], of the 1217 patients enrolled Asian patients with lung adenocarcinoma, *EGFR* mutation was found in 261 patients, an incidence rate of 21.4%. A separate study showed *EGFR* mutation rate was 35% in the Asian race, while non-smoking adenocarcinoma mutation rate was 59%[10]. In the Monaco race[11], lung adenocarcinoma mutation rate was 21% (28/137). In the present study, EGFR mutation rate was 55.4%. The high percentage might be attributed to the fact that policy decision changed since 2009 that Asian female non-smoker adenocarcinoma patients are screened more aggressively for somatic mutations. Contrary to this, fewer squamous carcinoma cases or smoking males are subjected to mutation screening. In this study, among 799 cases non-squamous cell carcinoma accounted for a high 85.9%.

In the current study the complex mutation rate was 4.97%. An earlier study has reported an incidence rate of 15% complex mutations (11 out of 79 cases with mutations)[9]. In yet another study, the rate was 8.4% (140 total cases)[12]. Our observed rate mimics closely to a study of 627 cases that detected complex mutation in 20 cases (3.19%)[8]. It must be noted that complex mutations being rarer coverage detection limit in sequencing and subsequent validation will dictate the incidence rate of complex mutations.

Of the 22 cases of complex mutation patients in the current study, 12 cases were males and 10 cases were females. Of the 22 cases, 20 were adenocarcinoma, and 5 cases had prolonged history of smoking. Male: female ratio of common mutations was 1 : 0.8. It was earlier reported that the proportion of men and women in common mutations was 1 : 1.8, rare mutations the proportion of men and women was 1 : 1.3[8], which is consistent with the current study.

In this study, *EGFR* mutations were detected by direct sequencing, which is consistent with most studies. Liu et al.[12] suggested that using either fine-needle aspiration or surgical specimens did not affect *EGFR* mutation detection rate. However, for the source of 22 cases of complex specimens mutations found in this study, 19 cases were surgical specimens. This is suggestive that detection of complex mutations is reliant not only on the integrity of specimens but also on the amount of specimen; in fact it was earlier shown that the inverse relationship between amount of biopsy specimens and detection of rare mutations is related to the error in detection method[13]. Cumulatively, our results emphasizes that for *EGFR* mutation detection, specimen source is an important factor affecting the test results, clinicians should collect enough tissue samples, in order to avoid detection error.

In this study, the majority of complex mutations (20/22) contained at least one common mutation; therefore, complex mutation was classified and analyzed based on common mutations. Complex mutations containing L858R or exon 19 deletion mutation showed higher efficacy with EGFR-TKI treatment and prognosis in these patients were better, and close to the efficacy outcomes of common mutations.

Efficacy of patients containing T790M complex mutation after using the TKI was different; PFS of 2 patients in this study were more than six months, while the data reported in other literatures suggested poor efficacy and prognosis. Earlier studies on the T790M mutation showed that it was closely related to acquire resistance of TKI drug[14,15]. In a prospective review of 2774 cases of untreated specimens, 20 cases with the T790M mutation associated with mutations in other exons, of which 16 cases were T790M + L858R, and 4 cases were delE746-A750 + T790M[16]. In another study, one case of T790M + L858R patient among 68 cases of non-smoking lung cancer patients was PD after using of TKI treatment a month, PFS time was a month, OS time was 17.4 months[17]. In the current study, the mutation types of 2 patients of complex mutation contained T790M (T790M + delE746-A750 and T790M + L858R). The best efficacy of patients using TKI was SD, PFS time was respectively 8 months, 10 months, OS time was respectively 29 months, 18 months (still alive). Cumulatively, the difference in efficacy and prognosis in patients with lung cancer using TKI was not only closely related to *EGFR* mutations, but also other members of the signaling pathway may be involved in the efficacy and influenced efficacy outcomes. By downstream gene detection and efficacy follow-up of some patients, Kim et al[17] found that mutations of *EGFR* downstream genes are closely related to the efficacy of TKI, which also reflected *EGFR* mutations complexity from a side reaction.

Complex mutation inclusive of exon 18 is a special group because exon 18 mutations itself are relatively rare, while complex mutations often exist, TK1 efficacy and prognosis was below common mutation only by the cases analysis reported in the literature. Elucidation of the reason for the same need to be further investigated. One limitation of the current study is that given that it is a retrospective it did not allow potential unification of the timing of TKI treatment. Some patients chose to use TKI as first-line, while others used it for second or third-line therapy. Cumulatively, the current study will potentially help replenish *EGFR* mutation data and help clinical development of rational treatment strategies.

Table 1 | Details of the 22 lung cancer patients with complex *EGFR* mutations

N	Sex	Age	Histopathology	Stage	Source of specimens	Exon	Specific mutations
1	Male	35	Moderately differentiated adenocarcinoma, partly mucus adenocarcinoma	T2aN1M0	Excision	18, 19	18missenseG719A, 19missenseTTA-TCA,L747S
2	Female	39	Poorly differentiated adenocarcinoma	T1N0M0	Excision	18, 20	18missenseG719A, 20missenseCGC-CAC,R776H, 20synonymous CAG-CAA,Q787Q
3	Male	48	Moderately differentiated adenocarcinoma	T4N2M0	Excision	18, 20	18 missenseG719A, 20missense(CGC-CAC,R776H)
4	Female	59	Highly and moderately differentiated adenocarcinoma, most partly fine counts	T1N0M0	Excision	18, 20	18missenseGGC-GCC,G719A, 20missenseAGC-ATC,S768I
5	Male	50	Moderately differentiated adenocarcinoma, partly fine counts	T1N0M0	Excision	18, 21	18missense(GGC-GCC,G719A), 21missense(TTG-TTT,L833V; 21missenseGTG-TTG,V834C)
6	Male	58	Moderately differentiated papilla adenocarcinoma	T1N0M0	Excision	18, 21	18missenseGAA-AAA,E709K, 21missense(CTG-CGG,L858R)
7	Male	70	Moderately differentiated adenocarcinoma	T2N2M0	Excision	19, 20	19deletions delE746-A750 20missense:T790M,ACG-ATG
8	Male	74	Moderately and poorly differentiated adenocarcinoma	T1N1M0	Excision	19, 21	19deletions delE746-A750 21missenseCTG-CGG,L858R
9	Male	49	Moderately differentiated squamous carcinoma	T2N1M0	Excision	19, 21	19deletions delE746-A750, 21missenseTTG-GTG,L833V, 21missenseCAC-CTC,H835L
10	Female	47	Moderately differentiated papilla adenocarcinoma	T2bN0M0	Excision	19, 21	19missenseCCG-TCG,P753S 21missenseCTG-CGG,L858R
11	Female	56	Moderately differentiated adenocarcinoma fine counts	T1N0M0 double primary	Excision	19, 21	19deletions delS752-I759, 21missenseCTG-CGG,L858R
12	Female	53	Moderately differentiated squamous carcinoma or adenosquamous carcinoma and fine counts	T2N2M0	Excision	19, 21	19deletions delE746-A750, 21missenseL834L, L858R.
13	Male	70	Moderately and poorly differentiated adenocarcinoma	T3N2M0	Excision	20, 21	20missenseAGC-ATC S768I, 21missenseCTG-CGG L858R
14	Female	64	Moderately and highly differentiated adenocarcinoma partly fine counts	T1N0M0	Excision	20, 21	20missenseAGC-AGT,S768I 21missenseCTG-CGG,L858R
15	Female	50	Highly and moderately differentiated adenocarcinoma	T1N0M0	Excision	20, 21	20 synonymous CAG-CAA,Q787Q, 20 missenseAGC-ATC S768I 21 missenseCTG-CGG,L858R
16	Female	48	Moderately differentiated adenocarcinoma	T3N1M1	Fine needle aspiration biopsy	20, 21	20missenseCAC-CAT,T790M 20synonymous CAG-CAA,Q787Q. 21missenseCTG-CGG,L858R.
17	Male	56	Adenocarcinoma	T1N0M0	Excision	20, 21	20missenseACC-ATC T783I, 21missenseCAA-CTC H855C
18	Female	66	Moderately differentiated adenocarcinoma partly fine counts	T1N0M0	Excision	18, 19	18 missenseACA-GCA,T639A; 19 deletions delL747-S752; 21 synonymous CTA-TTG,L858, 21 synonymous CTG-TTG,L861L
19	Male	68	Moderately differentiated adenocarcinoma little fine counts	T1N2M1	Fine needle aspiration biopsy	18, 21	18missenseGAA-AAA,E709K, 20synonymous CAG-CAA,Q787Q, 21missenseCTG-CGG,L858R
20	Male	76	Poorly differentiated adenocarcinoma	T2N1M0	Excision	18, 21	18missenseGGC-GCC,G719A; 20synonymous CAG-CAA,Q787Q; 21missenseTTG-TTT,L833F,
21	Male	48	Moderately differentiated adenocarcinoma	T1N0M0	Excision	18, 21	18 missenseGAA-AAA,E709K; 20:synonymous CAG-CAA,Q787Q, 21missenseCTG-CGG,L858R
22	Female	75	Adenocarcinoma	T2N3M0	Fine needle aspiration biopsy	19, 21	19 deletions del(L747-S752); 20 synonymous CAG-CAG,Q787Q 21 missenseCAC-CTC,H835L

Table 2 | Outcome of EGFR-TKI therapy in 11 lung cancer patients harboring complex *EGFR* mutations. SD, stable disease; CR, complete response; PR, partial response; SAE, serious adverse effects; PFS, progression free survival, OS, overall survival

Sex	Age	Histopathology	Smoking history (months)	Treatment	Response	PFS (months)	OS (months)	Mutation
Male	35	Moderately differentiated adenocarcinoma partly mucus adenocarcinoma	32	Gefitinib	SD	3	8	G719A, L747S
Male	50	Moderately differentiated adenocarcinoma partly fine counts	28	Gefitinib	CR	16+	29+	G719A, L833V, V834C
Male	70	Moderately differentiated adenocarcinoma	0	Gefitinib	SD	8	29	delE746-A750, T790M
Male	74	Moderately and poorly differentiated adenocarcinoma	0	Gefitinib	SD	8	8+	delE746-A750, L858R
Male	49	Moderately differentiated squamous carcinoma	0	Gefitinib	SD	6	16	delE746-A750, L833V, H835L
Female	47	Moderately differentiated papilla adenocarcinoma	10	Gefitinib	SD	21	39	P753S, L858R
Female	53	Moderately differentiated squamous carcinoma adenosquamous carcinoma and fine counts	0	Gefitinib	PR	15	58+	delE746-A750, L858R
Male	70	Moderately and poorly differentiated adenocarcinoma	20	Gefitinib	SD	6	6.5	S768I, L858R
Female	48	Moderately differentiated adenocarcinoma	0	Gefitinib	SD	10	18+	T790M, L858R
Male	68	Moderately differentiated adenocarcinoma little fine counts	80	Gefitinib	SD	10	26	E709K, L858R
Male	76	Poorly differentiated adenocarcinoma	80	Gefitinib	SAE	0.5	13	G719A, L833F

Methods

Patient Selection. The study was approved by the Institutional Review Board of the Chinese PLA General Hospital and all experiments performed were strictly in accordance with the approved guidelines. All patients enrolled in the current study provided signed informed consent. Study population was limited to lung cancer patients with *EGFR* mutations treated in the Department of Pathology at our Hospital between August 1, 2009 and June 1, 2012. Inclusion criteria were confirmed detection of lung cancer (pathology, lungs and other abdominal head CT, blood tests), availability of complete medical records (records of patients general, family history, smoking history, pathology, immunohistochemistry, operation time and surgical name, medication records, tumor response assessment), and compliance with follow-up. Source of detection of *EGFR* mutation was tissue of primary tumor or metastases after resection or fine-needle aspiration biopsy. Pathological diagnosis and clinical staging (according to NCCN Guidelines) was performed by a pathologist and oncologist, respectively, in blinded fashion. Signed informed consent from patients or family members (where patients were dead) were obtained for all enrolled patients.

Evaluation of EGFR-TKI Efficacy and end-points tested. For advanced patients treated with gefitinib or erlotinib (11 of 22 patients with more than one *EGFR* mutations), the attending physician decided the regimen according to the patient's condition. Gefitinib was administered orally at 250 mg daily, and erlotinib was administered orally at 150 mg daily, until tumor progression, death, or patient refusal. All patients had a pretreatment tumor assessment by computer tomography scan, which was repeated to assess tumor response after 4 weeks from the beginning of the treatment, then every 1 to 2 months until treatment discontinuation. Tumor Response was evaluated using Response Evaluation Criteria In Solid Tumors (RECIST). Stable disease (SD) was defined as disease control maintained for at least 4 weeks. The duration of progression-free survival (PFS) was calculated from the date of initiation of EGFR-TKIs to the date of disease progression. Overall survival (OS) time was determined from the date of initiation of EGFR-TKIs to the date of death.

***EGFR* mutation detection.** *EGFR* mutation analysis was performed using standard DNA sequencing techniques with direct sequencing of exons 18 to 21 of EGFR. In brief, DNA was isolated from the sample, quantified and amplified by polymerase chain reaction (PCR) using primers to exons 18 to 21 of *EGFR*. PCR products were analyzed by bidirectional direct DNA sequencing. Tumor gene type was performed in baseline diagnostic specimens before patient exposure to *EGFR* TKIs.

Data Collection. Data regarding the treatment process, imaging results and assessment records, communication, and follow-up efficacy and survival time of patients meeting the inclusion criteria were retrieved. Database inputs were general condition of patients, family history, smoking history, pathology test results, immunohistochemistry, operation time and surgical name, previous chemotherapy regimens, targeted medication records, tumor efficacy evaluation, and follow-up survival time records.

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Author contributions

L.P. and S.C.J. conceived the study; Z.G.S. helped with the pathological diagnosis; L.P. did most of the experiments; L.P., Z.G.S. and S.C.J. analyzed the data and wrote the manuscript.

Additional information

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