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EGFR-tyrosine kinase inhibitor treatment in a patient with advanced non-small cell lung cancer and concurrent exon 19 and 21 EGFR mutations: A case report and review of the literature

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Abstract. Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are considered to be effective treatments for advanced non-small cell lung cancer (NSCLC) patients with sensitizing EGFR mutations, including exon 19 deletion and exon 21 L858R mutations. However, with the development of EGFR mutation detection assays, patients with complex EGFR mutations are emerging, and their response to EGFR-TKIs remains unclear. The present study reports a case of a 62-year-old, non-smoking female patient with advanced NSCLC, presenting with concurrent EGFR 19+21 sensitizing mutations, who had a poor response to the first-line EGFR-TKI erlotinib and succumbed 5 months subsequent to diagnosis. Furthermore, the present study performed a literature review, and 18 patients with complex EGFR 19+21 mutations that had received EGFR-TKIs were identified. The majority of these patients responded well to EGFR-TKIs. To the best of our knowledge, the present case is the first to report a patient with lung adenocarcinoma with complex EGFR 19+21 sensitizing mutations that had a poor clinical response to a first-line EGFR-TKI. Despite the 70% response rate of sensitizing EGFR-mutant NSCLCs to EGFR-TKIs, there is still a proportion of patients that experience *de novo* resistance, and heterogeneity is likely to be important in this resistance mechanism. Therefore, comprehensive genomic detection assays and multi-targeted therapies for patients with NSCLC with complex EGFR mutations require additional investigation.

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Key words: EGFR mutation, complex mutations, EGFR tyrosine kinase inhibitors, non-small cell lung cancer

Introduction

Lung cancer remains one of the most common types of cancer worldwide (1.8 million novel cases were diagnosed in 2012, 13% of the total cancer cases diagnosed), and it was the leading cause of cancer-associated mortality in 2012 (1.59 million mortalities; 19.4% of all cancer-associated mortalities in 2012) (1). Conventional platinum-based chemotherapy has reached a plateau in improving patient survival and disease control obtained with classic doublet chemotherapy in patients with advanced lung cancer is usually restricted to a few months (2-4). Fortunately, developments in molecular biology have attracted attention to the molecular subtypes of lung cancer, resulting in great improvements in understanding the disease, including the identification of mutations in the epidermal growth factor receptor (EGFR).

The majority of EGFR mutations are located at exon 19, which are deletions (Del-19), and exon 21 (L858R), and mutations at these sites account for 45 and 40% of all EGFR mutations, respectively (5). These mutations are considered to be sensitizing mutations that confer sensitivity to EGFR-tyrosine kinase inhibitors (EGFR-TKIs), including gefitinib and erlotinib (6-8). Clinical trials have demonstrated that EGFR-TKIs are associated with a significantly increased response rate (RR) and progress free survival (PFS) rate for patients harboring EGFR sensitizing mutations, as compared with chemotherapy alone (9,10). To date, the detection of EGFR mutations has been performed prior to the treatment of advanced non-squamous NSCLC, while an increasing number of rare types of EGFR mutations have been discovered, which are not only limited to the mutation of a single exon, but the concurrence of two mutations on different exons, primarily exons 18-21, in a single tumor sample (11,12). These mutations are termed 'complex mutations'. However, the response of patients harboring the complex EGFR mutations to first-line EGFR-TKIs remains unclear. The present study reports a case of a patient with advanced NSCLC and complex EGFR mutations, and the patient's response to first-line EGFR-TKI treatment. Written informed consent was obtained from the patient for the publication of the present study.

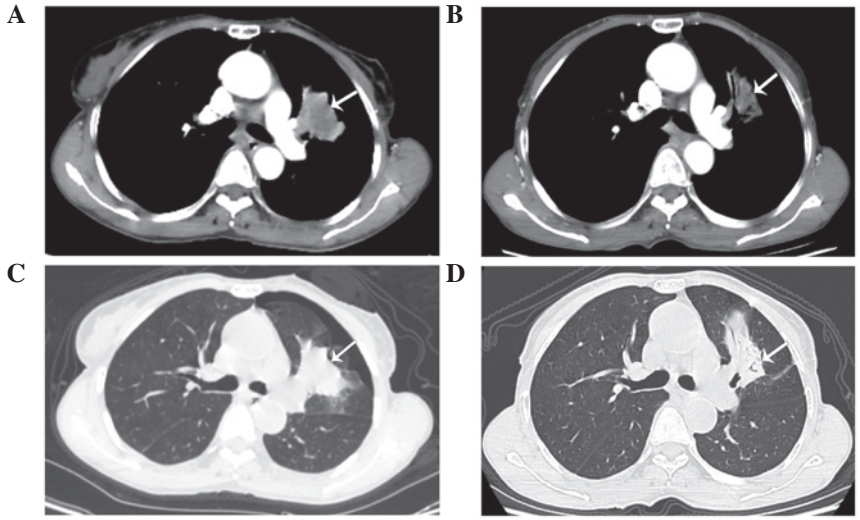

Figure 1. Chest computed tomography scans. Presence of a left upper lobe primary mass with multiple small pulmonary nodules in both lungs was observed prior to erlotinib treatment; a left upper lobe primary mass was identified on the (A) mediastinal window and (B) pulmonary window, respectively (arrows). Following 8 weeks of erlotinib treatment the mass had shrunk on the (C) mediastinal and (D) pulmonary windows, respectively (arrows).

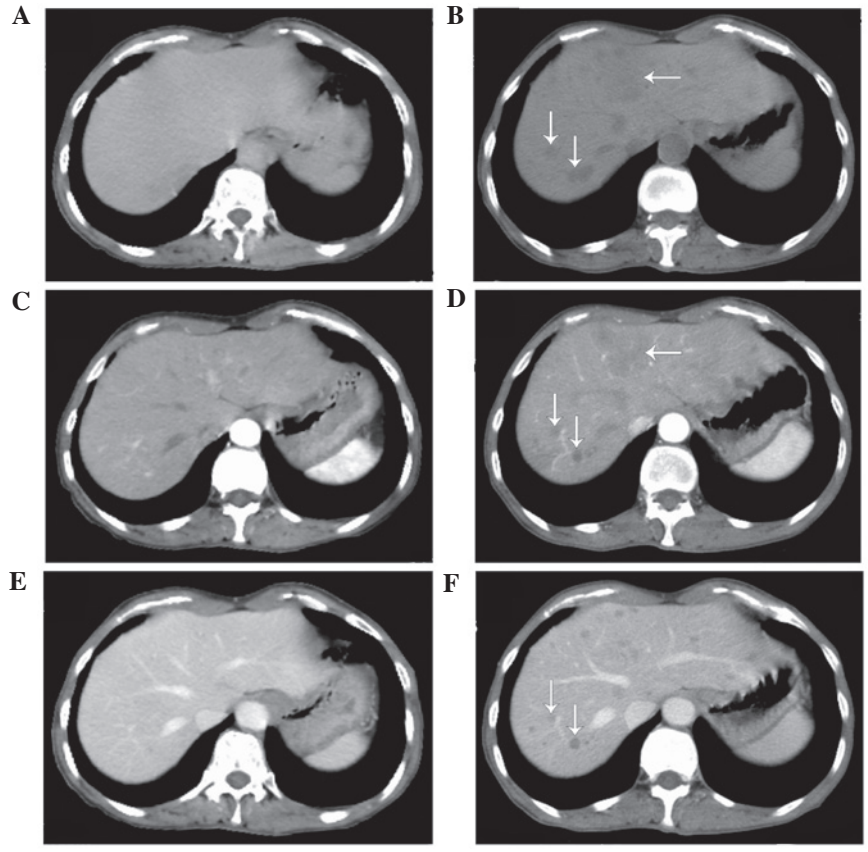

Figure 2. Hepatic computed tomography scans. Absence of hepatic metastasis prior to erlotinib treatment was observed on a (A) plain, (B) venous phase and (C) arterial phase scan (arrows). Presence of hepatic metastasis following 8 weeks of erlotinib treatment was observed on a (D) plain, (E) venous phase and (F) arterial phase scan (arrows).

Case report

On March 10, 2014, a 62-year-old, non-smoking woman with an Eastern Cooperative Oncology Group Performance Status score of 2 (13) presented to the Department of Oncology, Comprehensive Cancer Center of Drum-Tower Hospital (Nanjing, China) with a cough that had been ongoing for 2 months. Computed tomography (CT; Discovery CT750 HD; GE Healthcare Life Sciences, Shanghai, China) revealed a primary mass in the left upper lobe of the lung (Fig. 1A and B), with multiple small pulmonary nodules (maximum diameter, 4 mm) observed bilaterally. A CT-guided fine needle aspiration biopsy of the primary lesion was performed on the left lung, which revealed the presence of a middle-differentiated

Table I. Characteristics of 18 patients with EGFR Del-19 + L858R mutations that were treated with EGFR-tyrosine kinase inhibitors.

Patient	Author, year	Gender	Smoking status	Histology	Response	PFS, months	OS, months	(Ref.)
1	Zhang *et al*, 2007	M	Yes	Adenocarcinoma	CR	18	20	(17)
2	Zhang *et al*, 2007	M	No	Adenocarcinoma	PR	>19	>19	(17)
3	Zhang *et al*, 2007	M	Yes	Adenocarcinoma	NE	2	2	(17)
4	Masago *et al*, 2009	F	No	Adenocarcinoma	PR	14	23	(18)
5	Masago *et al*, 2009	F	No	Adenocarcinoma	PD	1	11	(18)
6	Masago *et al*, 2009	F	No	Adenocarcinoma	PR	11	11	(18)
7	Masago *et al*, 2009	F	No	Adenocarcinoma	PD	2	>39	(18)
8	Masago *et al*, 2010	M	No	Adenocarcinoma	NE	>2	>2	(19)
9	Keam *et al*, 2014	F	No	Adenocarcinoma	PR	4	12	(20)
10	Peng *et al*, 2014	M	No	Adenocarcinoma	SD	8	>8	(21)
11	Peng *et al*, 2014	F	No	Adenosquamous carcinoma	PR	15	>58	(21)
12	Hata *et al*, 2010	F	No	Adenocarcinoma	CR	31	>41	(22)
13	Hata *et al*, 2010	F	No	Adenocarcinoma	PR	7	>33	(22)
14	Hata *et al*, 2010	F	Yes	Adenocarcinoma	PR	>23	>23	(22)
15	Hata *et al*, 2010	M	Yes	Adenocarcinoma	PR	12	>13	(22)
16	Hata *et al*, 2010	M	Former	Adenocarcinoma	PR	>10	>10	(22)
17	Hata *et al*, 2010	M	No	Adenocarcinoma	PR	>9	>9	(22)
18	Hata *et al*, 2010	M	Yes	Adenocarcinoma	SD	>13	>13	(22)

Patients were assessed based on the Response Evaluation Criteria in Solid Tumors (15). EGFR, epidermal growth factor receptor; PFS, progress free survival; OS, overall survival; M, male; F, female; Del-19, deletion on exon 19; L858R, mutation on exon 21; NE, not evaluated; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

adenocarcinoma. EGFR mutation analysis was performed on the biopsied tissue within exons 18 and 21. EGFR mutations were detected by amplification refractory mutation system in multiple quantitative polymerase chain reaction analysis with the Human EGFR Mutation Detection kit (YuanQi Bio-Pharmaceutical Co., Ltd., Shanghai, China), as previously described (14), which demonstrated a co-mutant of Del-19 (del 2239_2248+insC) and L858R. In addition, anaplastic lymphoma kinase rearrangement analysis was performed using fluorescence *in situ* hybridization, with negative results. The patient was orally administered erlotinib, at a dose of 150 mg/day. Two months later, no considerable relief of the cough was noted; however, a CT scan revealed that the primary lung mass had shrunk (Fig. 1C and D), but the bilateral multiple pulmonary nodules remained. In addition, prior to erlotinib treatment there was no hepatic metastases (Fig. 2A-C); however, following treatment multiple hepatic metastases were observed (Fig. 2D-F). Based on the Response Evaluation Criteria in Solid Tumors (RECIST) (15), the treatment response of the patient was assessed as progressive disease (PD). The patient refused to undergo a biopsy of the liver. Treatment with erlotinib was stopped when PD was detected, and 2 cycles (3 weeks/cycle) of chemotherapy with pemetrexed (500 mg/m^2) plus cisplatin (75 mg/m^2) were administered to the patient intravenously. However, the hepatic metastases continued to progress under the assessment of CT scans, which were performed monthly. The patient succumbed 2 months subsequent to the detection of PD, with the best supportive care possible. The patient's overall survival was ~5.3 months.

Discussion

The use of first-line EGFR-TKIs in NSCLC patients with EGFR sensitizing mutations has been demonstrated to be superior to chemotherapy in terms of PFS, RR and quality of life; however, previous phase III clinical trials primarily enrolled patients harboring common EGFR mutations, including exon 19 deletion or exon 21 L858R (16). For patients who exhibit uncommon mutations, particularly complex 19+21 mutations, the efficacy of first-line EGFR-TKIs remains unknown. Despite the fact that the present patient harbored Del-19 and L858R, which are sensitizing EGFR mutations, the patient experienced PD following treatment with first-line erlotinib. In order to acquire an improved understanding of this, the present study performed a comprehensive search of relevant studies published on the PubMed database (www.ncbi.nlm.nih.gov/pubmed) using keywords, including 'complex mutations', 'double mutations', 'compound mutations', 'multiple mutations', 'rare mutations' and 'uncommon mutations', with 'EGFR' added to each keyword. All studies with relevant abstracts (1,949 abstracts) were retrieved first, and the full texts of those that were considered valuable, including studies that contained patients carrying complex, double,

compound, multiple, rare or uncommon mutations of EGFR, were carefully reviewed (27 studies). Relevant references from the searched studies (6 references) were also reviewed.

In total, 18 patients with EGFR Del-19 + L858R mutations that received EGFR-TKIs were identified between January 2002 and January 2015 (Table I) (17-22). Of these 18, 16 patients were assessed based on RECIST (15) and 2 patients were not evaluated. The 18 patients presented in Table I exhibited the following responses: 2 (11.1%), complete response; 10 (55.6%), partial response; 2 (11.1%), stable disease; 2 (11.1%), PD; and 2 patients (11.1%) that were not evaluated experienced a relief of their symptoms. Only 2 out of the 18 patients experienced PD following the initiation of EGFR-TKIs, and these patients were administered EGFR-TKIs as second-line treatment, following first-line carboplatin and paclitaxel regimens. Compared with patients harboring EGFR mutations that were administered first-line EGFR-TKIs, those who received EGFR-TKIs as second-line treatment following platinum-based chemotherapy exhibited a lower response rate (23,24). A study by Bai *et al* (25) demonstrated that prior chemotherapy may reduce the EGFR mutation rate in the plasma, suggesting that the poor response of the 2 aforementioned patients with PD may be associated with the first-line chemotherapy each received.

The present patient had concurrent EGFR19+21 sensitizing mutations and received EGFR-TKI as a first-line treatment; however, the patient had PD following treatment. Previously, genetic heterogeneity of tumors has received considerable attention. Studies on clear cell renal carcinoma have demonstrated substantial intratumor heterogeneity of mutations in known cancer genes (26,27), which suggests that a single biopsy of a tumor may be inadequate to detect all cancer gene mutations, resulting in inconsistencies between detection and clinical efficacy. By contrast, a recent study by Zhang *et al* (28) on lung adenocarcinoma demonstrated that 76% of mutations were detected in various regions of the same tumor, indicating that single-region biopsy may compromise the intratumor heterogeneity to an acceptable extent, therefore identifying the majority of cancer gene mutations in localized lung adenocarcinomas.

In the present case, the EGFR gene detection assay on the core biopsy tissue of the primary lesion in the left lung revealed Del-19 and L858R mutations. Notably, following the initiation of EGFR-TKI treatment the primary lung mass shrunk and hepatic metastases appeared extensively. The present study hypothesizes that the genomic features of the hepatic metastases varied from those of the primary tumor, indicating that intertumor heterogeneity may be responsible for the PD observed in the present patient. In addition, de Bruin *et al* (29) revealed evidence of branched evolution with driver mutations arising prior to and following subclonal diversification, which supports the hypothesis of the present authors. Considering that the majority of NSCLC driver mutations occur in the early phases of tumor evolution (29), the Del-19 + L858R mutations of the present patient may have been truncal mutations of the ancestral clone, existing only in the primary lung lesion. With regard to the hepatic metastases, it is likely that they consisted of sectional and predominant subclones with branch gene alternations that confer *de novo* resistance to EGFR-TKIs, including phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α, phosphatase and tensin homolog, protein kinase B and serine/threonine kinase 11 alterations, c-met amplification, hepatocyte growth factor overexpression and Kirsten ras mutations (30-34). Under circumstances when re-biopsy is not feasible, comprehensive genomic detection and multi-targeted therapies may be used. It has been suggested that liquid biopsy may reveal relatively comprehensive gene alternations and provide predictive information on the identification of therapeutic targets and resistance mechanisms (35).

To the best of our knowledge, this is the first study of a treatment-naive NSCLC patient harboring complex EGFR 19+21 sensitizing mutations presenting with *de novo* resistance to first-line EGFR-TKI treatment. Previous studies have suggested that NSCLC patients harboring complex EGFR 19+21 mutations usually have good response to EGFR-TKIs; however, in order to understand the complexity of EGFR mutations and determine the efficacy of EGFR-TKIs against them, large-scale clinical trials are required.

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