Nonsmall cell lung cancer with rare exon 7 p. A289V mutation in the EGFR gene responds to Icotinib treatment
A case report
Limeng Dai, PhD, Xuejiao Su, MD, Lin Lu, MD, Donglai Lv, MD, PhD*

Abstract
Rationale: Mutation p.A289V involving extracellular region of epidermal growth factor receptor (EGFR) exon 7 has not yet been reported in nonsmall cell lung cancer (NSCLC). Studies have shown p.A289V mutation responding to tyrosine kinase inhibitors (TKIs) in glioblastoma cell lines suggesting the point mutation as a potential therapeutic target. However, sufficient evidence of the effect of TKI treatment on the p.A289V mutation involved in NSCLC is not available.

Patient concerns: An 80-year-old nonsmoker male with lung mass was suffering from severe bone pain.

Diagnosis: Needle biopsy and positron emitted tomography/computed tomography were performed. The patient was diagnosed with advanced NSCLC adenocarcinoma with bone and lymphatic metastasis. Next-generation sequencing of circulating tumor DNA was performed, which identified a p.A289V mutation in the EGFR gene of the patient.

Interventions: Our patient refused to receive chemotherapy and tried Icotinib treatment.

Outcomes: Our patient had a partial response to Icotinib after treatment for 5 months during the therapeutic trial by TKIs. The patient showed adverse symptoms of mild diarrhea and rash (Common Terminology Criteria for Adverse Events grade 1) during the treatment.

Lessons: In this case, Icotinib prevented completion of the signal transduction cascade of p.A289V mutant in NSCLC. Our patient had a partial response to Icotinib after treatment for 5 months during the therapeutic trial by TKIs. The patient refused to receive chemotherapy and tried Icotinib treatment.

Abbreviations: EGFR = epidermal growth factor receptor, NSCLC = nonsmall cell lung cancer.

Keywords: case report, EGFR mutation, Icotinib, nonsmall cell lung cancer, tyrosine kinase inhibitors

1. Introduction
In recent years, targeted drugs are widely used in the treatment of nonsmall cell lung cancer (NSCLC) based on the pathological subtype and target drug-related gene mutations of the patients.
2. Case report
An 80-year-old nonsmoker male was diagnosed with advanced NSCLC adenocarcinoma involving the left upper lobe of lung along with bone and lymphatic metastasis as revealed from the Positron Emission Tomography–Computed Tomography. Bone destructions were found in the posterior section of the left 6th rib, 2nd lumbar vertebral body, 3rd sacral vertebral body, and middle segment of right femur, which lead to severe pain for the patient. Multiple lymphadenectomy was found in the mediastinum and left supraclavicular fossa (Fig. 1A). Moreover, the patient underwent a needle biopsy of the primary lung mass. Histopathology assay suggested an invasive adenocarcinoma with histological features of mucinous adenocarcinoma (Fig. 1B).

Next-generation sequencing (NGS) of the circulating tumor DNA identified the mutations in the \textit{EGFR}, \textit{ALK}, \textit{KRAS}, \textit{ROS1}, \textit{MET}, \textit{ERBB2}, \textit{BRAF}, \textit{RET}, and other genes.\cite{5} The NGS data (Repu Gene Technology Company, Hangzhou, China) indicated that a p.A289V (c.866C>T) mutation was in the \textit{EGFR} gene and the minor allele T accounted for 0.61% (Fig. 1C). Because of the old age and history of underlying diseases of diabetes, hypertension, and chronic obstructive pulmonary disease, the patient and his family refused to receive chemotherapy for treatment. Thus, we started a trial of icotinib (125mg/tid, Beida Pharmaceutical Company, Hangzhou, China) treatment after the informed consent. The accompanying treatment plan consisted of zoledronic acid injection, and radiotherapy for femoral and vertebral metastases (DT 3000cGy/10F).

After 1 month of treatment, tumor growth was observed to be controlled. The tumor volume and mediastinal lymph nodes were slightly decreased. The pain caused by bone metastases was also greatly relieved. After 5 months of treatment, tumor volume significantly decreased (Fig. 2) suggesting that the \textit{EGFR} p.A289V (c.866C>T) mutation in the patient had positive response to icotinib. Notably, the volume of primary tumor decreased 60% compared with the baseline levels of treatment initiation after 5 months. The patient obtained the efficacy of PR according to the Response Evaluation Criteria in Solid Tumors scores. Moreover, the adverse events of the patient were less during the treatment involving mild diarrhea and rash. According to the Common Terminology Criteria for Adverse Events grade, our patient was at grade 1. An informed consent was obtained from the patient for the publication of the case study.

3. Discussion
EGFR is a receptor-type tyrosine kinase that contains an extracellular ligand binding region, transmembrane region, and an intracellular region. Dysregulated EGFR signaling contributes to the formation of many epithelial malignancies and closely relates to neovascularization, tumor invasion, and metastasis in NSCLC.\cite{6} The mechanisms of dysregulated EGFR signaling could be from cell-surface overexpression, autocrine activation, \textit{EGFR} gene mutation, or rearrangements involving extracellular/intracellular regions.\cite{7}

The p.A289V (c.866C>T) mutation locates on exon 7 of \textit{EGFR} gene and encodes an amino acid of extracellular domain of \textit{EGFR} protein (Fig. 3). To date, p.A289V mutation is only reported in glioblastoma, low-grade glioma, head and neck neoplasms according to the published studies and tumor databases such as the Cancer Genome Atlas, and the International Cancer Genome Atlas.\cite{8}
Figure 3. *EGFR* exon 7 p.A289V missense mutation in the extracellular domain. It shows dimerized EGFR molecules bound by the EGF ligand. The position of the common sensitive mutations is indicated along with the downstream effectors activated by EGFR autophosphorylation—STAT3, MAP kinase (MAPK), and AKT. The newly discovered p.A289V mutation in nonsmall cell lung cancer is shown in orange. EGFR = epidermal growth factor receptor.

Figure 2. Computed tomography scans of the chest showed: (A) before Icotinib therapy; (B) tumor volume and mediastinal lymph nodes slightly decreased after 1 month of Icotinib; (C) tumor volume and mediastinal lymph nodes significantly decreased, and the patient had a partial response after 5 months of Icotinib. Yellow arrow indicated primary lung mass; yellow arrow with rectangle indicated multiple metastatic lymph nodes.
Moreover, the mutation is rare and collected as rs149840192 with pathogenic allele in dbSNP database. To our knowledge, this is the first report of p.A289V mutation in NSCLCs, and its corresponding treatment with the first-generation EGFR-TKI (Icotinib) for more than 5 months. It is surprising that the patient obtained the efficacy of PR. In the previous study, a glioma cell line carried p.A289V mutation was inhibited by another TKI (Erlotinib) in an in vitro assay. These in vitro/in vivo results suggest that the exon 7 p.A289V mutation on extracellular region may be a TKI-sensitive site especially in the NSCLCs.

For the response mechanisms of EGFR p.A289V mutation to Icotinib, we speculate that the mutation may activate the oncogenic signaling pathway from abnormal binding between the ligand and EGFR protein while the mutation has not disturbed the binding between the ATP-binding site of the EGFR protein and TKIs. As a result, Icotinib and Erlotinib could prevent completion of the signal transduction cascade of p.A289V mutant. However, the result from this case should be explained carefully, as some cases respond to TKI regardless of the mutation status of EGFR. The molecular mechanisms need to be further explored in NSCLC cell lines and in vivo experiments. Moreover, histopathology of our case suggested primary pulmonary mucinous adenocarcinoma which is a rare subtype of NSCLC and accounts for only 0.2% of lung adenocarcinoma. Our finding needs to be confirmed in more patients at a larger scale with different tissue types of NSCLC in Chinese and other populations. To summarize, we are the first to present a case of NSCLC with EGFR exon 7 p.A289V mutation and a corresponding treatment showing PR to Icotinib. This finding may expand the EGFR mutation spectrum for using TKI as a treatment in NSCLC.

Author contributions

Data curation: Lin Lu, Donglai Lv.
Funding acquisition: Donglai Lv.