## CASE REPORT

# Detection and monitoring of driver mutations by next-generation sequencing in squamous cell lung cancer patient and possible predictive biomarker of third generation EGFR-tyrosine kinase inhibitors

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## Abstract

Driver mutation detection and the development of targeted drugs have significantly improved survival of advanced lung adenocarcinoma patients with driver mutations. However, we still lack understanding of druggable mutations in patients with advanced squamous cell lung cancer (SQCLC). Less than 10% of SQCLC patients have *EGFR* gene mutations, thus we have limited knowledge of biological molecular changes with first generation EGFR-tyrosine kinase inhibitor (TKI) resistance. We report a case of an SQCLC patient treated with first-line platinum-doublet chemotherapy. After disease progression, the patient was administered first generation EGFR-TKI gefitinib based on next generation sequencing results. After five months, a second biopsy was performed and both the tumor and plasma samples indicated an acquired *EGFR* exon 20 T790M mutation. The patient was subsequently administered AZD9291, which resulted in disease control for a time. Our results indicate that a *TP53* exon 8 mutation might act as a negative predictive biomarker for third generation EGFR-TKIs.

## Introduction

Lung cancer remains the most common cause of cancer death worldwide.[1] With the detection of druggable mutations, treatments for advanced adenocarcinoma have made great progress.[2] Squamous cell lung cancer (SQCLC) accounts for about 25–30% of lung cancers,[3] and FGFR1 amplification, PTEN deletions or mutations, and point mutations in PIK3CA, PDGFRA, and DDR2 are considered the most common driver mutations in SQCLC.[4,5] Unfortunately, no effective targeted drugs have been approved for SQCLC, which makes precision therapy in this field difficult.

In recent years, next-generation sequencing (NGS), which allows massive parallel sequencing with small tumor samples, has played an increasingly important role in determining driver mutations in different cancers.[6] Herein, we report a case of an advanced stage SQCLC patient who experienced disease progression after first-line chemotherapy and palliative radiation therapy, who subsequently gained survival benefit by NGS and corresponding treatment.

## Case report

In November 2015, a 39-year-old male heavy smoker presented with a severe cough and hemoptysis and was admitted into our department. Computed tomography

(CT) revealed two lesions in the right upper lung lobe (Fig 1a). Immunohistochemical staining of a bronchoscopic biopsy sample was positive for p63, p40, and CK 7, and negative for TTF-1 and CK 20, favoring a diagnosis of poorly differentiated squamous cell carcinoma (Fig 2a–d). Together with the results of bone scanning and magnetic resonance imaging, the patient was diagnosed with stage IV squamous cell lung cancer with multiple bone metastases. He was administered platinum-based chemotherapy with pamidronate disodium and palliative radiation therapy of 2–5 lumbar vertebra as first-line treatment. The patient achieved a partial response (PR) and hemoptysis disappeared after two cycles of chemotherapy (Fig 1b). However, six cycles later, the disease progressed and the tumor mass was enlarged (Fig 1c). Fortunately, NGS detected an *EGFR* 19 exon deletion in the bronchoscopic biopsy sample. The patient was administered first generation EGFR-TKI gefitinib (250 mg per day) as second-line treatment from June 2016. A PR was achieved and the cough was relieved after one month of gefitinib treatment (Fig 1d). Two months later, the PR was further confirmed by CT scan; therefore, targeted therapy with gefitinib continued. However, the patient suffered explosive disease progression after five months of gefitinib treatment. He had a severe cough and airway obstruction (Fig 1e) and new metastasizing lesions had appeared, including subcutaneous, intracranial, and intramuscular metastases. A needle re-biopsy was performed at the subcutaneous lesion, in which pathologists found small cells (Fig 2e). Immunohistochemical staining results positive for CK5/6 and negative for Synaptophysin A, CD56, and chromogranin verified the diagnosis of SQCLC (Fig 2f–i). NGS results indicated *EGFR* 19 exon deletion and exon 20 T790M mutation in both tissue and plasma samples (Table 1). This could explain the mechanism of failure of the first generation EGFR-TKI and the sensitivity of the

third generation EGFR-TKI. The patient was admitted to the ASTRIS clinical trial and started taking AZD9291 (80 mg per day). The disease remained stable for six weeks (Fig 1f), and clinical symptoms such as fatigue, anhelation, and coughing were relieved; his performance status score decreased; and all subcutaneous lesions reduced in size. Unfortunately, after eight weeks of AZD9291 treatment, the patient was sent to hospital with respiratory failure, and died one week later. He achieved overall survival of 15 months.

## Discussion

With the closure of the AURA/AURA EX, AURA2, AURA3 clinical trials, the United States Food and Drug Administration, the European Medicines Agency, and the China Food and Drug Administration have approved AZD9291 for non-small cell lung cancer patients with an *EGFR* T790M mutation resistant to first generation EGFR-TKIs.[7–9] To the best of our knowledge, this is the first report of a SQCLC patient harboring an *EGFR* exon20 T790M mutation after first generation EGFR-TKIs, administered the third generation EGFR-TKI AZD9291 as subsequent therapy. The patient's symptoms were relieved during the first six weeks of AZD9291 treatment, with a stable evaluation on imaging tests. With developments in targeted therapy, traditional treatment based on histopathological diagnosis has become limited, while precision treatments based on molecular markers have attracted increasing attention. NGS, with the advantages of high sensitivity, high throughput, and low sample quantity, plays an irreplaceable role in precision therapy. Particularly in patients diagnosed via small samples, NSG can provide maximal tumor genomic assessment, determine potential therapeutic targets, and evaluate tumor heterogeneity at the gene level.[6,10] Some researchers believe that it



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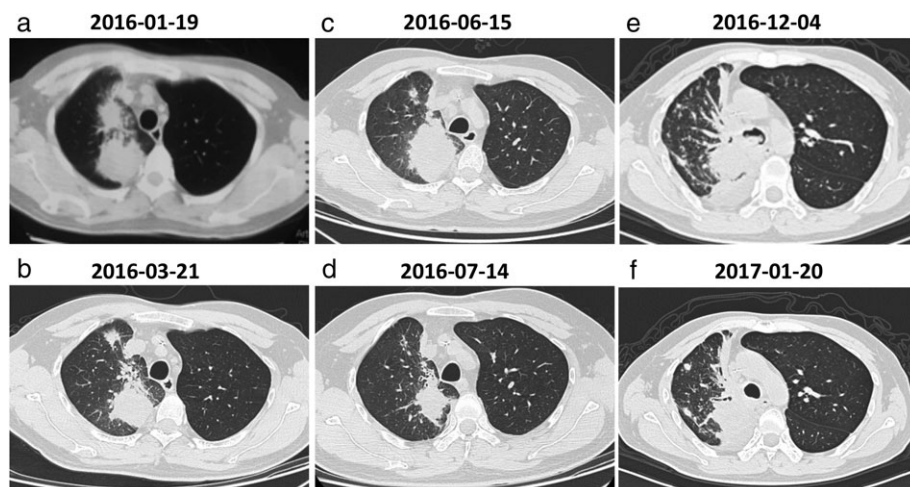
**Figure 1** Chest computed tomography scanning. (**a**) Two primary lesions in the right upper lung lobe; (**b**) the disease reached partial remission after two cycles of chemotherapy; (**c**) disease progression was observed after six cycles of chemotherapy; (**d**) lesions were significantly reduced after taking Iressa for one month; (**e**) disease progression after TKI administration for five months; and (**f**) lesions in the lung remained stable after administration of AZD9291 for six weeks.

**Figure 2** Hematoxylin and eosin (H&E) ×200 and immunohistochemical staining of biopsy tissues. (**a**) H&E staining of bronchoscopic biopsy tissue. Tumor cells were positive for (**b**) p63 and (**c**) p40 and negative for (**d**) TTF1, indicating poorly differentiated squamous cell carcinoma. (**e**) H&E staining of re-biopsy tissues in subcutaneous metastases, small cells were observed in the sample. Immunohistochemical staining of the tissue shows (**f**) positive for CK5/6 and (**g**) negative for Synaptophysin A, (**h**) CD56, and (**i**) chromogranin, which verified the diagnosis of squamous cell lung cancer.
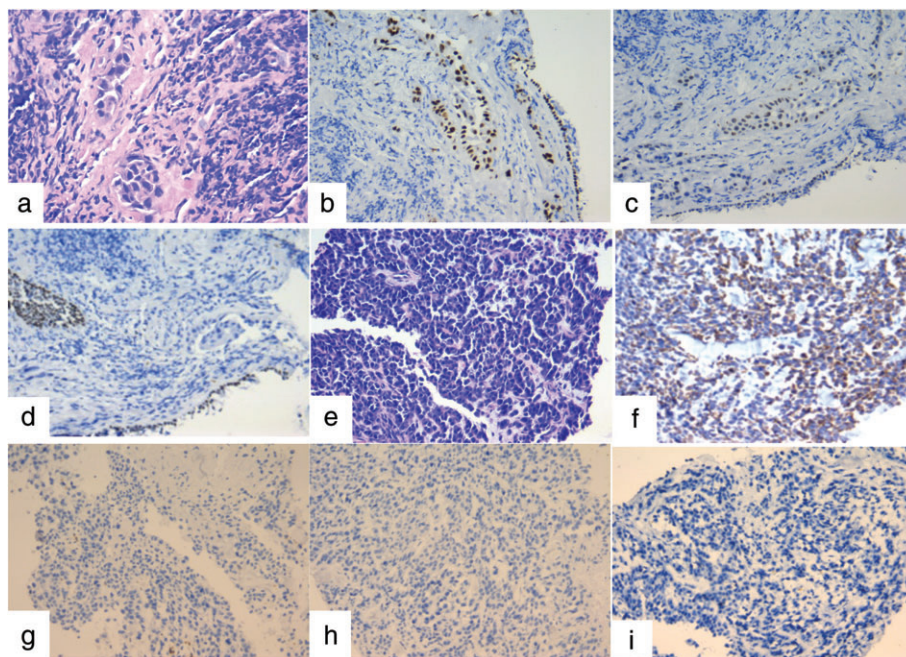


**Table 1** Mutations revealed by NGS in blood and biopsy tissue samples

| Gene point | Primary lesion (Feb 5 2016) | Subcutaneous metastasis (15 Dec 2016) | cfDNA at disease progression (15 Dec 2016) |
|---|---|---|---|
| *EGFR* | Exon 19: p.745_750del | Exon 19: p.745_750del, exon20: p.T790M | Exon 19: p.745_750del, exon 20: p.T790M |
| *TP53* | P72R polymorphism | Exon 8: p.R282Q | Exon 8: p.R282Q |
| *RB1* | Copy number deletion | — | — |
| *SOX2* | Gene amplification | — | — |
| *FGFR1* | — | Exon 9: p.K400N | Exon 9: p.K400N |
| *NTRK1* | — | Exon 12: p.S465Y | Exon 12: p.S465Y |
| *KIT* | — | Exon 11: p.N566T, p.WKVVEEINGN557delinsY, p.Q556H | — |

cfDNA, circulating free DNA; NGS, next-generation sequencing.

is impossible for patients with pure SQCLC to have an *EGFR* mutation.[11] However, because of tumor heterogeneity, National Comprehensive Cancer Network guidelines recommend *EGFR* and *ALK* assessment in nonsmokers or patients diagnosed via small biopsies or with mixed histological SQCLC. The patient reported in our case was diagnosed with SQCLC through a bronchoscopic biopsy sample, therefore, NGS was recommended to determine precise therapy at the onset of the disease. NGS was performed again after disease progression, with re-biopsy of tissues and simultaneous circulating free DNA (cfDNA) in plasma for the dynamic monitoring of gene status (Table 1). Somatic mutations in blood and tissue samples yield similar results, as both can detect *EGFR*-sensitive mutations and patients with acquired resistance to EGFR-TKIs. When a tissue sample is inadequate or difficult to obtain, NGS on cfDNA can reflect the gene status of the tissue sample and can also be used

to monitor driver mutations during different courses of the disease.

Intriguingly, although both the tissue and blood samples in this case revealed an abundance of *EGFR* exon 20 T790M mutations after first generation EGFR-TKI resistance (24.11% and 13.48%, respectively), the response of different lesions to AZD9291 varied. In this case, the symptoms were relieved, performance status improved, and all subcutaneous lesions reduced while lung lesions remained stable, and the patient finally died of respiratory failure. We suggest that discrepancies in the responses of different lesions may be caused by tumor spatial heterogeneity. In the AURA3 clinical trial, the objective response rate of *EGFR* T790M (+) patients reached only 71%,[9] indicating that further predictive biomarkers of AZD9291 need to be determined. It should be noted that this patient harbored both *TP53* and *EGFR* gene mutations in re-biopsy and plasma samples. Researchers have claimed that in *EGFR*-mutated NSCLC patients treated

with first generation EGFR-TKIs, patients with a simultaneous *TP53* mutation have poorer prognosis than those with *TP53* wild type. In particular, the disease control rate is decreased and progression-free survival is significantly reduced in patients with *EGFR* exon 19 deletions/*TP53* exon 8 mutations.[12] It is possible that the presence of a *TP53* exon 8 mutation may serve as a negative predictive biomarker for third generation EGFR-TKIs. Further research is required to confirm this observation.

To the best of our knowledge, herein we report the first case of a lung cancer patient with squamous histology harboring an *EGFR* exon 20 T790M mutation after administration of first generation EGFR-TKIs, treated with AZD9291 as subsequent therapy. The SQCLC patient's NGS results indicated that a *TP53* exon 8 mutation might serve as a negative predictive biomarker for both first and third generation EGFR-TKIs. In addition, we can consider cfDNA in plasma samples to monitor the status of driver mutations, especially for those whose tissue sample is inadequate or difficult to acquire.

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## Disclosure

No authors report any conflict of interest.

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