Newly emergent acquired EGFR exon 18 G724S mutation after resistance of a T790M specific EGFR inhibitor osimertinib in non-small-cell lung cancer: a case report

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Background: T790M mutation is well known as the most common mechanism for resistance to the first- and second-generation tyrosine kinase inhibitors (TKIs) for EGFR mutation in non-small-cell lung cancer. Several third-generation EGFR TKIs, such as osimertinib, have been explored and approved for conquering this resistance; however, acquired resistance to osimertinib is evident and the resistance mechanisms remain complex and incompletely explored.

Case presentation: A non-smoking 58-year-old female patient was initially diagnosed with lung adenocarcinoma harboring EGFR exon 19 deletion and clinically responded to initial gefitinib treatment. The patient progressed on gefitinib after >1 year and a T790M mutation was detected in tissue biopsy by next-generation sequencing (NGS). Osimertinib treatment was administrated for several months and an acquired rare EGFR G724S mutation was detected via NGS blood sample after osimertinib resistance.

Conclusion: The specific mechanisms of acquiring drug resistance for EGFR-TKIs have not been fully explored. EGFR G724S mutation might be associated with osimertinib resistance but more studies about the mechanism should be explored.

Keywords: NSCLC, EGFR mutation, tyrosine kinase inhibitor, next-generation sequencing

Background
EGFR gene mutations in lung adenocarcinoma are closely related to the clinical efficacy of EGFR tyrosine kinase inhibitors (TKIs). Commonly used EGFR-TKIs include gefitinib, erlotinib (and icotinib) as the first-generation EGFR-TKIs, and afatinib (and dacomitinib) as the second generation, and osimertinib as the third generation. Furthermore, many clinical trials have demonstrated that EGFR-TKIs significantly increased clinical benefits compared with the standard chemotherapies in non-small-cell lung cancer (NSCLC) patients.1-2 However, most NSCLC patients eventually developed resistance to these first-generation TKIs within 1 year. The EGFR exon 20 T790M mutation has been well known as the major resistant mutation for the first- and second-generation TKIs.3 Approximately 50% of the patients with TKI resistance were detected of harboring the EGFR T790M mutation.4 Several third-generation EGFR-mutant selective TKIs, such as osimertinib (AZD9291), olmutinib (HM61713), and rociletinib, are being developed for EGFR T790M mutation in NSCLC. Osimertinib targets both sensitive EGFR mutation and resistant T790M, and a better benefit of progression-free survival (PFS) was observed in osimertinib (mPFS 18.9 months)
when compared to a standard first-generation EGFR-TKI (gefitinib or erlotinib, 10.2 months). Even so, most patients who were given the third-generation EGFR-TKIs would eventually develop resistance after several months. Acquired resistant C797S mutation, also located in EGFR exon 20, has been reported as one of the most important resistance mechanisms of osimertinib,\(^6,7\) and C797S mutation was also reported as acquired resistance to another T90M-specific third-generation EGFR-TKI (HM61713) in NSCLC.\(^8\) Other mechanisms of resistance to osimertinib are reported, such as MET and HER2 amplification and small-cell lung cancer transformation.\(^9\) EGFR L718Q has also been found in T90M-positive patients after osimertinib resistance.\(^8\) Here, we report an extremely rare and complicated acquired mutation of EGFR exon 18 G724S after osimertinib resistance.

### Case presentation

A non-smoking 58-year-old female was diagnosed with lung adenocarcinoma (pT2N0M0, according to seventh TNM classification) after right lower lobe lobectomy. A SurPlex™ liquid chip test (SurPlex™-xTAG, Surexam, P.R. China) was performed on the resected tumor tissue, which showed an activated mutation of EGFR exon 19 deletion (19 Del, p.E746_S752>V), no KRAS, BRAF, PIK3CA mutation. The patient was administered with gefitinib at a dose of 250 mg/day for 1 year, during which the patient was stable (according to the Response Evaluation Criteria in Solid Tumors). Gefitinib therapy was discontinued after appearance of liver metastasis in December 2013, and second-line chemotherapy (pemetrexed 850 mg, d1+ cisplatin 40 mg, d1–d3) and percutaneous microwave coagulation therapy of liver were administrated. However, the patient discontinued the chemotherapy after two cycles due to serious adverse effects of nausea and vomiting and continued with gefitinib treatment again. New liver metastases and a pulmonary mass were detected in March 2015. Wedge resection of left upper lobe was carried out by video-assisted thoracoscopic surgery and pathological diagnosis was adenocarcinoma (pT2NxM1, IV stage). Next-generation sequencing (NGS, Langqing™, Burning Rock Dx, P.R. China) of the tumor biopsy presented EGFR 19 Del and T790M mutation, MAP2K1 and TP53 mutation, no ALK and ROS1 mutation. The patient was enrolled in the Phase II clinical trial of osimertinib (40 mg/day) instead of gefitinib therapy. The patient progressed with brain metastasis in June 2015 and withdrew from the clinical trial and changed dose of osimertinib from 40 mg/day to standard 80 mg/day, complementary with stereotactic brain irradiation. The patient developed multiple metastases after several months and the peripheral blood NGS test (Langqing™) showed rare EGFR G724S on 2 June 2016 (in addition to EGFR 19 Del and T790M). Then, the therapy combined osimertinib 80 mg/day and gefitinib 250 mg/day. Moreover, the patient’s family purchased pembrolizumab and cabozantinib (XL184) from outside China, which also failed to slow down the progress of the disease. Two additional peripheral blood NGS tests revealed MET amplification in August and September 2016. However, the disease worsened quickly and the patient died of respiratory failure on 25 September 2016. All the processes of diagnosis and treatment are shown below (Figure 1 and Table 1).

### Discussion

T790M mutation is well known as the most common resistant mutation for the first- and second-generation TKIs for EGFR mutation.\(^11\) Osimertinib as a selective third-generation TKI has been explored and approved for treating EGFR T790M in NSCLC patients. A better clinical response was also observed in osimertinib group compared to gefitinib and/or erlotinib group in untreated EGFR-mutant advanced NSCLC.\(^5\) However, similar to patients treated with first- or second-generation EGFR TKIs, resistance eventually occurred in patients treated with osimertinib after no >10 months.\(^12\) Several mechanisms of resistance including EGFR-dependent and EGFR-independent were reported. EGFR exon 20 C797S was reported to be a major resistance mechanism to osimertinib as an EGFR-dependent mechanism.\(^8,13,14\) EGFR C797S codon, located in the kinase-binding pocket, results in loss of binding of osimertinib to EGFR. Similarly, C797S has also been detected in EGFR T790M patients with rociletinib, olmutinib, and nazartinib treatment.\(^8,15,16\) However, there are still numerous patients who have acquired resistance to these third-generation TKIs without acquiring C797S mutation.\(^13\) More recently, a novel EGFR C797G mutation has been reported after osimertinib resistance.\(^17\) Other rare EGFR mutations, including G796S/R, L792F/H, L798I, and L718Q, have also been reported to be possible resistance mutations to osimertinib.\(^10,18–20\) EGFR T790M loss was often seen early after osimertinib treatment and was accompanied by osimertinib resistance in a shorter time compared to those with maintained T790M.\(^21\) EGFR-independent mechanisms have been reported in tumor or plasma samples, including MET and HER-2 amplification,\(^22\) MAPK activation,\(^22\) mutations in KRAS,\(^15\) PI3KCA,\(^16\) BRAF,\(^24\) and transformation to small-cell lung cancer.\(^25\)

Herein, we report that EGFR exon 18 G724S may serve as an acquired potential resistance mutation in a
lung adenocarcinoma post progression of osimertinib. In this case report, G724S mutation clearly emerged after osimertinib resistance, as the same genomic profiling assay was performed in the tumor tissues before osimertinib treatment and this mutation was not detected. G724S mutation was detected in two cases of NSCLC after osimertinib resistance.26 In another case report of lung adenocarcinoma, even though EGFR G724S was detected before osimertinib treatment, this mutation was not detected. G724S mutation was detected in two cases of NSCLC after osimertinib resistance.

Table 1 Gene mutations from tumor biopsy specimen or peripheral blood

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Amino acid</th>
<th>Nucleotide</th>
<th>December 2012 (tumor tissue: SurPlex™ liquid chip test)</th>
<th>AF%, April 2015 (left lung mass: NGS)</th>
<th>AF%, June 2016 ( peripheral blood: NGS)</th>
<th>AF%, August 2016 ( peripheral blood: NGS)</th>
<th>AF%, September 2016 ( peripheral blood: NGS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>19</td>
<td></td>
<td>2237-2255del ATTAA GAGAAGCACAACATTCinsT</td>
<td>Exon 19 del (p.E746_S752=V)</td>
<td>15.2</td>
<td>5.33</td>
<td>7.97</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>T790M 2369C&gt;T</td>
<td></td>
<td>11.5</td>
<td>1.34</td>
<td>5.37</td>
<td>8.42</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td>G724S 2170G&gt;A</td>
<td></td>
<td>1.56</td>
<td>0.89</td>
<td>0.89</td>
<td>0.97</td>
</tr>
<tr>
<td>MAP2K1 (MEK1)</td>
<td>2</td>
<td>D67E</td>
<td>201C&gt;A</td>
<td></td>
<td>16.1</td>
<td>2.88</td>
<td>11.9</td>
<td>21.8</td>
</tr>
<tr>
<td>TP53</td>
<td></td>
<td></td>
<td>c.764-766del</td>
<td></td>
<td>13.4</td>
<td>20.42</td>
<td>10.6</td>
<td>3.33</td>
</tr>
<tr>
<td>MET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CN =2.46</td>
<td>CN =3.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AF, allelic frequency; NGS, next-generation sequencing.
Table 2 General clinical characteristics of patients harboring the EGFR G724S mutation after TKIs treatment

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/age</th>
<th>Smoking status</th>
<th>Stage</th>
<th>Firstly detected EGFR mutation/TKIs</th>
<th>Secondly detected EGFR mutation/TKIs</th>
<th>Thirdly detected EGFR mutation</th>
<th>G724S (MAF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our case</td>
<td>F/58</td>
<td>Never</td>
<td>II→IV</td>
<td>Exon 19 Del/gefitinib</td>
<td>T790M/osimertinib</td>
<td>G724S</td>
<td>1.56/0.89/0.97</td>
</tr>
<tr>
<td>Oztan26</td>
<td>F/47</td>
<td>_</td>
<td>IV</td>
<td>Exon 19 Del/erlotinib</td>
<td>T790M/osimertinib</td>
<td>G724S</td>
<td>5%</td>
</tr>
<tr>
<td>Oztan26</td>
<td>M/74</td>
<td>_</td>
<td>IV</td>
<td>Exon 19 Del/erlotinib</td>
<td>_/osimertinib</td>
<td>G724S</td>
<td>0.7%</td>
</tr>
<tr>
<td>Peled27</td>
<td>F/49</td>
<td>Never</td>
<td>IIIA</td>
<td>Exon 19 Del/gefitinib</td>
<td>T790M/G724S</td>
<td>_</td>
<td>2.9%</td>
</tr>
<tr>
<td>Li28</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>Exon 19 Del/gefitinib or erlotinib</td>
<td>T790M/G724S</td>
<td>_</td>
<td>10.1%</td>
</tr>
<tr>
<td>Li28</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>Exon 19 Del/gefitinib or erlotinib</td>
<td>_</td>
<td>G724S</td>
<td>1.4%</td>
</tr>
<tr>
<td>Li28</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>Exon 19 Del/gefitinib or erlotinib</td>
<td>_</td>
<td>G724S</td>
<td>80.5%</td>
</tr>
<tr>
<td>Li28</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>Exon 19 Del/gefitinib or erlotinib</td>
<td>_</td>
<td>G724S</td>
<td>37.9%</td>
</tr>
<tr>
<td>Li28</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>Exon 19 Del/gefitinib or erlotinib</td>
<td>_</td>
<td>G724S</td>
<td>59.9%</td>
</tr>
<tr>
<td>Fassunke29</td>
<td>F/59</td>
<td>_</td>
<td>IIIA</td>
<td>Exon 19 Del/erlotinib</td>
<td>T790M/osimertinib</td>
<td>G724S</td>
<td>6.3%</td>
</tr>
<tr>
<td>Fassunke29</td>
<td>F/47</td>
<td>_</td>
<td>IV</td>
<td>Exon 19 Del/erlotinib</td>
<td>T790M/EGF816</td>
<td>G724S</td>
<td>71.1%</td>
</tr>
<tr>
<td>Fassunke29</td>
<td>F/68</td>
<td>_</td>
<td>IV</td>
<td>Exon 19 Del/erlotinib</td>
<td>T790M/G724S/ osimertinib</td>
<td>_</td>
<td>5.3%/49.6%</td>
</tr>
<tr>
<td>Fassunke29</td>
<td>_/69</td>
<td>_</td>
<td>IV</td>
<td>Exon 19 Del/erlotinib</td>
<td>T790M/G724S/ osimertinib</td>
<td>_</td>
<td>_/39.3%</td>
</tr>
</tbody>
</table>

Abbreviations: TKIs, tyrosine kinase inhibitors; F, female; M, male; MAF, mutant allele frequency; _, not mentioned.

treatment, G724S clone significantly increased after osimertinib resistance, which further implicated G724S mutation could potentially be resistant to osimertinib. Li et al subsequently reported 5 cases of G724S mutation in 1,170 NSCLC patients. Surprisingly, these G724S mutations were exclusively presented in patients after resistance to gefitinib or erlotinib, and the G724S mutation frequencies varied drastically. The allelic frequency for tissue-based assay presents the percentage of DNA obtained from mutation-containing tumor, which is easy to be affected by which tumor biopsy was performed. Very recently, Fassunke et al also reported four cases with G724S mutation, two of whom acquired G724S mutation after third-EGFR TKIs, including osimertinib and EGF816. Another two cases were detected with G724S in the same time with T790M after gefitinib or erlotinib resistance (Table 2). It can be assumed that EGFR G724S mutation may be an extremely rare mutation induced by EGFR-TKIs, including different generations of TKIs. EGFR G724S may be assumed as an oncogenic mutation as it is also within the ATP binding-loop of the kinase, which confers drug resistance to TKIs by either changing the protein structure, or enhancing ATP affinity or stabilizing active mutation. Fassunke et al demonstrated that EGFR G724S limited the activities of third-generation EGFR TKI inhibitors both in vitro and in vivo. Structural analyses and computational modeling indicated that EGFR G724S might induce conformation of the glycine-rich loop, which disrupted the binding with third-generation TKIs. More studies about structure and mechanisms should be performed to determine the precise effects conferred by this mutation.

Conclusion

In conclusion, we should make best use of the highly advanced tools for lung cancer, like ctDNA and NGS, to discover therapeutic benefits of drugs and variations of the diseases. EGFR G724S has been reported to show potential resistance to osimertinib, but more studies should be performed to explore the mechanisms conferred by this mutation in regards to osimertinib resistance.

Ethics approval and consent to participate

Research reported in the study was approved by Review Board of Xiangya Hospital, Central South University.
Consent for publication
Documented written consent from the patient was obtained to report the details of the case.

Data sharing statement
All data generated or analyzed during this study are included in this published article.

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Author contributions
All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure
The authors report no conflicts of interest in this work.

References