CASE DESCRIPTION

The patient was a 78-year-old woman, a nonsmoker, with a history of a stage IIIC2 low-grade endometrial carcinoma, endometrioid type, in 2011, which was treated with surgery, vaginal cuff brachytherapy, and adjuvant chemotherapy. Her medical history also included hyperthyroidism and hypertension.

In December 2013 she presented to another hospital with nausea and a posterior occipital headache. Head computed tomography demonstrated a cerebellar mass and she was transferred to our institution for further workup. Imaging also showed an approximately 5-cm left lung perihilar mass suspicious for a primary bronchogenic malignancy, an ill-defined approximately 3-cm right hepatic lobe mass concerning for malignancy, either primary or metastatic, and multiple sclerotic osseous metastases in the thoracolumbar spine. A brain MRI was performed to further characterize the metastatic lesions. There was a 3.0-cm mixed solid and cystic mass in the medial and inferior right cerebellar hemisphere with marked mass effect upon the fourth ventricle. An additional enhancing mass was present in the left occipital lobe (1.7 cm).

The right cerebellar tumor was excised and on pathology showed a moderately differentiated adenocarcinoma, TTF-1 (thyroid transcription factor 1)-positive and PAX-8 negative, consistent with a metastatic adenocarcinoma of pulmonary origin.

PATIENT FOLLOW-UP

The patient’s tumor was genotyped on the Ion AmpliSeq™ Cancer Hotspot Panel v2 and was found to harbor an epidermal growth factor receptor (EGFR) exon 20 9-bp insertion (c.2311_2312insGCGTGAGCA, p.D770_N771insSVD) and an incidental tumor protein p53 (TP53) mutation (c.403C>T, p.R135W); another testing method was not used to confirm the genotype.

DISCUSSION

Molecular genotyping of lung adenocarcinoma is the current standard of care, the results of which are used to direct personalized patient care. Recent guidelines from International Association for the Study of Lung Cancer/Association for Molecular Pathology/College of American Pathologists recommend at minimum EGFR and anaplastic lymphoma receptor tyrosine kinase (ALK) testing on all tumors with adenocarcinoma histology (including adenosquamous, adeno–small cell neuroendocrine, and poorly differentiated) (1). EGFR mutations that occur at a frequency of >1% should be tested in routine care (1).

The most common EGFR mutations occur in exons 18–21 which encode for the receptor kinase domain. These mutations are activating or driver mutations and lead to constitutive activation of the downstream MAPK (mitogen activated kinase-like protein) and PI3K/AKT (phosphatidylinositol 3-kinase/protein kinase B) pathways. Although common sensitizing and resistance mutations are well described in the literature, there is relatively little knowledge of rarer mutations that may present a management challenge for clinicians in their daily practice. In contrast to the more common EGFR exon 21 p.L858R point mutation and exon 19 in-frame deletions, which are sensitizing or activating mutations, EGFR exon 20 insertions are generally associated with primary (de novo) resistance exhibited as a lack of or decreased response to tyrosine kinase inhibitors (TKIs) (2). Another exon 20 mutation, p.T790M, is a well-described cause of secondary acquired resistance to TKIs in patients with activating EGFR mutations who initially...

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4 Human genes: EGFR, epidermal growth factor receptor; TP53, tumor protein p53; ALK, anaplastic lymphoma receptor tyrosine kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog, ERBB2, erb-b2 receptor tyrosine kinase 2; BRAF, B-Raf protooncogene, serine/threonine kinase.

5 Nonstandard abbreviations: TKI, tyrosine kinase inhibitor; PFS, progression-free survival; NSCLC, non–small cell lung cancer.
respond to TKIs but then develop resistance and recurrent disease due to a second EGFR resistance mutation (p.T790M) in the tumor. Table 1 lists the most common sensitizing and resistance mutations in EGFR exons 18–21 (3).

EGFR exon 20 insertion mutations, reported in approximately 2%–6% of lung adenocarcinoma patients, are the third most common EGFR mutation (5%–11% of EGFR mutations) and like other EGFR mutations are associated with never-smoker history (2, 4, 5, 6). These are heterogeneous in-frame insertions involving codons 763–775 at the proximal region of the exon; upwards of 20 different insertions have been reported (5). The insertion mutations occur at the N-lobe of EGFR after the C-helix; this region has a critical role in the EGFR tyrosine kinase catalytic activity (7). In one study, an in silico computation analysis predicted that some mutations directly affect the drug-binding pocket and others likely reduce drug-binding affinity; this heterogeneity likely accounts for the varied response to erlotinib in these patients (4, 7).

Overall, in a study of 839 patients with advanced stage lung cancer (stage IV or recurrent) with a median follow-up of 30 months, the 24 patients with exon 20 insertions had a median survival of 16.5 months, which was shorter than the patients with common EGFR mutations (median, 33.0 months; \( P = 0.06 \)) but similar to patients who lacked EGFR mutations (median, 20.0 months; \( P = 0.60 \)) (5). Eight of these patients received erlotinib, and of those with imaging studies, none demonstrated an objective response (5).

Several second-line TKIs are under assessment in clinical trials. Afatinib is a potent irreversible inhibitor of all members of the ERBB family of receptor tyrosine kinases. It is designed to covalently bind to cysteine 797 within the catalytic domain of EGFR, thus blocking the ATP binding site. In tumor xenograft models, afatinib has shown activity in tumors resistant to first-line TKI, including those harboring the EGFR p.L858R/p.T790M double mutant (8). Subsequently, the safety and efficacy of afatinib has been studied in a series of clinical trials referred to as the LUX-Lung program. Several of the trials have focused on afatinib treatment for advanced lung cancer patients with acquired resistance to first-line gefitinib/erlotinib (8).

In the LUX-Lung trials 2, 3, and 6, a total of 75 patients with uncommon EGFR mutations were treated with afatinib. In an independent review, the uncommon mutations were broken down into 3 groups: de novo p.T790M (n = 14), exon 20 insertions (n = 23), and all other mutations (n = 38). The response rate was low in tumors with exon 20 insertions [median progression-free survival (PFS), 2.7 months], similar to the p.T790M mutant tumors (median PFS, 2.9 months); however, durable tumor control was achieved in some patients. Afatinib was active in the group of other uncommon EGFR mutations (such as p.G719X and p.L861Q), and the rate and duration of response was comparable with that observed in patients with common mutations (9). The response of exon 20 mutations to other irreversible EGFR inhibitors (neratinib and PF00299804) has been similarly low (10). Therefore, identification of treatment strategies, unique to these exon 20 insertion mutations, is needed. Currently, there is an ongoing phase 2 clinical trial of AUY922 [an HSP90 (heat shock protein 90) inhibitor] in non–small cell lung cancer (NSCLC) patients with EGFR exon 20 insertion mutations (NCT01854034); the mechanism of action of this drug is to induce proteasome-mediated degradation of client proteins in the oncogenic pathways. Alternative approaches, such as combination therapies and novel compounds with a structural composition different from current EGFR inhibitors, as are being studied in EGFR p.T790M tumors, may be applicable to exon 20 insertion mutations as well (3, 8).

EGFR exon 20 insertion mutations are mutually exclusive from other well-identified oncogenic driver mutations seen in lung adenocarcinoma [e.g., KRAS (Kirsten rat sarcoma viral oncogene homolog), ERBB2 (erb-b2 receptor tyrosine kinase 2), BRAF (B-Raf protooncogene, serine/threonine kinase), and ALK fusions] (4, 6). Patients with these insertions comprise a distinct subset of lung adenocarcinoma that shows primary resistance to common first-line TKIs (gefitinib and erlotinib) and low but some (perhaps dependent on the insertion transcript) limited response to second-generation irreversible TKIs (e.g., afatinib).

In our case, the patient’s brain metastases were treated with radiosurgery to the surgical bed and the left occipital mass (as opposed to whole brain irradiation). She was treated by an outside medical oncologist initially

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Table 1. Some of the most common EGFR tyrosine kinase domain sensitizing and resistance mutations in exons 18–21.

<table>
<thead>
<tr>
<th>EGFR exon</th>
<th>Mutations</th>
<th>Sensitizing</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>p.G719</td>
<td>p.E709</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>In-frame deletions</td>
<td>p.D761Y</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Rare</td>
<td>Insertions, p.Q787R, p.T790M</td>
<td></td>
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</tbody>
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* Adapted from Massarelli et al. (3).

\(^b\) EGFR exon 21 p.L861Q mutation appears to be resistant to erlotinib and gefitinib but can be effectively inhibited by a second-generation irreversible EGFR inhibitor (2).
Clinical Case Study

with 6 cycles of pemetrexed. Five months later there was no evidence of new brain metastases; however, because there was not significant improvement in her systemic disease she was switched to an alternative chemotherapy agent. Optimal strategies for targeting these EGFR exon 20 insertion mutations are under investigation. Comprehensive mutational analysis, such as the Ion AmpliSeq™ Cancer Hotspot Panel v2 used in our laboratory, can readily identify patients with less common EGFR mutations and significantly direct the selection of therapeutic drugs or enrollment into clinical trials.

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Commentary

Geoffrey R. Oxnard*

Tumor genotyping has become a fundamental component in the care of advanced NSCLC and is growing significantly contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References


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Lung cancers with EGFR activating mutations do not all have the same response to TKIs; there are sensitizing and either primary or secondary resistance mutations. Therefore, tumor genotyping is important to direct selection of therapy; currently, there are recommendations to routinely test lung adenocarcinomas for EGFR mutations and ALK rearrangements.

Tumors that initially respond to TKIs usually develop drug resistance (a secondary resistance mutation like p.T790M is one mechanism); second-generation irreversible TKIs and other drugs and combination therapies are being evaluated in clinical trials to address drug resistance.

Comprehensive mutational analysis, such as next-generation sequencing gene panels, can be used to identify patients with less common EGFR mutations and help direct the selection of targeted therapeutics or enrollment into clinical trials.

POINTER TO REMEMBER

- Lung cancers with EGFR activating mutations do not all have the same response to TKIs; there are sensitizing and either primary or secondary resistance mutations. Therefore, tumor genotyping is important to direct selection of therapy; currently, there are recommendations to routinely test lung adenocarcinomas for EGFR mutations and ALK rearrangements.
- Tumors that initially respond to TKIs usually develop drug resistance (a secondary resistance mutation like p.T790M is one mechanism); second-generation irreversible TKIs and other drugs and combination therapies are being evaluated in clinical trials to address drug resistance.
- Comprehensive mutational analysis, such as next-generation sequencing gene panels, can be used to identify patients with less common EGFR mutations and help direct the selection of targeted therapeutics or enrollment into clinical trials.