Case Report

A patient with a Germline (p.R776H) *EGFR* Mutation With Multiple Lung Cancers Harboring Different Somatic *EGFR* Mutations

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Clinical Practice Points

- There are not standard guidelines for the selection and treatment of patients with synchronous multiple nodules with ground glass/lepidic features.
- The presence of different *EGFR* mutations in the various resected nodules with ground glass/lepidic features suggests the existence of multifocal primary tumors.
- The results suggest the possibility that a germline mutation in an oncogene could facilitate the development of a tumor with additional mutations in the same oncogene.

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Introduction

In July 2022, a 48-year-old female was referred to our center due to the discovery of multiple bilateral lung nodules on a chest X-ray preformed for persistent cough. Therefore, she underwent a chest computed tomography (CT) scan, which confirmed the presence of multiple bilateral ground glass opacities (GGO) and a solid nodule measuring 18 mm in the basal lateral segment of the lower right lobe. The bronchoscopy revealed normal findings, but Staphylococcus aureus colonization was detected in the bronchial washing. She received treatment with 400 mg daily of moxifloxacin for 5 days, resulting in the resolution of her cough. The patient has no family history of cancer; she has never smoked and has no known exposure to carcinogenic agents.

The GGOs and the nodule in the lower right lobe persisted in a follow-up chest CT scan, and they exhibited a modest 18-fluorodeoxyglucose uptake on positron emission tomography (PET) with value ranging between 3 and 3.4. In December 2022, the patient underwent 3 wedge resections comprising the nodule and some GGOs in the upper and lower right lobes through video-assisted thoracoscopy.

Macroscopic evaluation of the lower right lobe revealed multiple nodules, ranging from 10 to 3 mm, and diffuse areas of increased consistency. No visible lesions were in the specimen of the right upper lobe. Histopathological examination showed 2 invasive adenocarcinomas in the lower right lobe; the largest was 10 mm, well-differentiated, with prevalent lepidic growth and pleural invasion. The other was a poorly differentiated adenocarcinoma (6 mm) with predominant solid growth pattern (Figure 1). Furthermore, multiple minimally invasive and in situ adenocarcinomas were found in the further samples from the lower lobe. The wedge resection from right upper lobe included 2 adenocarcinomas in situ (the largest 2 mm) and a meningothelial nodule.

Molecular testing was performed by next generation sequencing (NGS) on formalin-fixed and paraffin-embedded (FFPE) tumor samples from the resections of upper (1) and lower (2) right lobes. Briefly, for each specimen three unstained FFPE sections, with a thickness of 10 µm, underwent standard deparaffinization in xylene and a rehydration in graded ethanol. Each sample was enriched for cancer cells by manual macrodissection, DNA was then purified by the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. DNA fragmentation and concentration were determined using a quantitative real-time PCR assay (Diatech Pharmacogenetics, Jesi, Italy), 25 ng of total DNA was used for

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Figure 1 (A) CT scan of lung parenchyma at the diagnosis reveals multiple lung cancers; the largest have been surgically removed. (B) Sanger sequencing on blood DNA revealed a germline heterozygous R77H mutation of EGFR. (C and D) Inferior right lobe (invasive adenocarcinoma G3; 40% tumor cells): EGFR exon 18 mutation c.2155G>A p.(G719S), VAF 18,84%. (E and F) Inferior right lobe (invasive adenocarcinoma G1; 50% tumor cells): EGFR exon 20 mutation c.2327G>A p.(R776H) VAF 50.66; exon 21 mutations c.2573T>G p.(L858R) VAF 8,71% and c.2582T>G p.(L861R) VAF 2,47%. (G and H) Superior right lobe (in situ adenocarcinoma; 20% tumor cells): EGFR exon 20 mutation c.2327G>A p.(R776H) with a variant allele frequency (VAF) equal to 44.4%; and exon 21 mutation c.2582T>A p.(L861Q) VAF 2,76%; negative for PD-L1 expression.

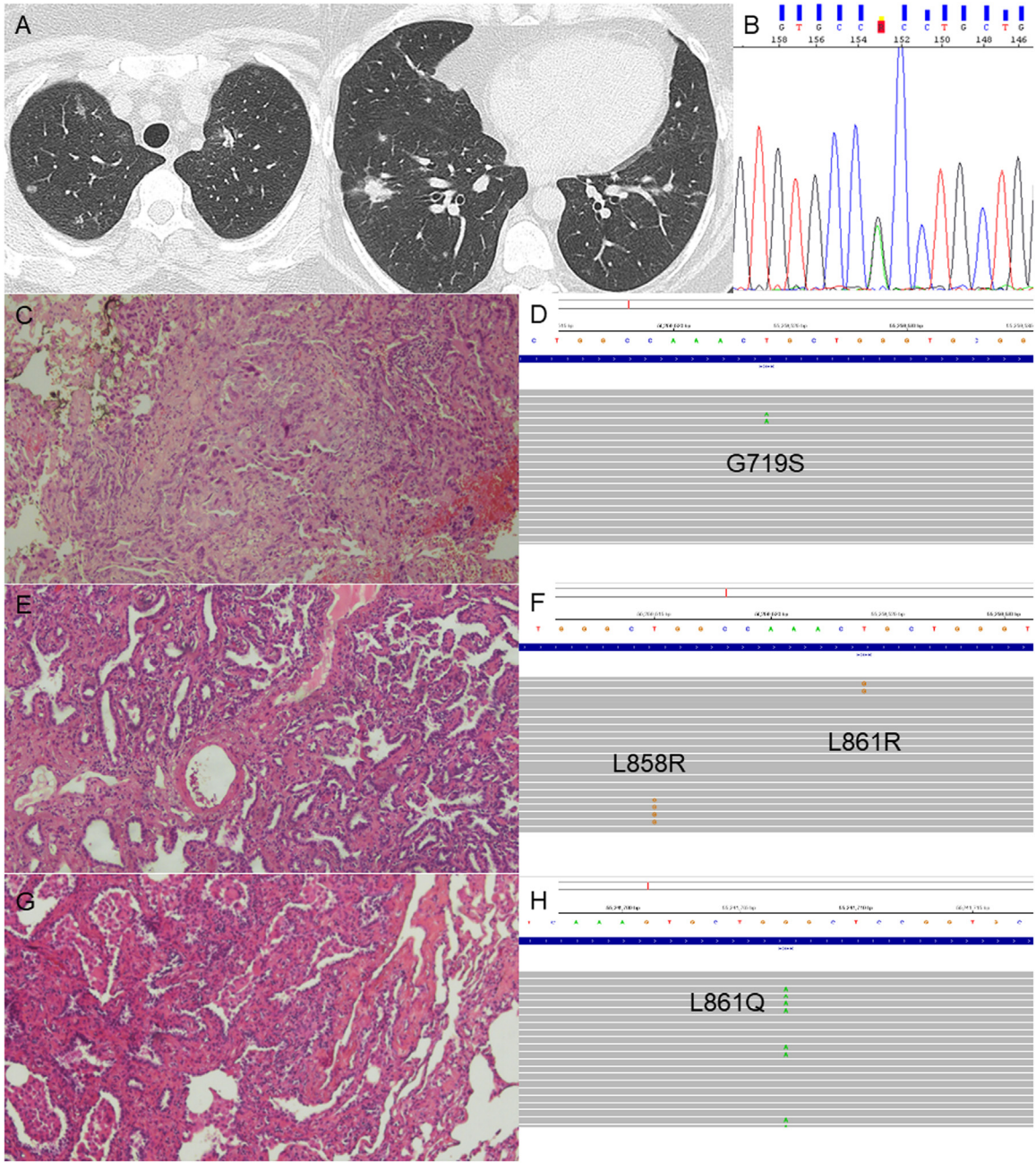

Table 1 EGFR Mutations of Resected Lung Cancer Nodules

EGFR mutations	Nodule of Right Inferior Lobe	Nodule of Right Inferior Lobe	Nodule of Right Superior Lobe
Exon 18	G719S (VAF 18.8%)		
Exon 20	R776H (VAF 53.6%)	R776H (VAF 50.7%)	R776H VAF (44.4%)
Exon 21		L858R (VAF 8.7%)	
Exon 21		L861R (VAF 2.5%)	L861Q VAF (2.8%)

VAF, Variant allele frequency.

the NGS analysis, according to manufacturer's instructions. The NGS amplicon-based panel, Myriapod®-NGS Cancer panel DNA (Diatech Pharmacogenetics, Jesi, Italy), covering clinically relevant regions within 17 oncogenes (i.e *EGFR, KRAS, BRAF*), was used. Sequencing was executed on the MiSeq platform (Illumina, San Diego, California).

Germline alterations within *EGFR* exon 20 were evaluated by direct Sanger sequencing on DNA purified from whole blood using the EZ1 SPD DNA blood Kit version 3 according to the manufacturer's protocol (Qiagen, Valencia, CA, USA) via automated EZ1 Advanced QIAGEN extractor. Sequencing was performed on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystem - ThermoFisher Scientific, San Francisco, CA, USA), with the Sequencing Analysis 5.0 Software (Applied Biosystem - ThermoFisher Scientific, San Francisco, CA, USA).

All adenocarcinomas harbored the *EGFR* exon 20 mutation c.2327G>A (p.R776H) and the same variant was confirmed as germline on DNA from whole blood. Each tumor presented also different *EGFR* activating mutations (Table 1) (Figure 1). No alterations were detected in the other analyzed oncogenes and PD-L1 immunohistochemical expression was negative in all the lesions.

In March 2023, a CT scan revealed the enlargement of 2 lung nodules. Consequently, she underwent chemotherapy treatment with cisplatin at a dosage of 75 mg/sqm and pemetrexed at a dosage of 500 mg/sqm, administered every 21 days. After completing 6 cycles of treatment, the nodules decreased in size, while the other GGOs remained stable. During the treatment, the patient experienced grade 3 anemia, for which she received a blood transfusion once, and Grade 2 neutropenia and thrombocytopenia. Currently, the patient continues to receive maintenance treatment with pemetrexed.

Discussion

This patient, with a germline (p.R776H) mutation of *EGFR*, has several lung adenocarcinomas harboring multiple somatic *EGFR* mutations. The different types of mutations observed in different nodules support the idea of multiple different primary tumors of the lungs. This observation suggests a predisposing role of (p.R776H) for the development of lung adenocarcinomas with *EGFR* mutations.

Lung tumors with a multifocal presentation are increasing in the last years from 3% to 14%.[1] In the VIII edition of the AJCC-TNM 4 major types of multifocal presentations have been depicted[2] and this patient belonging to the category of multifocal pulmonary adenocarcinoma with GG/L features.[3] These lesions are more frequently lepidic-predominant adenocarcinoma, minimally invasive adenocarcinoma, in situ adenocarcinoma, adenomatous alveolar hyperplasia or mucinous adenocarcinomas.[2] Patients with these tumors have peculiar demographic characteristics: mainly never smoker, female, excellent outcomes, and infrequent recurrences outside the lung parenchyma.[4] The histology of the nodules in our patient were lepidic-predominant invasive adenocarcinoma, minimally invasive adenocarcinoma and in situ adenocarcinoma. More nodules with similar radiologic characteristics remain in both lungs of the patient and have been followed radiologically until progression. There are not standard guidelines for the selection and treatment of patients with synchronous multiple nodules with GG/L features.

The demonstration of different primary tumors could be challenging without an extensive molecular characterization showing different somatic mutations in different tumors. In our patient, all the 3 nodules, with a molecular characterization, showed *EGFR* mutations. However, the mutations were different in each nodule except for (p.R776H). The variant allele frequency of (p.R776H) mutations was around 50% and, therefore, suggestive of a heterozygous germline mutation. Indeed, the variant allele frequency of the other *EGFR* somatic mutations was much lower ranging from 2.47% to 18.84%, supporting the idea of somatic mutations. We observed the presence of the (p.R776H) on the DNA extracted from the whole blood of the patient confirming its heterozygous germline origin.

The most frequent (75%) missense mutations affecting the residue 776 involve the substitution of arginine (R) with histidine (H). Other possibilities include substitutions with serine (S) or glycine (G).[5-9]

Frequently, the (p.R776H) mutation of *EGFR* is a germline mutation,[5-9] but somatic (p.R776H) mutations have also been reported in lung adenocarcinomas.[10-13] Genomic profiling is commonly performed using tumor DNA; therefore, it can be difficult to distinguish between germline or somatic mutations in some reports.[5,14-15]

The alteration (p.R776x) is frequently linked with a secondary EGFR mutation; indeed, whether germinal or somatic, when detected, the mutation (p.R776x) is often 1 of the 2 genetic alterations found in lung cancer with compound EGFR mutations.[5,10] The most common associated alterations are missense mutations involving G719, L858, L861, residues.[5,10,14-15] Even a third co-mutation of *EGFR* has been described in tumors harboring (p.R776S), (p.G719S), and (p.T790M) mutations.

The germline mutations (p.R776H), (p.T790M), (p.V834I), and (p.P848L) within *EGFR* increase the risk to develop lung cancer.[16] (p.T790M) has been associated with familial NSCLC.

Among never-smoking women who carry the (p.T790M) germline mutation, there is a 31% probability of developing lung cancer.[17] However, the incidence of (p.T790M) germline mutation is rare with few cases described in the literature (about 10 families).[16] It has been hypothesized that *EGFR* with the germline (p.T790M) mutation is a weak oncogene that requires an additional mutation, such as (p.L858R), to induce the neoplastic transformation.[16] Indeed, the presence of an additional mutation of *EGFR* such as (p.L858R) or (p.L861Q) are frequently observed in combination with (p.T790M). The mutations interest the same allele of *EGFR* similarly to what observed in cases of acquired mutation to first and second generations anti EGFR tyrosine kinase inhibitors. Similar considerations have been made for (p.V834I) and (p.P848L) germline mutations: they are uncommon and associated with an increased risk to develop lung cancer with additional *EGFR* mutations.[16]

EGFR with (p.R776G) mutation but not wild type *EGFR* determines the phosphorylation of Y1045 in the absence of receptor ligands when transfected into COS7 or 293 EBNA cells.[6] Therefore, it is reasonable to expect that the (p.R776H) mutation induces EGFR autophosphorylation and constitutive activation. Similarly, *EGFR* with the (p.R776H) mutation, either alone or in combination with additional mutations, such as (p.G719x), led to increased autophosphorylation of the receptor and its downstream signaling cascade.[9] Sequencing of *EGFR* mRNA clones from patients with combined mutations, for example (p.R776H) and (p.G719x), revealed the presence of both mutations within the same transcript, showing that the mutations were located in cis, on the same *EGFR* allele.[9] When *EGFR* with the (p.R776H) mutation is transfected into fibroblasts, the cells undergo transformation and exhibit increased proliferation, but only when the intracellular pH rises. The reduced protonation of histidine at residue 776 under alkaline conditions induces conformational changes in the alphaC helix, which could potentially stabilize the active form of the kinase.[18]

In vitro, the (p.R776H) mutation promotes the activation of EGFR in a dimerization-dependent manner, primarily augmenting the acceptor function rather than the donor function.[19]

Preclinical models in which *EGFR* with the (p.G719x) and (p.R776H) mutations were introduced into the BaF3 cell line exhibited sensitivity to gefitinib and erlotinib.[9]

Patients with metastatic lung adenocarcinomas carrying (p.R776x) mutations have received treatment with various TKIs, including gefitinib, erlotinib, afatinib, and osimertinib.[5,7-8,11,13-15] The results showed 6 partial responses, 3 stabilization of the disease, 1 progressive disease, and 1 case without a radiological evaluation of the response, with a median progression-free survival of 17.5 months (range 0.25-23 months). None of the patients with the germline (p.R776x) mutation of *EGFR* experienced abnormal toxicities to EGFR TKIs. A single patient with a lung adenocarcinoma, harboring the (p.R776H) mutation without additional *EGFR* mutations, was treated with osimertinib and achieved a partial response lasting more than 6 months.[11] The patient that progressed to gefitinib treatment had a (p.R776G) plus a (p.L858R) mutation of *EGFR*.[15] Anti-EGFR TKIs appear to be valuable treatment options for tumors with combined *EGFR* mutations, including those with a (p.R776H) germline missense mutation. First, second, and third generation TKIs have demonstrated anecdotal efficacy. In the presented case, progression occurred simultaneously in three nodules. As the histological characterization of the mutation was not feasible in all the progressing nodules, we opted for chemotherapy. Furthermore, re-assessment of circulating DNA did not reveal the presence of known activating *EGFR* mutations. The use of TKIs remains a valuable alternative for further progression.

Conclusion

This case presents several intriguing aspects. The presence of different *EGFR* mutations in the various resected nodules demonstrates the existence of multifocal primary tumors. The coexistence of multifocal primary tumors in a patient with the (p.R776H) germline mutation supports the idea of a hereditary syndrome predisposing to lung cancer. The presence of an *EGFR* mutation in all the resected nodules suggests the intriguing possibility that a germline mutation in an oncogene could facilitate the development of a tumor with additional mutations in the same oncogene.

Disclosure

The authors declare no potential conflicts of interest.

CRediT authorship contribution statement

Iacopo Petrini: Writing – original draft. **Rossella Bruno:** Methodology, Formal analysis. **Iosè Di Stefano:** Writing – original draft, Investigation. **Vittorio Aprile:** Methodology, Data curation. **Eleonora Pardini:** Writing – original draft. **Antonio Chella:** Writing – review & editing. **Greta Alì:** Writing – review & editing.

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