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CASE REPORT

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Next-generation sequencing clarified why first-line treatment with osimertinib was ineffective in an autopsied case of EGFR-mutated lung squamous cell carcinoma

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Abstract

Epidermal growth factor receptor (EGFR)-mutated squamous cell carcinoma (SCC) is less common than adenocarcinoma. The third-generation EGFR-tyrosine kinase inhibitor, osimertinib, is effective in EGFR-mutated lung adenocarcinoma, but its efficacy in EGFR-mutated lung SCC is unclear. The patient was an 83-year-old male. He was diagnosed with SCC of the lung, and molecular analysis revealed that the tumor was positive for EGFR exon19 deletion. He was treated with osimertinib 80 mg/day. No adverse events were observed, but after 18 days of therapy, he complained of dyspnea, and a computed tomography scan showed enlarged lung cancer. The case was categorized as a progressive disease. The patient died 3 weeks later. The autopsy findings confirmed the diagnosis of lung SCC, with morphology and immunohistochemical staining identical to the tumor obtained by bronchoscopy. Next-generation sequencing showed the presence of *TP53 R158L*, *CDK6*, and *KRAS* amplifications. The current case report shows that next-generation sequencing can explain why osimertinib is ineffective in EGFR-mutated SCC.

KEYWORDS

autopsy, EGFR mutation, next-generation sequencing, non-squamous cell lung cancer, osimertinib

INTRODUCTION

Epidermal growth factor receptor gene (EGFR) mutations are detected in lung adenocarcinomas in 47.9% of Asian and 19.2% of Western patients.[1] The EGFR-mutated lung squamous cell carcinoma (SCC) is less frequent. EGFR mutations are detected in lung SCC in 4.6% of Asian and 3.3% of Western patients.[1] Treatment with osimertinib is associated with better progression-free survival (PFS) and overall survival (OS) than first- and second-generation EGFR-tyrosine kinase inhibitors in non-small cell lung cancer patients.[2,3] However, the efficacy of osimertinib in lung SCC is unclear. There are only a few reports of treatment of lung SCC patients with osimertinib. Here, we report an autopsied case of lung SCC that was unresponsive to osimertinib as a first-line treatment. A next-generation sequencing (NGS) study provided clues to understanding why the tumor was unresponsive to osimertinib.

CASE REPORT

The patient was an 83-year-old male. He had a history of bronchial asthma, type 2 diabetes mellitus, and a smoking status of 30 pack-years. The Eastern Cooperative Oncology Group Performance Status (ECOG PS) was 0. A chest

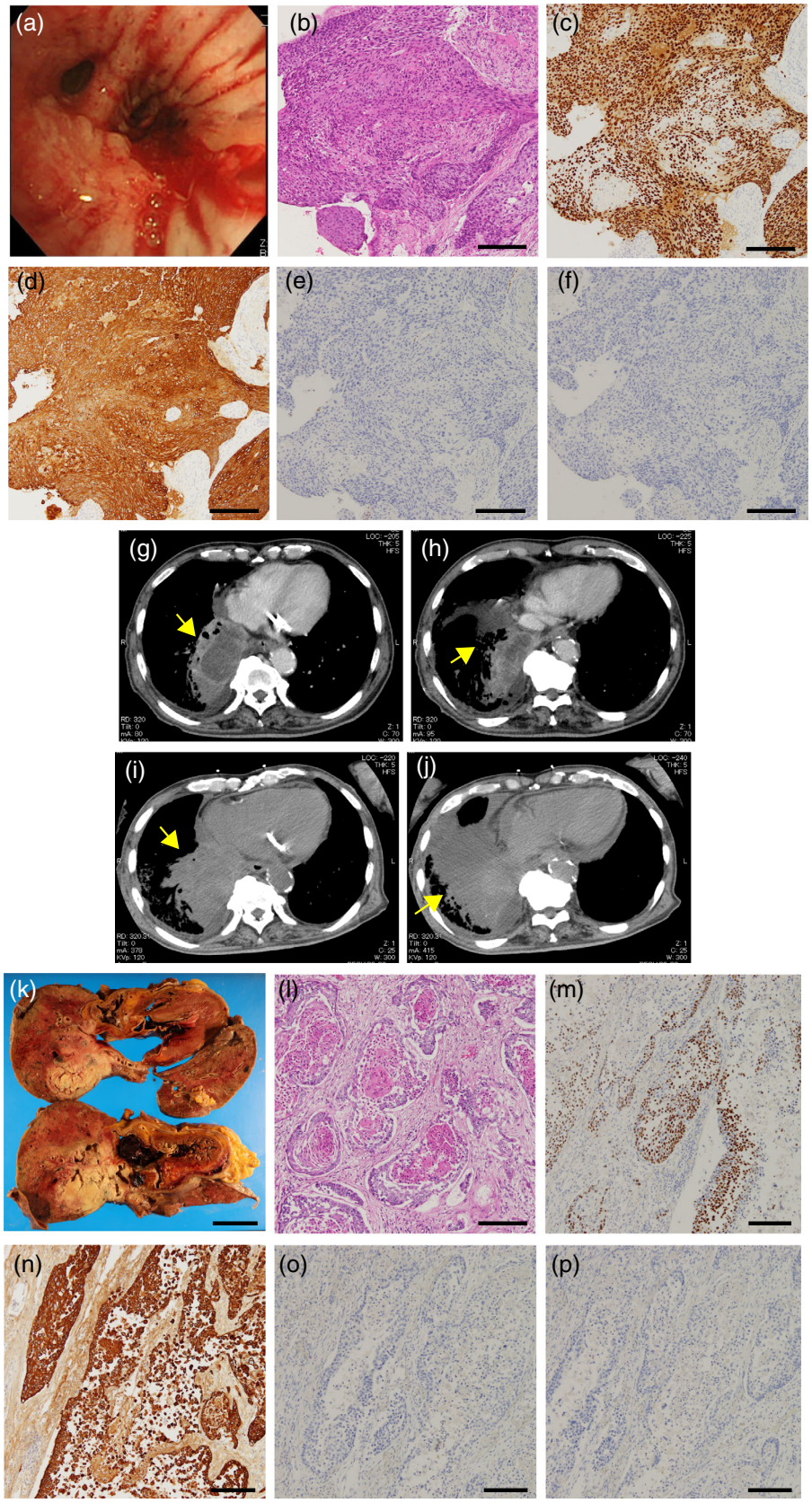

FIGURE 1 Bronchoscopy study, chest computed tomography scan and macroscopic examination of the lungs. The tumor was observed in the truncus intermedius (a). Morphological findings were suggestive of squamous cell carcinoma (b), and immunohistochemical staining revealed positive expression for p40 (c), and ck5/6 (d), and negative expression for TTF-1 (e) and napsin A (f). Computed tomography findings before (g), (h) and after osimertinib (i), (j). Tumor enlargement can be detected in the lower lobe of the right lung with increased pleural and pericardial effusion. Yellow arrows indicate the tumor. Pericardial invasion by the tumor (k). Morphological findings are suggestive of squamous cell carcinoma (l), and immunohistochemical staining revealed positive expression for p40 (m), ck5/6 (n), and negative expression for TTF-1 (o) and napsin A (p). Scale bar: 50 mm in panel a. Scale bar: 200 μm in panels (b), (c), (d), (e), (f), (l), (m), (n), (o), (p). TTF-1, thyroid transcription factor-1.

computed tomography (CT) revealed pleural effusion and a mass in the lower lobe of the right lung. The serum levels of tumor markers were as follows: carcinoembryonic antigen (CEA) 10.0 ng/mL, cytokeratin 19 fragment (CYFRA) 54.2 ng/mL, and SCC 5.7 ng/mL. Bronchoscopy with tumor biopsy was performed. A tumor was visible in the tracheal

TABLE 1 Somatic genetic mutations in tumor tissue detected by next-generation sequencing

Gene name	SMO	PTEN	IRS2	TP53	CDKN2A
Chromosome	chr.7	chr.10	chr.13	chr.17	chr.9
Genomic position	128850877	89720799	110435231	7578457	21970900
Reference name	A	TACT	C	C	C
Alternative name	T	–	T	A	T
Variant frequency	0.36015	0.26667	0.28826	0.32325	0.36313
Depth	261	180	281	529	358
Transcript ID	NM_005631.5	NM_000314.8	NM_003749.3	NM_000546.6	NM_000077.5
Function	Missense	Frameshift	Missense	Missense	Splice donor site
CDS change	c.1724A > T	c.950_953delTACT	c.3170G > A	c.473G > T	c.457 + 1G > A
Amino acid change	p.K575M	p.V317fs	p.G1057D	p.R158L	
Variant type	SNV	DEL	SNV	SNV	SNV
Cosmic IDs	COSV50827853	COSV64288418	COSV65478662	COSV52676395	COSV58690553

Abbreviations: CDKN2A, cyclin-dependent kinase inhibitor 2A; CDS, coding sequence; Cosmic, catalogue, of somatic mutations in cancer; IRS2, insulin receptor substrate-2; PTEN, phosphatase and tensin homolog deleted in chromosome 10; SMO, smoothened; TP53, tumor protein 53.

lumen (Figure 1(a)). The immunohistochemical studies revealed thyroid transcription factor-1 (−), napsin A (−), p40 (+), and cytokeratin 5/6 (+) (Figure 1(b)–(f)). The diagnosis was lung SCC. The clinical stage was cT4N2M1a (PLE) stage IVA. Molecular analysis revealed EGFR exon 19 deletion, T790M negative, and programmed death-1 with a tumor proportion score of <1%. [Correction added on 3 February 2023, after first online publication: in Case Report section, the first paragraph has been inserted.]

The patient refused treatment with cytotoxic agents. Therefore, osimertinib (80 mg/day) was indicated as first-line therapy. No adverse event was observed. However, 18 days after starting treatment with osimertinib, he consulted our emergency room for progressive dyspnea. A CT scan showed an enlarged lung cancer (Figure 1(g)–(j)). The diagnosis was disease progression. He, then, consented to receive only palliative treatment and died 3 weeks later. The patient's family agreed to perform an autopsy. The histopathological study of the autopsied samples confirmed the diagnosis of lung SCC without an adenocarcinoma component (Figure 1(k)–(p)). NGS (Illumina TruSight Oncology 500) of the autopsy specimens was performed. The results showed the following mutations (Table 1): cyclin-dependent kinase inhibitor 2A with splice site 457 + 1G > A, insulin receptor substrate-2 G1057D, phosphatase and tensin homolog deleted in chromosome 10 V317fs, smoothened K575M mutation, tumor protein 53 R158L, and the following gene amplifications (Table 2): fibroblast growth factor 10, cyclin-dependent kinase 6, fibroblast growth factor receptor 1, MYC, ret proto-oncogene, fibroblast growth factor 23, fibroblast growth factor 6, and KRAS.

TABLE 2 Amplifications of somatic genes in tumor tissue detected by next-generation sequencing

Genes	Fold change	Locus
FGF10	1.59	chr.5:44305095–44388786
CDK6	1.553	chr.7:92243233–92462639
FGFR1	1.489	chr.8:38271144–38326324
MYC	1.566	chr.8:128743614–128758574
RET	1.492	chr.10:43572705–43623989
FGF23	1.712	chr.12:4476292–4490788
FGF6	1.608	chr.12:4539807–4559806
KRAS	1.695	chr.12:25359177–25401358

Abbreviations: CDK6, cyclin-dependent kinase 6; FGF10, fibroblast growth factor 10; FGF23, fibroblast growth factor 23; FGF6, fibroblast growth factor 60; FGFR1, fibroblast growth factor receptor 1; RET, ret proto-oncogene.

DISCUSSION

PFS of 1.4 months and OS of 14.6 months have been reported in lung SCC patients treated with first-generation EGFR tyrosine kinase inhibitors.[4] This improvement in PFS and OS in lung SCC is worse than that reported in patients with lung adenocarcinoma.[4] Several reports have shown the efficacy of third-generation EGFR tyrosine-kinase inhibitors, including osimertinib, in lung SCC,[5–11] as described in Table 3. However, osimertinib was used as first-line therapy only in three cases.[10,11]

The poor clinical outcome of patients with SCC despite receiving molecular-targeted therapy has also been demonstrated in a previous study.[12] Multiple EGFR mutations may affect the efficacy of tyrosine-kinase inhibitors in SCC.[13] Cortiula et al.[6] reported a good response to osimertinib when they used NGS analysis as a guide to indicate this tyrosine kinase inhibitor in patients with EGFR-mutated lung SCC. This observation suggests the usefulness of the NGS technology as a guide to predict therapeutic response to tyrosine-kinase inhibitors in EGFR-mutated lung SCC.[6] Excellent reviews have been published on the mechanism of therapeutic resistance to osimertinib as first-line therapy.[14–18] Resistance to osimertinib may occur via bypass mechanisms and

TABLE 3 Reported cases of lung squamous cell carcinoma treated with osimertinib

First author, year	Timing of osimertinib	Age	Sex	Smoking status	EGFR mutation	Specimen	Stage	Resp
Bruno et al., 2017[5]	2nd line (SQT)	44	F	8 pack/year	Ex 19 del/T790M	TBB	IVB	PR
Okabe et al., 2017[9]	2nd line (SQT)	69	M	Former smoker	Ex 19 del/T790M	CTNB	IVA	PR
Izumi et al., 2017[7]	3rd line (SQT)	78	M	50 pack-year	Ex 21 L858R/T790M	CTNB	IB (Rec)	PR
Kong et al., 2018[8]	3rd line (SQT)	64	F	Never smoker	Ex 21 L858R/T790M	Unknown	IVA	PR
Cortiula et al., 2019[6]	2nd line	54	M	3 pack/year	Ex 19 del/T790M	LB	Unknown	PR
Shoji et al., 2020[11]	1st line	67	F	23 pack/year	Ex 19 del	Unknown	IIIB	PR
Shoji et al., 2020[11]	1st line	63	M	45 pack/year	Ex 21 L858R	Bone meta	IVB	PR
Peng et al., 2020[10]	1st line	50	M	10 pack/year	Ex 19 del/T790M	CTNB	IIIB	PR
Present case	1st line	83	M	30 pack/year	Ex 19 del	TBB	IVA	PD

Abbreviations: CTNB, computed tomography-navigated biopsy; EGFR, epidermal growth factor receptor; Ex, exon; F, female; LB, liquid biopsy; M, male; PD, progressive disease; PR, partial response; Resp, response; SQT, squamous cell carcinoma transformation; TBB, transbronchial biopsy.

activation of downstream pathways.[14] Genetic abnormalities detected by NGS, including *EGFR* mutations (*C797X*, *G796X*, *L92X*, *G724S*, *L7118Q*), amplifications (*MET*, *HER2*, *KRAS*, *NRAS*, *YES1*, *CDK*), and gene rearrangements (*RET*, *NTRK*, *ALK*, *BRAF*, *ROS1*, *FCFR3*) have been associated with resistance to osimertinib as first-line therapy.[14,17]

Here, we performed NGS to clarify why osimertinib was ineffective in EGFR-mutated lung SCC. The analysis revealed multiple genetic abnormalities, including *TP53* mutation and amplification of *CDK6* and *KRAS*, which have previously been associated with resistance to osimertinib.[15,16,18–20] These tumor cell-associated gene abnormalities might explain the ineffectiveness of osimertinib in our case. Intriguingly, EGFR mutations were not detected in the autopsy specimens. Biopsy specimen sampled by bronchoscopy is just a small portion of the entire tumor.[19] EGFR mutations were not detected in the autopsy specimens, probably because of previous treatment with osimertinib.Therefore, we believe that osimertinib was ineffective in our case because of other genetic abnormalities or bypass signals rather than EGFR gene alteration. This observation illustrates the importance of evaluating other gene mutations by NGS in addition to EGFR mutations.

Our patient is the first autopsied case of EGFR-mutated lung SCC treated with osimertinib. Previous studies reported a good therapeutic response to osimertinib in EGFR-mutated lung adenocarcinoma patients. However, only a partial response to osimertinib has been reported in EGFR-mutated lung SCC patients (Table 3). The explanation for this differential response is unclear. This report presented the first case of osimertinib-resistant EGFR-mutated SCC because of other gene abnormalities, including *TP53* mutation and amplifications of *CDK6* and *KRAS*, as demonstrated by NGS. We learned from the current case that NGS is warranted in EGFR-mutated SCC patients to identify additional targetable gene mutations and select more effective therapeutic agents.

In conclusion, the therapeutic strategy for patients with EGFR mutant-positive SCC should include an analysis of coexisting genetic abnormalities by NGS. Therapy with EGFR tyrosine kinase inhibitor should be recommended if no additional gene associated with resistance to tyrosine kinase inhibitor is detected. However, if a gene associated with resistance to therapy is detected, we believe that chemotherapy alone or combined with immune checkpoint inhibitors should be indicated.

AUTHOR CONTRIBUTIONS

Conceptualization and study design: Tadashi Nishimura and Hidenori Ibata. *Resources*: Hirotoshi Tarumi, Chikashi Tsuji, Soichi Iwanaka, Yasumasa Sakakura, Masahiro Naito, Hidenori Ibata, and Yoshinaga Okugawa. *Interpretation of the data*: Hajime Fujimoto, Hidenori Ibata, Tadashi Nishimura, Tetsu Kobayashi, Takumi Fujiwara, and Esteban Cesar Gabazza. *Original draft of the manuscript*: Tadashi Nishimura, Takumi Fujiwara, and Hajime Fujimoto. *Review and editing of the manuscript*: Yoshinaga Okugawa, Taro Yasuma, Tetsu Kobayashi, and Esteban Cesar Gabazza. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declared no conflicts of interest regarding this study.

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