Transformation of epidermal growth factor receptor T790M mutation-positive adenosquamous carcinoma of the lung to small cell carcinoma and large-cell neuroendocrine carcinoma following osimertinib therapy: an autopsy case report

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Keywords
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Abstract
A 59-year-old man with relapsed epidermal growth factor receptor (EGFR) exon 19 deletion-positive stage IA adenosquamous carcinoma after lobectomy was treated with erlotinib and bevacizumab for 1 year followed by erlotinib alone for 1 year. Because of mediastinal and supraclavicular lymphadenopathy and a nodule on the left anterior chest wall, the patient underwent repeat biopsy from the supraclavicular lymph node. Pathological analysis demonstrated adenosquamous carcinoma harbouring EGFR exon 19 deletion and T790M mutation. Osimertinib treatment was therefore started. Six months later, the patient underwent a second-repeat biopsy from the mediastinal lymph nodes and liver metastases. Both specimens showed small cell lung carcinoma (SCLC). After chemotherapies for SCLC, he died from lung cancer. An autopsy demonstrated tumour heterogeneity, including histological types of adenosquamous, SCLC, and large-cell neuroendocrine carcinoma. Repeat biopsies at the time of disease progression are useful to choose subsequent treatment for patients with EGFR mutation-positive lung cancer.

Introduction
Resistance mechanisms to osimertinib are of two types: epidermal growth factor receptor (EGFR) dependent, such as point mutations containing C797S, and EGFR independent, such as bypass signal pathway or histological transformation to small-cell lung carcinoma (SCLC) and large-cell neuroendocrine carcinoma (LCNEC) [1,2]. Here, we report an autopsy case with EGFR T790M-positive adenosquamous carcinoma that transformed to EGFR T790M-negative SCLC and LCNEC after osimertinib therapy.

Case Report
A 59-year-old man underwent video-assisted thoracic surgery of the left upper lobe with ND2a-1 lymphadenectomy 4 years prior to disease presentation and was diagnosed as having stage IA (T1bN0M0) adenosquamous carcinoma of the lung (Fig. 1A–C) harbouring EGFR exon 19 deletion based on the American Joint Committee on Cancer staging system, seventh edition. He was a non-smoker with no previous medical problems. Two years after the surgery, he was diagnosed as having recurrence in the mediastinal lymph nodes and left anterior chest wall. Erlotinib
(150 mg once daily) and bevacizumab (15 mg/kg every 3 weeks) were started as first-line therapy. After 19 months of the therapy, the lung cancer also metastasized to the right supraclavicular lymph node. He underwent percutaneous supraclavicular lymph node needle biopsy as the first-repeat biopsy. Adenosquamous carcinoma harbouring EGFR exon 19 deletion and T790M mutation was detected. Osimertinib (80 mg once daily) was started, and the patient achieved partial response. Seven months after starting treatment with osimertinib, the mediastinal lymphadenopathy recurred, and multiple new liver metastases were seen. The second-repeat biopsy for the subcarinal mediastinal lymph nodes was performed. The histological diagnosis was SCLC harbouring EGFR exon 19 deletion without T790M mutation (Fig. 1D–F). He was treated with combination chemotherapy of cisplatin (80 mg/m², day 1, every

Figure 1. Histology of lung surgical specimen (A–C); (A) haematoxylin and eosin, (B) TTF-1 and CK5/6, and (C) p40 and napsin stains. Histology of endobronchial ultrasound-guided transbronchial needle aspiration specimens from mediastinal lymph nodes (D–F); (D) Haematoxylin and eosin, (E) synaptophysin, and (F) CD56 stains. Histology of liver biopsy (G–I), (G) Haematoxylin and eosin, (H) synaptophysin, and (I) CD56 stains.
3 weeks) and etoposide (100 mg/m^2, days 1–3, every 3 weeks). After one cycle of chemotherapy, computed tomography (CT) imaging demonstrated that the mediastinal lymph nodes had shrunk, but the hepatic metastases had disseminated. Fine-needle aspiration biopsy of the liver was performed, and the histological diagnosis was also SCLC harbouring EGFR exon 19 deletion without T790M mutation (Fig. 1G–I). After four cycles of chemotherapy, the size of the mediastinal lymph nodes further decreased, but the hepatic metastases increased.

Two months later, liver metastases became worse, and new lesions of the bone metastases at spine developed. We administered amrubicin (35 mg/m^2, 75% on days 1–3, every 3 weeks) for the next treatment. However, the treatment was changed to irinotecan (100 mg/m^2, 75% on day 1, 8, and 15 every 4 weeks) after one cycle of amrubicin.

Figure 2. Histology of autopsy (A–H); (A) haematoxylin and eosin staining of adenosquamous carcinoma component, (B) TTF-1 and CK5/6 stains, (C) p40 and napsin stains, (D) haematoxylin and eosin staining of small cell carcinoma component, (E) haematoxylin and eosin staining of large-cell neuroendocrine carcinoma component, (F) TTF-1 stain, (G) synaptophysin stain, and (H) CD56 stain.
Table 1. Previous reports with transformation of non-small-cell lung adenocarcinoma to small-cell lung carcinoma (SCLC) and large-cell neuroendocrine carcinoma (LCNEC) following osimertinib therapy.

|   | Gender | Age | Stage     | Histology at diagnosis | EGFR status at diagnosis | EGFR-TKIs before first re-biopsy | Detection | Best response with osimertinib | Response duration (months) | Histology after second re-biopsy | EGFR status after second re-biopsy | Treatment |
|---|--------|-----|-----------|------------------------|--------------------------|---------------------------------|----------|-------------------------------|-----------------------------|-------------------------------|----------------------------------|-----------|
| 1 | Kim et al. [6] | Female | 57 | ND | Ad | 19 del | Gefitinib | 19 del + T790M + | Tissue | PR | 11 | SCLC | 19 del + T790M + | Radiation |
| 2 | Ham et al. [7] | Female | 57 | cT3N2M1a IVa | Ad | L858R | Erlotinib | L858R + T790M + | Tissue | PR | 14 | SCLC | L858R + T790M - | CBDCA + VP16 |
| 3 | Ham et al. [7] | Female | 58 | Recurrent | Ad | Wild | Erlotinib | Afatinib | 19 del + T790M + | Tissue | PR | 18 | SCLC | 19 del + T790M - | CBDCA + VP16 |
| 4 | Li et al. [8] | Female | 52 | Recurrent | Ad | 19 del | Erlotinib | 19 del - T790M - | Plasma | PR | 6 | SCLC | 19 del + T790M - | CBDCA + VP16 + osimertinib |
| 5 | Minari et al. [9] | Male | 69 | ND | Ad | 19 del | Afatinib | 19 del + T790M + | Plasma | PD | 3 | SCLC | 19 del - T790M - | BSC |
| 6 | Minari et al. [9] | Female | 70 | ND | Ad | 19 del | Afatinib | 19 del + T790M + | Plasma | PD | 2 | SCLC | 19 del + T790M - | BSC |
| 7 | Minari et al. [9] | Male | 55 | ND | Ad | 19 del | Gefitinib | 19 del + T790M + | Plasma | PD | 3 | SCLC | 19 del + T790M - | CBDCA + VP16 |
| 8 | Minari et al. [9] | Male | 68 | ND | Ad | L858R | Afatinib | L858R + T790M + | Plasma | PD | 3 | SCLC | L858R + T790M - | CBDCA + VP16 |
| 9 | Taniguchi et al. [10] | Female | 67 | Recurrent | Ad | 19 del | Gefitinib | Erlotinib | L858R + T790M + | Tissue | ND | 13 | SCLC | 19 del + T790M - | CBDCA + VP16 |
| 10 | Baglivo S, et al. [11] | Male | 57 | cT1bN2M1ab IVb | Ad | 19 del | Erlotinib | 19 del + T790M + | Tissue | I | PD | LCNEC | 19 del + T790M - | Radiation, Adrenalectomy, CDDP+VP16 |
| 11 | Ricordel et al. [12] | Male | 57 | cT3N2M1b IVb | Ad | 19 del | Gefitinib | 19 del + T790M + | Tissue | SD | 10 | LCNEC | 19 del + T790M + | Radiation, Osimertinib |
| This case | Male | 63 | Recurrent | Ad-Sq | 19 del | Erlotinib | 19 del + T790M + | Tissue | PR | 7 | SCLC and LCNEC | 19 del + T790M - | CDDP+VP16 |

19 del, exon 19 deletion; Ad, adenocarcinoma; Ad-Sq, adenosquamous cell carcinoma; BSC, best supportive care; CBDCA, carboplatin; CDDP, cisplatin; L858R, L858R point mutation; ND, not described; PD, progressive disease; PR, partial response; SD, stable disease; T790M, T790M point mutation; VP16, etoposide.
Discussion
We report a patient with EGFR T790M-positive adenosquamous carcinoma that transformed to EGFR T790M-negative SCLC and LCNEC after treatment with osimertinib. EGFR exon 19 deletion was detected in both adenosquamous carcinoma and SCLC.

SCLC transformation following treatment with first- and second-generation EGFR-tyrosine kinase inhibitors (TKIs) has been reported in 3–15% of patients [2,3]. As in cases of adenocarcinoma changing to SCLC, the inactivation of retinoblastoma 1 (RB1) and tumour protein 53 (TP53) were also reported [4,5].

SCLC transformation due to osimertinib is rare, and its frequency is still unknown. To our knowledge, only nine cases of SCLC transformation after osimertinib treatment have been reported [6–10] (Table 1).

In the autopsy findings of this case, LCNEC was also found along with SCLC. Several cases with transformation from adenocarcinoma to LCNEC were reported as a resistant mechanism to the first- and second-generation EGFR-TKIs along with two cases to osimertinib (Table 1) [11,12]. LCNEC was a very heterogeneous group. A next-generation sequencing (NGS) study of LCNEC from the Memorial Sloan Kettering Cancer Center showed that 18 of 45 LCNEC cases had SCLC-like molecular profile, but 25 had non-small cell lung carcinoma (NSCLC)-like molecular profile [13]. These data made sense to us because LCNEC coexisted in our case, transforming from adenosquamous carcinoma to SCLC.

Several studies showed heterogeneity of acquired resistance to EGFR-TKIs. Because the heterogeneity of expression of immune markers depends on the metastatic sites and histological transformation, and the biopsy of one lesion may not represent the immune marker status of all lesions [14]. In this case, we conducted second-repeat biopsies from two metastatic sites (mediastinal lymph nodes and liver), which resulted in an observation of SCLC with EGFR exon 19 deletion without T790M mutation. However, the results on both biopsies were a little different from autopsy findings, a strange mix of SCLC, LCNEC, and adenocarcinoma. This result showed limitation of small biopsies and usefulness of repeated biopsies from different sites to accumulate information for a clinical decision.

Identifying the histological diagnosis and driver mutations is important in deciding treatment options. Given the various mechanisms of acquired resistance, repeat biopsy at the time of disease progression is useful to guide the direction of subsequent treatment in patients with EGFR mutation-positive lung cancer.

Disclosure Statement
Appropriate written informed consent was obtained for publication of this case report and accompanying images.

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