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Case Report

Two Cases of ALK-Altered Cancers of Unknown Primary Diagnosed by Immunohistochemistry

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Cancers of unknown primary · Immunohistochemistry · ALK inhibitor

Abstract
Cancer of unknown primary (CUP) accounts for 5% of all malignancies. Patients with CUP may live averagely for 8 months after diagnosis, and thus, rapid and reasonable diagnosis is necessary. Among patients with CUP, anaplastic lymphoma kinase (ALK)-overexpressing CUPs, whose primary sites were confirmed to be the lungs (Lung-CUP) by using antibodies against cytokeratin 7, thyroid transcription factor-1, and Napsin A, along with clinical characteristics progressed rapidly and were very sensitive to the ALK inhibitor alectinib. The incidence of ALK alteration in Lung-CUP is 19%. Consequently, it is advised that Lung-CUP be examined by immunohistochemistry (IHC) with an anti-ALK antibody. Alternative examinations, such as a cancer genome test, require as much as 2 months to complete, whereas IHC can be completed within days. In this report, a rapid assessment by IHC led to alectinib treatment, which resulted in good outcomes in 2 cases of Lung-CUP. Alectinib was effective for ALK-altered Lung-CUPs.

Introduction

Cancer of unknown primary (CUP) is defined as a metastatic cancer without an identifiable primary lesion despite standard diagnostic measures. CUP accounts for 1–5% of all malignancies [1]. The primary sites cannot be detected even during autopsy in ~70% of cases [2].

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Because the prognosis of patients with CUP is poor and the median survival time of patients is only 6–9 months, it is not beneficial to spend substantial amounts of time to determine the primary site [2].

Systemic chemotherapy is the standard of care for CUP. Adenocarcinoma is the most frequent CUP. The diagnosis of the primary lesion is very helpful for selecting an appropriate chemotherapy. For this purpose, immunohistochemistry (IHC) is useful because, in addition to clinical information, it could be used to speculate the origin of the CUP in a matter of days [3].

Lung adenocarcinoma is reported to be detected in ~10% of CUP [4]. Lung adenocarcinoma is determined by IHC using antibodies against cytokeratin 7 (CK-7), thyroid transcription factor-1 (TTF-1), and Napsin A [3, 5, 6], along with clinical characteristics, including age over 50 years in males with mediastinal and/or cervical lymph node metastases [3].

Among CUPs whose origin is speculated to be the lung (Lung-CUPs), an anaplastic lymphoma kinase (ALK) alteration, was more frequently detected in 4 out of 21 cases (19.0%) [4], compared with the ALK alterations in the general nonsmall-cell lung cancers (NSCLCs) (6.7%) [7]. Alectinib, an ALK inhibitor, is a highly selective tyrosine kinase inhibitor of ALK that is approved for the first-line treatment of ALK-positive NSCLC [7, 8]. The response rate of alectinib was 83%, and the median survival time was 18.6 months [7] compared with 7.9 months with conventional chemotherapies [9]. Consequently, it is beneficial to investigate *ALK* alteration rapidly in Lung-CUPs. ALK overexpression is examined by ICH, fluorescence in situ hybridization, or rapid fluorescence in situ hybridization [10]. CUP is also a good target for cancer genome analysis, but it is time-consuming and takes ~2 months to be completed [11]. In this report, immediate alectinib administration for ALK-altered Lung-CUPs helped the patients' survival.

Case Report/Case Presentation

Case 1

A 44-year-old male patient had a palpable, hard mass in his left supraclavicular region in early September 2017 (Fig. 1). Subsequently, he experienced a persistent wet cough, at which point, he visited a local doctor. Computed tomography (CT) revealed lymph node swelling of

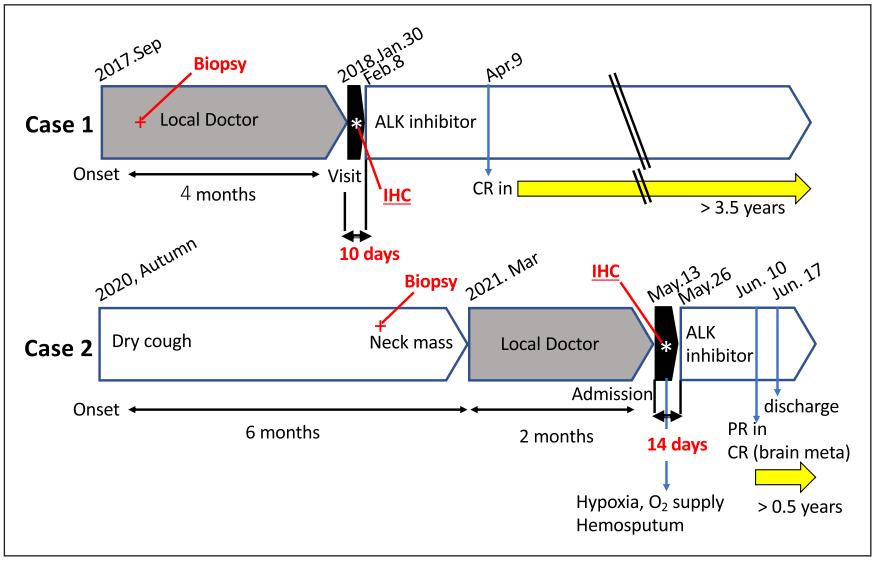

Fig. 1. Overview of the treatment courses.

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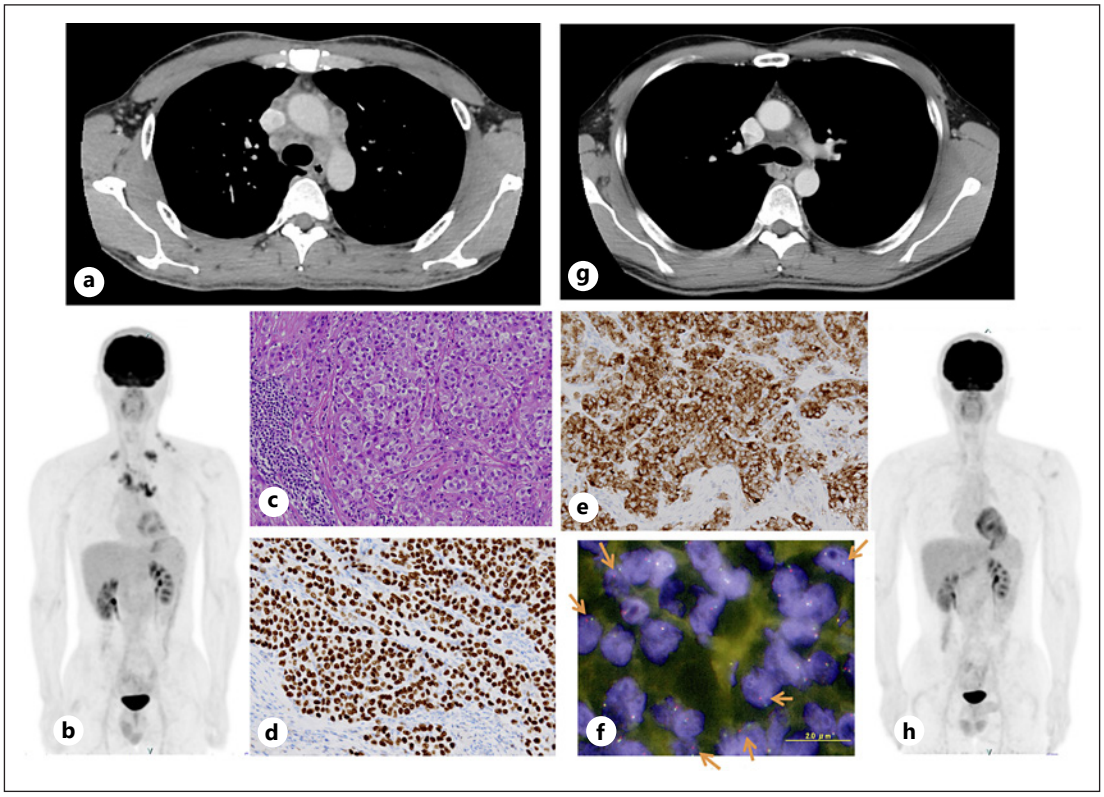

Fig. 2. Clinical features of case 1. **a** CT imaging of the mediastinal lymph node. **b** PET-CT imaging, frontal section. **c** H&E staining of the cervical lymph node. **d** IHC with an anti-TTF-1 antibody. **e** IHC with an anti-ALK antibody. **f** FISH imaging of ALK. The red and green fluorescence probes hybridize to the ALK gene. The breaking apart of these fluorescence signals, as represented by the orange arrows, indicates rearrangement. **g** Posttreatment CT imaging. **h** Posttreatment PET-CT imaging. FISH, fluorescence in situ hybridization.

the bilateral cervical, bilateral supraclavicular, mediastinal, and left pulmonary hilar regions (Fig. 2a). Positron emission tomography (PET)-CT also revealed tracer uptake in the same lesions (Fig. 2b). Histopathological analysis of the biopsy of the left cervical lymph nodes revealed a carcinoma not otherwise specified (Fig. 2c). No cancerous lesions were observed in his upper or lower gastrointestinal tract. Sputum cytology was negative. On January 30, 2018, he was referred to our department (Fig. 1). Laboratory data including the levels of tumor markers, including CEA, CA19-9, SCC, PSA, ProGRP, and NSE, were normal. Considering the clinical features, lung cancer was highly suspected. We performed IHC on his lymph node specimen using monoclonal antibodies against CK-7, TTF-1, and Napsin A, and the tissue was stained positive for all markers (Fig. 2d, e). The primary site was speculated to be the lung. Epidermal growth factor receptor (*EGFR*) mutation was negative. ALK overexpression was observed by IHC and rapid fluorescence in situ hybridization (Fig. 2e, f). Consequently, alectinib (600 mg/day) was administrated on February 8, 2018 (Fig. 1). On February 23, 2018, CT indicated a partial response (PR) in all targets, and on April 9, 2018, complete response (CR) was achieved (Fig. 1, 2g). CR has been maintained for 3 years and 4 months until now (Fig. 1, 2h) with no severe adverse events.

Case 2

A 57-year-old male patient experienced a dry cough in autumn 2020, with the sensation of a mass in the left side of his neck in February 2021 (Fig. 1). In March, he experienced hemosputum and visited his local doctor, who discovered an enlarged lymph node with poor mobility in

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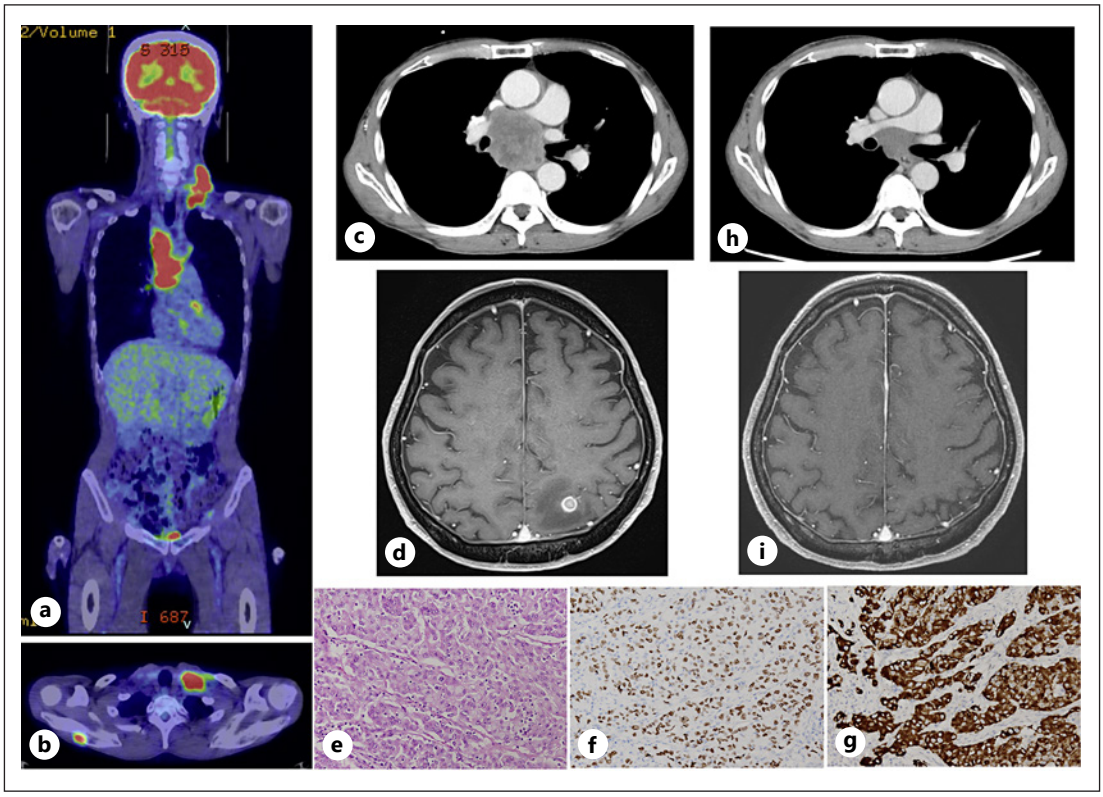

Fig. 3. Clinical features of case 2. **a** PET-CT imaging, frontal section. **b** Bone metastasis of the right scapula. **c** CT imaging of the mediastinal lymph node metastasis. **d** MRI imaging of the brain metastasis. **e** H&E staining of the cervical lymph node metastasis. **f** IHC with an anti-TTF-1 antibody. **g** IHC with an anti-ALK antibody. **h** CT imaging after alectinib treatment (day 16). **i** MRI imaging after alectinib treatment (day 23).

his left supraclavicular fossa. CT performed on April 19, 2021, indicated swelling of multiple lymph nodes from his cervical to mediastinal regions; however, no primary tumors were detected. The lymph nodes in the left side of his neck were 2–3 cm in diameter, whereas the lymph node in the mediastinum was 5 cm and had invaded the trachea. Histopathological analysis of the biopsy of the left cervical lymph node revealed poorly differentiated adenocarcinoma. On May 13, he was referred to the Department of Esophageal Surgery at our hospital, but upper gastrointestinal endoscopy showed no lesions in the esophagus (Fig. 1). The PET-CT showed tracer uptake in the left parietal lobe of the brain, right scapula, right fourth rib, right iliopsoas muscle, bilateral adrenal glands, and multiple lymph nodes from the cervical to the mediastinal regions in addition to those in the left outer iliac and the left inguinal regions (Fig. 3a, b). On May 17, he was admitted for the emission of a large amount of hemosputum (Fig. 1). CT revealed tumors corresponding to those observed on PET-CT, but no primary sites were detected (Fig. 3c). Magnetic resonance imaging (MRI) also revealed a 9-mm brain metastasis with edema (Fig. 3d). He also experienced dyspnea and the percutaneous oxygen saturation decreased to 88%. Consequently, oxygen therapy (O_2: 2 L/min) was initiated on May 21 (Fig. 1). The surgeons consulted with us, and IHC was conducted with CK-7, TTF-1, Napsin A, and ALK monoclonal antibodies (shown in Fig. 3e–g). CK-7 and TTF-1 were positive. ALK overexpression was also observed (Fig. 3g), and alectinib (600 mg/day) was initiated on May 26 before *EGFR* mutation data were obtained because *ALK* alterations and *EGFR* mutations are mutually exclusive. Later, no *EGFR* mutation was detected. After alectinib administration, his condition rapidly improved, and on day 4, oxygen therapy was unnecessary,

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and hemosputum improved. On June 10 (day 16), the brain metastasis was undetectable by CT, and all other targets had shrunken (Fig. 3h). MRI indicated that the brain metastasis had decreased in size from 9 to 4 mm by day 20. On June 17 (day 23), he was discharged (Fig. 1). On August 4, 2021, the levels of CEA and CA19-9 were normalized (from 10.2 to 4.7 ng/mL and from 43.2 to 8.7 U/mL, respectively), and MRI indicated that the brain metastasis had decreased to 1 mm in size (Fig. 3i). No adverse events have been observed thus far.

Discussion/Conclusion

In conclusion, *ALK* alterations are found 20 times more frequently (19%) in Lung-CUPs. The diagnosis of ALK alteration by IHC could be completed within 2 weeks. IHC could be used to speculate the origin of the CUP, as shown in the clinical guidelines [3]. TTF-1 and CK-7 are 88% and 100% specific, and 100% and 28.5% sensitive for lung cancer, respectively [5]. Napsin A is 96% specific and 65% sensitive for lung adenocarcinoma [6].

In a previous study, 4 Lung-CUP cases had *ALK* alterations (4/21 = 19.0%) [4], whereas *ALK* alterations generally account for 6.7% of NSCLCs [7]. Further, although *ALK* alterations were detected in 1% of 200 cases or in 0.7% of 303 cases of the general CUPs [12, 13], *ALK* alterations might be more frequent in Lung-CUP. Practically, we experienced 2 cases of Lung-CUP with *ALK* alterations over a 4-year period, during which we treated 32 cases of CUPs.

Case 1 achieved CR, and case 2 rapidly achieved overall PR with almost CR of the brain metastasis. These results are consistent with the trial outcomes of alectinib for ALK-positive NSCLC, where CR was 4% and PR was 79%, and the central nervous system CR was 38% [8]. In the literature, the effect of alectinib has been reported in only one among 4 Lung-CUP cases [4]. We describe another 2 cases in detail.

The advantage of ICH is its short diagnostic time. Cancer genome tests might be useful for these purposes. NSCLC could be predicted in 37 of 310 patients with CUP (12%) using the 92-gene molecular cancer classifier assay [4]. Two randomized trials indicated that the therapeutic outcomes according to RNA sequencing-based assay were not superior to those with empiric chemotherapy [14, 15]. Consequently, cancer genome tests are not recommended for the prediction of the primary site of CUP in Japan.

In several clinical guidelines, cancer genome tests are recommended to detect the molecular targets of CUP [3]. At least one gene alteration was found in 96% of CUP using the hybrid-capture-based assay approved in Japan [12]. However, this assay is time-consuming. Furthermore, ALK inhibitors are not approved for CUP in Japan. Concerning case 1, the cancer genome test was not approved at that time. In case 2, we had insufficient time to do the cancer genome test due to the rapidly worsening clinical condition of the patient. Although further investigation should be planned, it is recommended to test ALK alteration by IHC for Lung-CUP practically.

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Statement of Ethics

The Certified Clinical Research Review Board, Akita University, acknowledged this study, and ethical approval was not required. Written informed consent was obtained from each patient for publication of this case report and any accompanying images.

Conflict of Interest Statement

The authors have no conflict of interest relevant to this report to declare.

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Author Contributions

Kato Y., Shimazu K., Fukuda K., Yoshida T., Taguchi D., and Shinozaki H. treated the patients. Nanjyo H. performed histopathological analysis. Shibata H. overviewed this study.

Data Availability Statement

All data in this study are included in this article. Further inquiries can be directed to the corresponding author.

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