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CASE REPORT

Case Report: Structurally Rare *EML4-ALK* Identified by Next Generation Sequencing in a Patient with NSCLC with Bilateral Ovarian Metastases

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Abstract: The *EML4-ALK* oncogene is a fusion of the *EML4* and *ALK* genes and is found in approximately 5–6% of the cases of non-small cell lung cancer (NSCLC). Herein, we present a unique case of lung adenocarcinoma with metastases to the bilateral ovaries harboring a rare *EML4-ALK* fusion gene variant in a 52-year-old patient. The patient had initially received a diagnosis of ovarian cancer, then had undergone neo-adjuvant chemotherapy followed by a surgical resection. Despite two cycles of adjuvant chemotherapy consisting of carboplatin and gemcitabine, CT revealed that the pleural effusion had increased from it before chemotherapy, and the shortness of breath worsened. Molecular profiling revealed an *EML4-ALK* rearrangement containing *ALK -EML4* and *ALK -NPR2* fusion genes. The diagnosis was changed to primary lung adenocarcinoma with metastases to the bilateral ovaries based on a pathological reevaluation. Treatment with alectinib, a second-generation ALK-tyrosine kinase inhibitor, led to a partial response of 18 months' duration, and the shortness of breath improved. No adverse events related to the alectinib therapy occurred. To assess the unique structure of the fusion genes, RNA sequencing was performed. An intronic sequence from both *ALK* and *EML4* was found between *ALK* and *EML4* exon, possibly because of an unusual insertion of a gene fragment derived from *NRP2*, indicated by the panel sequencing results. Variations in the drug response among *EML4-ALK* fusion variants highlight the importance of understanding their molecular structure. Further investigation is warranted to refine fusion gene detection methods and assess the therapeutic implications of rare fusion variants.

Keywords: ALK, lung adenocarcinoma, metastatic ovarian tumors, RNA sequencing, next-generation sequencing, case report

Introduction

Anaplastic lymphoma kinase (*ALK*) fusion is a genetic alteration occurring in approximately 5–6% of the cases of advanced non-small cell lung cancer (NSCLC).[1] The *ALK* gene, which encodes a transmembrane tyrosine kinase receptor, fuses with various partner genes, *EML4* being the most common of these. The frequency of *EML4-ALK* in the Japanese population is reportedly 4.35%. This fusion leads to the production of an oncogenic fusion protein that drives the development of NSCLC. Several clinical trials have demonstrated the efficacy of ALK-tyrosine kinase inhibitor (TKI) against *EML4-ALK*-positive NSCLC. Currently, several methods are available for detecting an ALK fusion, including fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR), and next-generation sequencing (NGS). FISH and IHC are most commonly used to detect *EML4-ALK* fusions in clinical trials and in the real world setting.[2] NGS, the newest modality, has enabled the identification of various *ALK* fusions by breakpoints on the *EML4* gene. However, there is, as of yet, no established method of detecting fusion gene variants before initiating treatment.

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We herein report a case of lung adenocarcinoma with metastases to the bilateral ovaries harboring a EML4*ALK* fusion gene with rare structure detected by RNA sequencing.

The patient provided written consent for the publication of their case report, and ethical approval was waived since this is a case report of a single case.

Case Presentation

Clinical Course

A 52-year-old, female patient was referred to the Department of Gynecology at the study center for lower abdominal distention. Computed tomography (CT) revealed bilateral ovarian tumors, ascites, lung nodules, right-sided pleural effusion, and multiple hepatic masses (Figure 1).

She was a never-smoker and had no family history or past medical history of a malignancy but did have a history of uterine fibroid and Hashimoto's thyroiditis. Based on a clinical diagnosis of ovarian cancer with metastasis to the lung

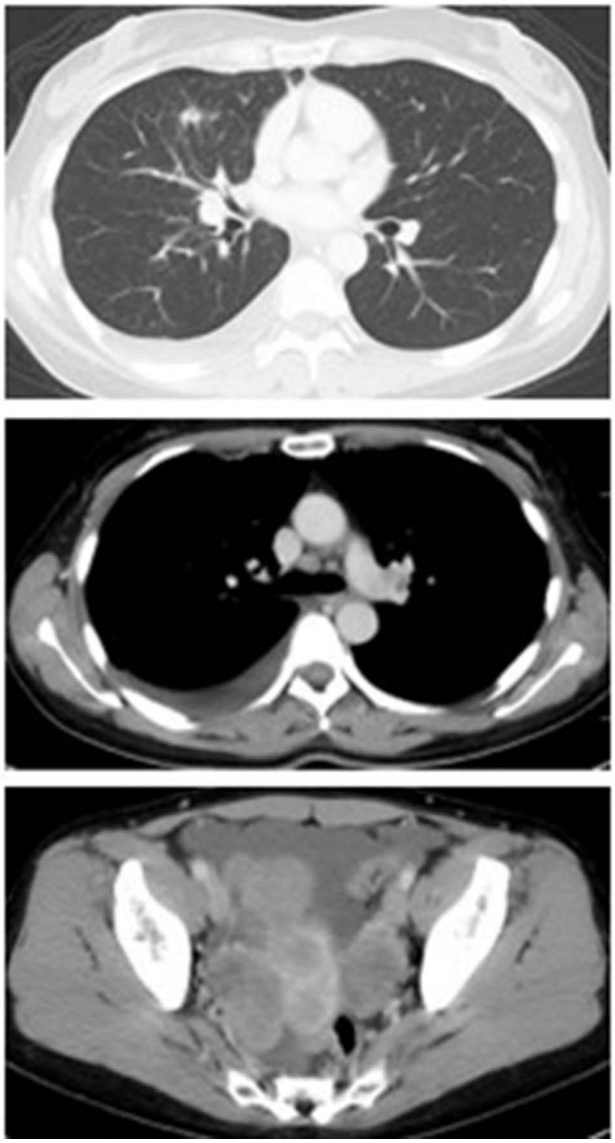

Figure 1 Preoperative CT. Upper: Nodule in the middle lobe of the lung, Middle: Pleural effusion and mediastinal lymphadenopathy, Lower: Multiple ovarian masses.

and liver, neo-adjuvant chemotherapy with carboplatin and paclitaxel was administered, after which a total hysterectomy, adnexectomy, epiploectomy, and peritoneal biopsy were performed.

Grossly nodular lesions were observed clustering on the bilateral ovaries, the surface of which was uneven. Pathologically, the right ovary demonstrated a proliferation of tumor cells with chromatin-enriched, irregularly round to spindle-shaped nuclei and acidophilic cytoplasm with varied histology as well as small, dilated or cord-like glandular ducts, some of which had a targetoid appearance with intracytoplasmic luminal secretions and dense tumor cell nests with abundant mucous granules in the cytoplasm (Figure 2a). Two cycles of carboplatin and gemcitabine therapy were administered as adjuvant chemotherapy. However, the pleural effusion increase observed on CT suggested that the chemotherapy had not been effective. Cancerous pleurisy was diagnosed clinically. Neither thoracentesis nor cytology was performed, but next-generation sequencing was done to identify the gene variant.

Molecular profiling, Foundation One CDx, found *EML4-ALK* chimeric gene with multiple rearrangement. An expert panel consisting of clinicians specializing in oncology and genetic medicine, a genetic counselor, pathologists, and a bio-informatician pointed out the possibility of an atypical *ALK* fusion expression. The pathologists therefore histologically re-analyzed the ovarian lesion. Immunostaining was positive for ALK, napsin A, and TTF-1 and negative for progesterone receptor, estrogen receptor, and PAX8. These findings indicated lung adenocarcinoma (Figure 2b).

There are very few reports of primary ovarian cancer with an *ALK* fusion gene,[3] and most are of high-grade serous carcinoma or clear cell carcinoma with partner genes other than *EML4*. In the present case, the diagnosis was changed to primary lung adenocarcinoma with metastases to the bilateral ovaries harboring an *EML4-ALK* fusion gene. Based on the expert panel's recommendation to administer ALK-TKI therapy, alectinib, a second generation ALK-TKI, was chosen. Three months after the start of the therapy, the primary and metastatic lesions shrank, and the pleural effusion decreased (Figure 3), and a partial response was achieved and maintained for 18 months.

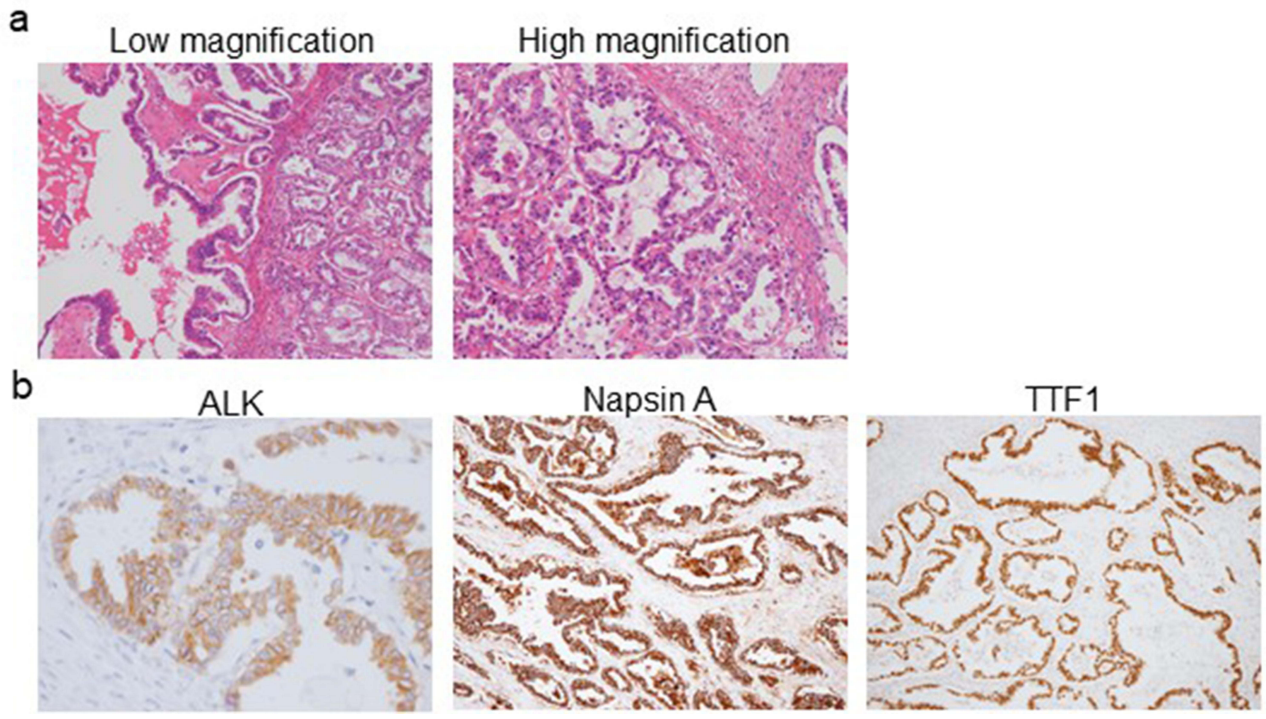

Figure 2 Pathological specimen of the ovarian tumors. (**a**) Hematoxylin-eosin staining (left: low power; right: high power), (**b**) Left: ALK staining (positive), Middle: Napsin A staining (positive), Right: Thyroid transcription factor-1 staining (positive).

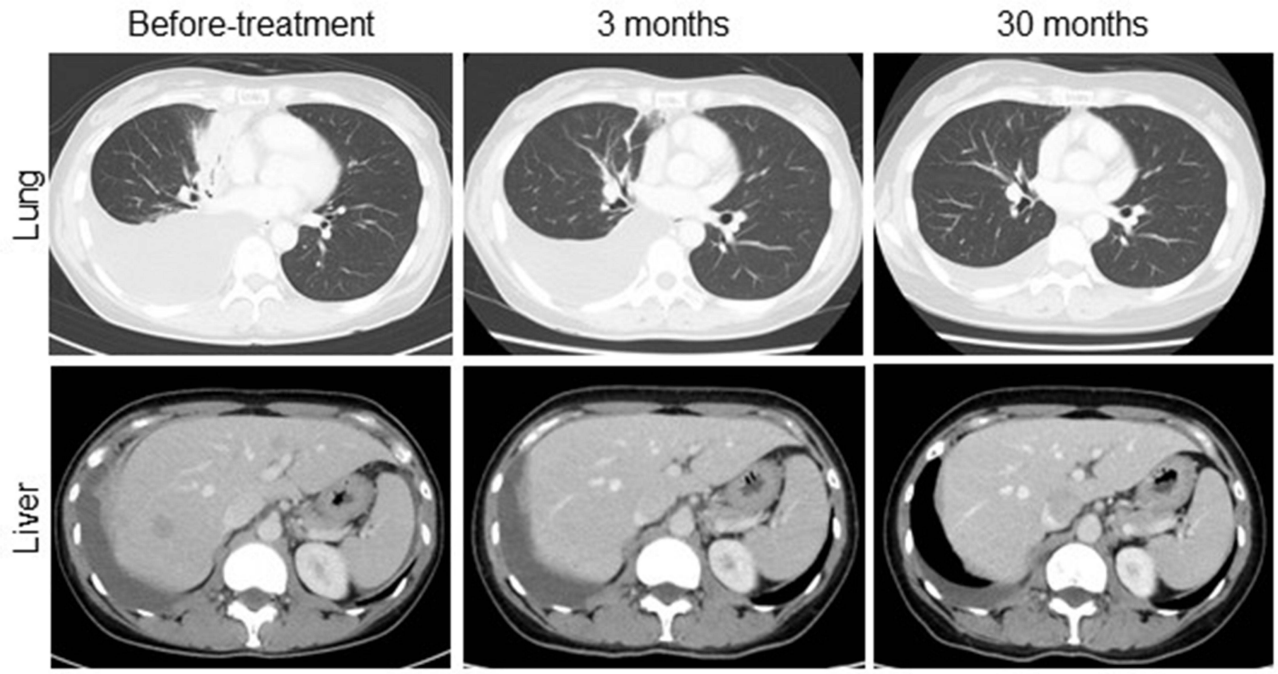

Figure 3 CT before and after alectinib therapy. Lung and liver image at before-, 3 months after-, and 18 months after-alectinib treatment were shown.

Molecular Findings

Further analysis of the molecular structure of *EML4-ALK* detected a fusion of *ALK* and *EML4* as well as a fusion of *ALK* and *NPR2* as shown in Table 1. *EML4-ALK* was thought to encode an out-of-frame product, but the structure of the *NPR2-ALK* fusion was unable to be analyzed because the *NRP2* region was not found on the gene body. This result suggested that the tumor cells had produced an *ALK* fusion gene with a unique structure. For further assessment, RNA sequencing was performed with extracted RNA from a formalin-fixed, paraffin-embedded (FFPE) sample after obtaining the patient's content. RNA was extracted using AllPrep DNA/RNA FFPE Kit (QIAGEN, Hilden, Germany), and the library for RNA sequencing was prepared with TruSeq RNA Exome (Illumina, San Diego, CA, USA). The synthesized library was sequenced by paired-end run using Illumina NovaSeq 6000 (Illumina) at Rhelixa, Inc. (Tokyo, Japan). With this output fastq files, the chimeric reads were analyzed using a previously reported method[4] with Arriba,[5] a fusion gene detector, which demonstrated the presence of an in-frame *EML4-ALK*-containing kinase activity domain (Figure 4). The chimeric read sequence was further analyzed by extracting sequencing reads containing the breakpoints and aligning them. The intronic sequence from both *ALK* and *EML4* had been inserted between the exons of *ALK* and *EML4* (Figure 4b and c) possibly as a result of an unusual insertion of a gene fragment derived from *NRP2*, as indicated by the panel sequencing results. This unique structure of the *EML4-ALK* fusion made accurate fusion gene prediction by targeted gene profiling using a DNA sample difficult.

Table 1 Detected Fusion Genes by Foundation One CDx Test

Predicted Structure	Gene1	Gene2	Position1	Position2	In-Frame	Type	Supporting Read Pairs
EML4-ALK (Exon19-Exon 20)	EML4	ALK	chr2:29446058–29446410	chr2:42547801–42548111	No	Fusion	259
NRP2-ALK (intron-Exon 20)	NRP2	ALK	chr2:29446347–29446536	chr2:206551468–206551812	Unpredictable	Rearrangement	244

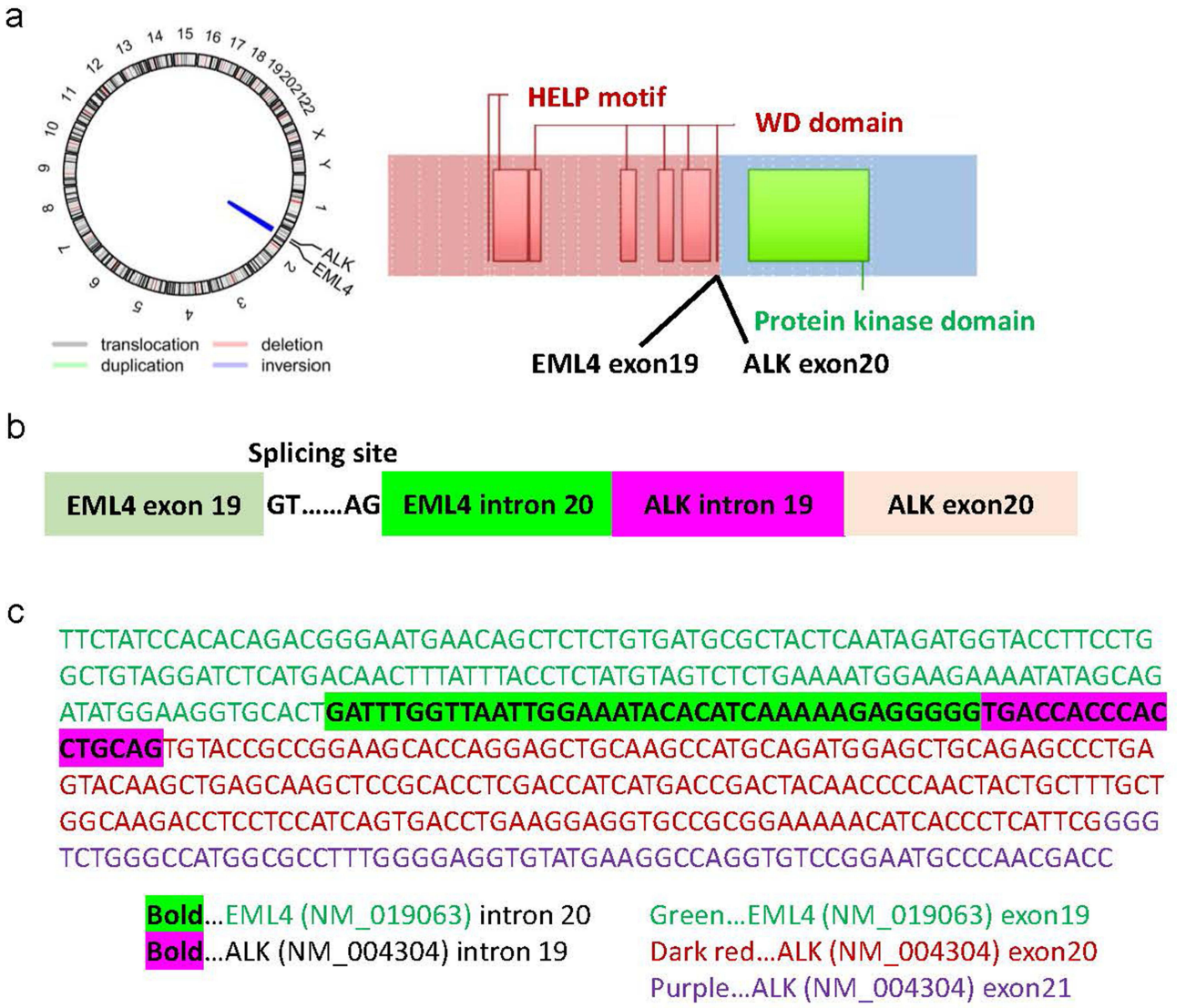

Figure 4 Result of fusion gene discovery using RNA sequencing and molecular structure of *EML4-ALK*. (**a**) Chimeric gene and structure of *EML4-ALK* detected in this case, (**b**) *EML4-ALK* fusion gene structure, (**c**) Estimated sequence of *EML4-ALK*.

Discussion

Metastatic ovarian tumors comprise 10–30% of all ovarian tumors.[3] In cases of metastatic ovarian tumor, commonly affected sites include the colon, stomach, appendix, breast, and pancreas. Metastasis to the lungs is rare, having a frequency of only 0.1% or 0.3–0.4%.[3] However, interestingly, ALK positivity in ovarian metastases of lung adenocarcinoma can be as high as 69%, with about 31% and 69% of the cases occurring in the bilateral ovaries and unilateral ovaries, respectively.[6] The mechanism underlying this metastasis is unknown. Some studies have reported ovarian metastases of ALK-positive lung adenocarcinoma, but in most cases, ALK was detected by FISH or IHC. Thus, the molecular characteristics of ALK-positive lung cancer with ovarian metastasis are also unclear. PFS and the median OS in patients with EML4-ALK-positive primary lung adenocarcinoma was reportedly 34.1 and 68.6 months, respectively.[7]

In the present study, RNA sequencing demonstrated that the *EML4-ALK* transcript contained *EML4* exon 19, *EML4-ALK* chimeric intron, and *ALK* exon20, which resulted in the formation of a unique structure. The data suggested that a micro-insertion between *EML4* and *ALK* had occurred, giving rise to the prediction difference between DNA- and RNA-based methods. Foundation One CDx suggested that the inserted DNA might have been from *NRP2*, but the RNA sequencing was unable to corroborate this hypothesis, and it is possible that intron mismapping might have given rise to

an image splicing forgery or noise. Similar microgene insertions into *EML4-ALK* fusions have previously been reported,[8] lending some support to this hypothesis. The details of the genomic DNA structure were unable to be fully ascertained in this study due to the lack of freshly frozen tumor samples, which would enable long-read sequencing.

Common breakage sites occur on exons 18 and 20 in *ALK* and exons 6, 18, and 20 in *EML4*.[9] A previous report found that the differences in length between *EML4* and *ALK* affected the response to ALK-TKI.[10] The study analyzed structural variants generated by differential fusion and found that the V1 variant of *EML4-ALK* was longer than any other variant and responded better to ALK-TKI. Pre-clinically and clinically, V1 responds better to ALK-TKI than V3. Other than these common variants, some rare variants of the EML4-ALK fusion gene, for which the efficacy of ALK-TKI is still unknown, have also been reported.[11] The *EML4-ALK* fusion gene detected in the present study is extremely rare but shared features with the V2 variant in having a breakage point at exon 20 in both EML4 and ALK and in responding to ALK-TKI. The difference in the drug response of each *EML4-ALK* fusion variant determines whether ALK-TKI should be administered; therefore, understanding the molecular structure of the ALK fusion gene is important. Considering that fusions with a unique structure that are unable to be detected accurately using DNA samples might be generated in tumors, a structural analysis using RNA sequencing may be more useful for a more accurate prediction of the drug response. Additionally, the kinase activity and/or proliferation assay using in vitro model would provide the information regarding the rare *EML4-ALK* function and could predict the responsibility for ALK-TKIs.

We reported a rare fusion of *EML4-ALK* in a patient with NSCLC with bilateral ovarian metastases. NGS can be used to diagnose the primary lesion with a relatively rare metastatic site and detect oncogenic fusion genes. The RNA sequencing in the present study confirmed that this rare fusion variant was functional. This report is a case report analyzed in a single case; thus, further study including structural analysis of multiple cases is warranted to determine the optimal method of detecting fusion gene variants and evaluating the efficacy of TK-I for each variant.

Abbreviations

ALK, anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein-like 4; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor. FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; RT-PCR, reverse transcription polymerase chain reaction; NGS, next-generation sequencing; CT, Computed tomography; FFPE, formalin-fixed, paraffin-embedded.

Disclosure

M.Y. received honoraria (lecture fee) from AstraZeneca, Takeda, MSD, Chugai Pharmaceutical, Ono Pharmaceutical, and Bristol-Myers Squibb. M.K. received honoraria (lecture fee) from AstraZeneca and Chugai Pharmaceutical. Y. H. received honoraria (lecture fee) from AstraZeneca, Eli Lilly Japan, Taiho Pharmaceutical, Chugai Pharmaceutical, Ono Pharmaceutical, Bristol-Myers Squibb, Kyowa Kirin, Nippon Kayaku, Takeda, Eisai, Novartis, Pfizer and MSD. The other authors declare that they have no conflicts of interest.

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