Case Report: iMDT Corner

An advanced NSCLC patient with *ALK-RNF144A* and *HIP1-ALK* fusions treated with *ALK*-TKI combination therapy: a case report

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Background: Anaplastic lymphoma kinase (*ALK*) rearrangement is one of the most important drivers in non-small cell lung cancer (NSCLC). Despite the effectiveness to canonical 3'-*ALK* fusions, the clinical efficacy of ALK inhibitors in patients with complex *ALK* fusions, such as nonreciprocal/reciprocal translocation remains uncertain. Exploring the optimal therapeutic regimens for this subset of patients is of crucial clinical significance.

Case Description: We reported a female patient diagnosed with stage IVB lung adenocarcinoma (LUAD) harboring a novel *ALK-RNF144A* fusion, concurrent with a Huntingtin-interacting protein 1 (*HIP1*)-ALK fusion and a *RB1* loss-of-function variant. The patient sequentially received multiple lines of treatment with *ALK*-tyrosine kinase inhibitor (TKI), chemotherapy, radiotherapy and *ALK*-TKI combined with anti-angiogenesis. Disease progression accompanied by a squamous cell carcinoma transformation was indicated after *ALK*-TKI combined with anti-angiogenesis and both *ALK-RNF144A* and *HIP1-ALK* fusions were retained in the tumor. The patient was subsequently treated with a third generation *ALK*-TKI, lorlatinib, in combination with albumin-bound paclitaxel and anlotinib, and then achieved stable disease. The patient remained on the treatment as of the last follow-up resulting in an overall survival (OS) of more than 18 months.

Conclusions: We have reported an advanced NSCLC patient with a complex nonreciprocal/reciprocal *ALK* translocation containing a novel *ALK-RNF144A* fusion, concurrent with a *RB1* loss-of-function mutation, who subsequently experienced pathological squamous cell carcinoma transformation. The combined treatment with *ALK*-TKI, chemotherapy, and anti-angiogenesis demonstrates clinical efficacy and may provide optional therapeutic strategies for this phenotype.

Keywords: Non-small cell lung cancer (NSCLC); nonreciprocal/reciprocal *ALK* translocation; ring finger protein 144A (*RNF144A*); combined treatment; case report

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Introduction

Non-small cell lung cancer (NSCLC) constitutes approximately 85% of all lung cancer cases (1,2). The rearrangement of the anaplastic lymphoma kinase (*ALK*) gene is one of the most important driver alterations in NSCLC, occurring in approximately 6.6% of adenocarcinomas (ADC) (3-5). *ALK* can be rearranged with various partner genes, resulting in either 5'-*ALK* or 3'-*ALK* fusions. To date, more than 90 different fusion partners have been reported, including *EML4*, *KIF5B*, *KLC1*, *TFG*, and Huntingtin-interacting protein 1 (*HIP1*), with *EML4* being the most common (6). 3'-*ALK* fusion has been confirmed as functional and responsive to crizotinib treatment in advanced NSCLC. However, the efficacy of *ALK* tyrosine kinase inhibitors (TKIs) can vary depending on the specific *ALK* fusion partner, fusion structure, and concurrent variants present in patients (7-9). Nonreciprocal/reciprocal *ALK* translocations refer to cases where 2 distinct partner genes fuse with *ALK* at different breakpoints, forming both 3'-*ALK* and 5'-*ALK* fusion products (10-12). Previous studies have demonstrated that 5'-*ALK* fusions exhibit limited sensitivity to ALK inhibitors duo to the absence of kinase domains (10,11,13), and also shown that patients with retained 5'-*ALK* in the genome during nonreciprocal/reciprocal translocation had a worse prognosis and a higher likelihood of baseline brain metastasis compared with those with a single *EML4-ALK* fusion (10). This suggests that certain *ALK* fusion partners and complex *ALK* fusion structures might lead to treatment failure or poor response. The therapeutic strategies and the effectiveness of *ALK*-TKIs for these complex fusions remain uncertain (14).

Herein, we report a case of a young Asian female with lung adenocarcinoma (LUAD) harboring nonreciprocal/reciprocal fusions of *HIP1-ALK* and a novel *ALK*-ring finger protein 144A (*RNF144A*), accompanied by a *RB1* loss-of-function mutation. The patient experienced squamous cell carcinoma transformation after treatment with ALK inhibitors and chemotherapy. She achieved stable disease (SD) after receiving a combination of ALK inhibitors and a multi-targeted inhibitor anlotinib, along with chemotherapy. The patient had an overall survival (OS) of 18 months at the time of submission of this report. We present this article in accordance with the CARE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-656/rc).

Highlight box

Key findings

- We reported an advanced non-small cell lung cancer (NSCLC) patient with a complex nonreciprocal/reciprocal anaplastic lymphoma kinase (*ALK*) translocation containing a novel ALK-ring finger protein 144A (*RNF144A*) fusion, concurrent with a *RB1* loss-of-function mutation, who subsequently experienced pathological squamous cell carcinoma transformation.
- The combined treatment with *ALK*-TKI, chemotherapy, and anti-angiogenesis demonstrates clinical efficacy and may provide optional therapeutic strategies for this phenotype.

What is known and what is new?

- Despite the effectiveness to canonical 3'-*ALK* fusions, the clinical efficacy of ALK inhibitors in patients with complex *ALK* fusions, such as nonreciprocal/reciprocal translocation remains uncertain.
- The combined treatment with *ALK*-TKI, chemotherapy, and anti-angiogenesis may achieve better efficacy for rare and complex *ALK* fusions.

What is the implication, and what should change now?

- The case report may provide new idea for the treatment of rare and complex *ALK* fusions.

Case presentation

A 33-year-old non-smoking female admitted to Jilin Cancer Hospital in August 2021 with a chief complaint of left-sided chest and back pain persisting for 1 month. A lung computed tomography (CT) scan revealed a mass in the left lung measuring 2.8 cm × 2.9 cm, along with enlarged mediastinal lymph nodes. A CT-guided percutaneous lung biopsy was performed synchronously, and the histopathologic evaluation showed poorly differentiated ADC with ALK (+, D5F3, Ventana), CK (+), Vimentin (partially +), CK5/6 (slightly +), P40 (–), TTF-1 (+), NapsinA (+), CD56 (–), Syn (–), Ki-67 (approximately 30%), and NUT (–) (*Figure 1A*). Imaging examination also found metastases in bilateral lungs, left hilar lymph nodes, mediastinal lymph nodes, bilateral supraclavicular lymph nodes, and bone. Based on the clinical and pathological findings, the patient was diagnosed with left lower lobe ADC at stage IVB (cT4N3M1c). The patient had an Eastern Cooperative Oncology Group Performance Status (ECOG PS) score of 1 (*Figure 1B*).

The patient received first-line treatment with alectinib 600 mg twice a day (bid) orally and achieved partial response (PR). Due to chest pain and aggravated bone destruction of the 8th thoracic vertebra, the patient underwent palliative local radiotherapy targeting the lesion of 7th to 9th thoracic vertebrae, with a tissue dose of 30 Gy/10 fractions/14 days.

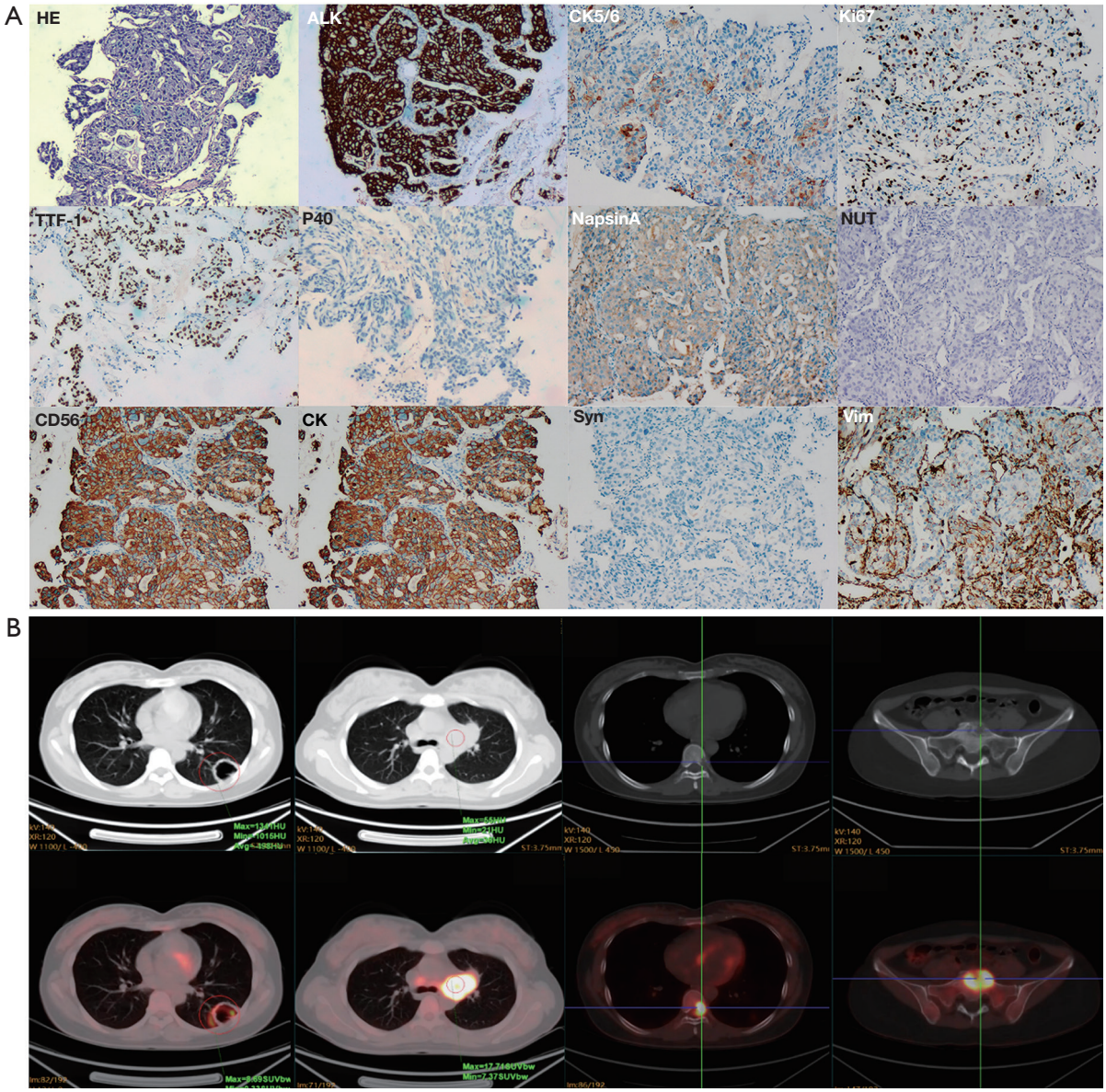

Figure 1 Baseline pathological examination and lung CT of the patient. (A) HE staining of the biopsy specimen showed a poorly differentiated tumor tissue. IHC experiments showed positive expressions of ALK, CK, CK5/6, TTF-1, NapsinA, Ki-67, and negative expression of P40, CD56, Syn, and NUT (×100), revealing an ADC origin of the lung cancer. (B) CT scan before treatment (Aug 2021). The locations of the lesions were marked with red circle. HE, hematoxylin and eosin; IHC, immunohistochemistry; ADC, adenocarcinoma; CT, computed tomography.

After 4.5 months, disease progression (PD) was indicated by CT scans, revealing enlarged mediastinal lymph nodes and new metastases in the 10th thoracic vertebra and the right occipital lobe (*Figure 2A*). A CT-guided mediastinal lymph node biopsy was performed, and histopathological evaluation indicated ADC based on immunohistochemistry (IHC) staining of ALK (+), CK7 (weakly +), CK20 (–), Villin (weak +), P40 (–), Ki-67 (approximately 20% +), TTF-1 (–), NapsinA (–), and CK (+) (*Figure 2B*). Next-generation sequencing (NGS) with a 520-gene panel (Burning Rock Biotech, Guangzhou, China) was performed with the biopsied tissue and revealed the presence of an

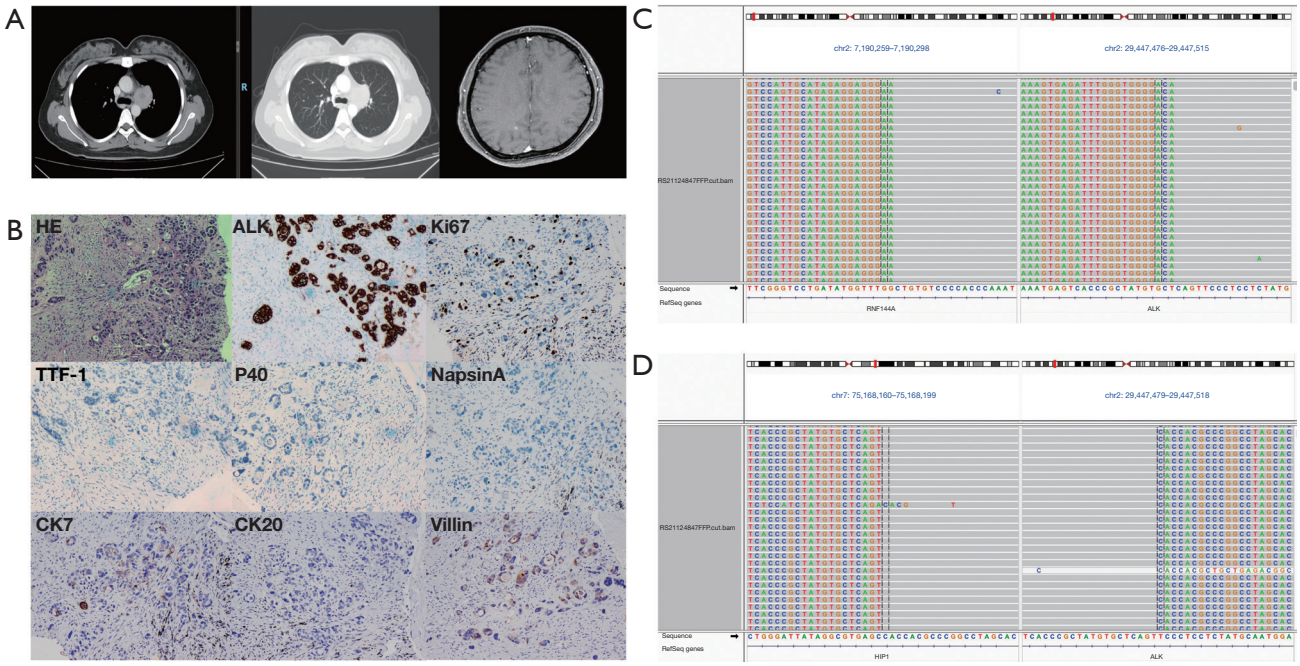

Figure 2 Imaging examination, pathological examination and NGS results after first-line treatment with aletinib and palliative local radiotherapy. (A) A CT scan and brain MRI after treatment with alectinib for 4.5 months was administered (Dec 2021). (B) HE staining of the biopsy specimen showed a poorly differentiated tumor tissue. IHC experiments showed positive expressions of ALK, CK7, Villin, Ki-67, CK, and negative expression of CK20, P40, TTF-1, and NapsinA (×100). (C) Coexistence of *ALK-RNF144A* and *HIP1-ALK* fusions by NGS in a female patient with lung adenocarcinoma. *ALK-RNF144A* fusion, with breakpoint positions of *ALK* at chr2 p23.2 and *RNF144A* at chr2 p25.1. (D) *HIP1-ALK* fusion, with breakpoint positions of *HIP1* at chr7 q11.23 and *ALK* at chr2 p23.2. NGS, nest-generation sequencing; CT, computed tomography; MRI, magnetic resonance imaging; HE, hematoxylin and eosin; IHC, immunohistochemistry.

ALK-RNF144A fusion [allele frequency (AF) 36.05%], a *HIP1-ALK* fusion (AF: 27.16%) (*Figure 2C,2D*), and a *RB1* splice variant in exon 14 (AF: 26.1%). NGS also showed the tumor mutation burden (TMB) of 1.0 mutations/Mb and a microsatellite stable (MSS) status. The IHC of programmed cell death ligand 1 (PD-L1) showed expression <1%.

Based on these data, the patient was enrolled in a phase I clinical trial (ChiCTR20210263), which investigated the efficacy of a third-generation dual *ALK/ROS1* TKI, TGRX-326, for advanced NSCLC patients with positive *ALK* or *ROS1*. The patient received second-line treatment with TGRX-326 80 mg once a day (qd) orally but experienced rapid PD characterized by enlarged mediastinal lymph nodes, extensive sacral soft tissue destruction, and significant sacrococcygeal pain. The ECOG PS score increased to 2 (*Figure 3A*). Subsequently, she received third-line treatment with pemetrexed, carboplatin, and bevacizumab for 3 cycles and palliative radiotherapy was also performed on the sacral soft tissue metastasis. The best response was SD. On May 24, 2022, imaging examination indicated PD by enlargement of brain metastases in the right occipital lobe and a new metastasis in the right lung (*Figure 3B*). The patient received docetaxel combined with temozolomide. However, after 1 cycle of treatment, imaging examination indicated the emergence of new brain metastases in the left parietal lobe (*Figure 3C*).

Given the absence of standard regimen beyond third-line treatment and limited efficacy of traditional chemotherapy drugs in controlling brain metastases, a re-challenge with a combination of an ALK inhibitor and targeted therapy with other mechanisms was considered, which has the advantage of penetrating the blood-brain barrier. The patient received crizotinib 250 mg (qd) combined with a multi-target TKI, anlotinib 12 mg (qd) orally on days 1–14 for 5 months, with the best response evaluated as SD.

On November 15, 2022, follow-up imaging revealed the enlargement of mediastinal lymph nodes and brain metastases (*Figure 3D*). Subsequently, the patient was treated

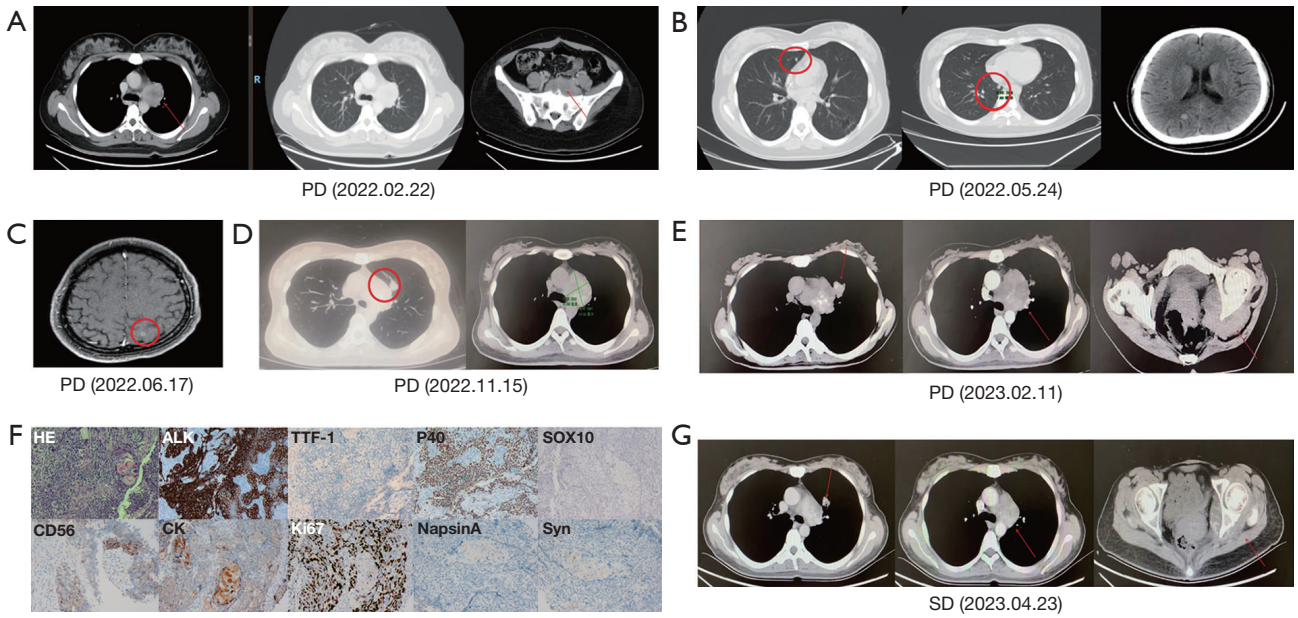

Figure 3 The representative lung CT, brain MRI scans, and pathological examination after second-line treatment. (A) A CT scan after treatment with TGRX-326 for 1.9 months (Feb 2022). The locations of the lesions were marked with red arrow. (B) A CT scan and brain MRI after treatment with pemetrexed + carboplatin + bevacizumab for 3 cycles (May 2022). The locations of the lesions were marked with red circle. (C) A computed tomography scan and brain MRI after treatment with 1 cycle of docetaxel combined + temozolomide (Jun 2022). The locations of the lesions were marked with red circle. (D) A CT scan after treatment with crizotinib + anlotinib cycles for 5.1 months (Nov 2022). The locations of the lesions were marked with red circle. (E) A CT scan after treatment with ensartinib + anlotinib cycles for 3.3 months (Feb 2023). The locations of the lesions were marked with red arrow. (F) HE staining of the biopsy specimen showed a poorly differentiated tumor tissue. IHC experiments showed positive expressions of ALK, P40, Ki-67, CK, CD56, and negative expression of TTF-1, NapsinA, Syn, and SOX10 (×100), revealing a SQC transformation of the lung cancer. (G) A CT scan after treatment with 3 cycles of albumin-bound paclitaxel + lorlatinib + anlotinib (Apr 2023). The locations of the lesions were marked with red arrow. PD, disease progression; SD, stable disease; CT, computed tomography; HE, hematoxylin and eosin; MRI, magnetic resonance imaging; SQC, squamous cell carcinoma; IHC, immunohistochemistry.

with a second generation *ALK*-TKI, ensartinib (225 mg, qd), combined with anlotinib (12 mg, qd, orally, on days 1–14). The regimen was continued for over 3 months. However, during the treatment, the metastases on the mediastinal lymph nodes and left upper lobe of lung enlarged, and new lesions appeared in the left acetabular (*Figure 3E*). The patient also experienced left lower limb pain and mobility disorder. The ECOG PS score remained at 2.

A CT-guided percutaneous biopsy was performed on the left acetabulum lesion. The pathological analysis showed ALK (+), P40 (+), Ki-67 (approximately 70% +), TTF-1 (–), NapsinA (–), CK (+), CD56 (weakly +), Syn (–), and SOX10 (–), implying a squamous cell carcinoma transformation from the original ADC (*Figure 3F*). NGS testing based on 8 lung cancer driver gene (Burning Rock Biotech) revealed the tumor retaining *ALK-RNF144A* (A19:R9) fusion (AF: 36.98%) and *HIP1-ALK* (H30:A20) fusion (AF: 44.04%).

Starting from February 28, 2023, the patient received albumin-bound paclitaxel 400 mg on day 1, lorlatinib 100 mg (qd), combined with anlotinib 12 mg orally on days 1–14 for 3 cycles. Additionally, local radiotherapy was administered to the left acetabular soft tissue. Imaging review after 2 months showed a 17% reduction in the lesion size (*Figure 3G*). The pain in the left lower limb was significantly relieved, and there was no activity impairment. The ECOG PS score dropped to 1. As of the last follow-up on April 27, 2023, the treatment is still ongoing and the patient had achieved an OS of more than 18 months. *Figure 4* shows the patient's treatment history.

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the

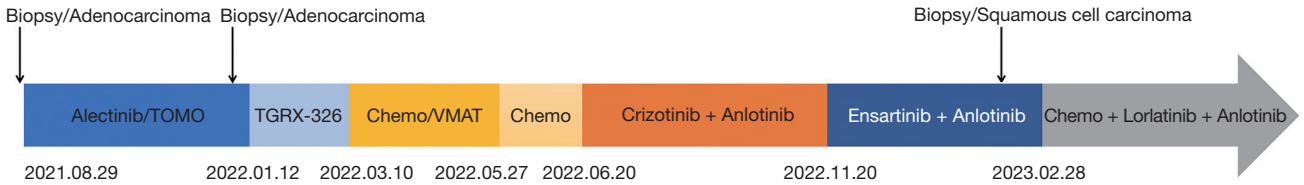

Figure 4 The timeline and treatment history of the patient (from Aug 2021 to Apr 2023). TOMO, Tomotherapy; VMAT, volumetric modulated arc therapy; chemo, chemotherapy.

Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

International multidisciplinary team (iMDT) discussion

Discussion among physicians from Jilin Cancer Hospital

Our patient achieved a short progression-free survival (PFS) of 4 and 1.5 months after receiving alectinib and TGRX-326 (lorlatinib deuterated compound), respectively. It is noteworthy that chemotherapy combined with anti-angiogenic drugs (bevacizumab) or other chemotherapy regimens following 1st and 3rd generation *ALK*-TKI failed to bring significant clinical improvement to the patient. Instead, the disease progressed quickly with the appearance of new brain metastases. Given the limited efficacy of *ALK*-TKI single agent in first-line and second-line treatment as well as the evidence that the emergence of new bypass-activating mutation sites or multi-clonal resistance sites can be treated using combination therapy strategy (15), we chose anlotinib as a component of the combination therapy. Anlotinib is a multi-target vascular endothelial growth factor receptor (*VEGFR*)-TKI and capable of penetrating the blood-brain barrier and better controlling intracranial lesions. The patient achieved a PFS of 8 months and her clinical symptoms and physical status significantly improved, suggesting a favorable clinical benefit for the patient after the failure of multi-lines of therapy.

Besides non-reciprocal/reciprocal translocation of *ALK*, this case also had a concurrent *RB1* loss-of-function variant. *RB1* is the first tumor suppressor gene discovered in humans and encodes the retinoblastoma protein. *RB1* variations, including copy number loss, nonsense mutations, splice abnormalities, and loss of wild-type *RB1* alleles are highly common in small cell lung cancer (SCLC) (16), but rare in NSCLC. *RB1* mutations are associated with poor prognosis and have been correlated with a reduced response to immunotherapy in advanced NSCLC (17). In this case, the patient experienced PD after 8 months of re-challenging with *ALK*-TKI and we speculate that the poor prognosis may be attributed, at least in part, to the concurrent *RB1* variant.

Furthermore, *RB1* inactivation has been associated with an increased risk of lung ADC transforming into a more invasive histological type (18). This phenomenon was observed in our case, where the patient experienced pathological transformation from ADC to squamous cell carcinoma after developing resistance to *ALK*-TKI re-challenge therapy. There are two possible explanations for histopathologic transformation. Firstly, *ALK*-TKI treatment may induce temporal evolution, similar to the transformation of epidermal growth factor receptor (*EGFR*)-mutant NSCLC to SCLC phenotype induced by *EGFR*-TKI. Secondly, the primary tumor may have had mixed components, and the resistant clone with squamous cell carcinoma features became predominant after treatment. Currently, there is limited clinical data available to guide the optimal treatment for patients who undergo squamous cell transformation after targeted therapy. In this case, we added albumin-bound paclitaxel for squamous cell carcinoma to the *ALK*-TKI (lorlatinib) and anlotinib combination. The treatment yielded a favorable outcome, with reductions observed in lung lesions, bone metastases, and brain metastases. The patient also experienced a significant improvement in clinical symptoms and physical status, and maintained an ECOG PS score of 1.

Re-biopsy and genetic testing after the development of drug resistance to *ALK* the inhibitor were very crucial in this case. These steps help identify the mechanism of resistance and guide the selection of subsequent therapies. The timing of applying first-generation, second-generation, and third-generation *ALK*-TKI, and the development of individualized treatment strategies for subsequent lines, represents a novel concept of the comprehensive

management of *ALK*-rearranged NSCLC. Due to the persistence of *ALK-RNF144A* and *HIP1-ALK* in the patient's second biopsy after multiline therapy, the ALK inhibitor was considered to re-challenge, and better survival benefits were achieved by combination therapy strategy. Although this case is only a single case, in the patient with complex *ALK* fusions, we observed the survival benefits of combination treatment, which brings some thinking and inspiration. In the future, the combination therapy strategy based on ALK-TKI can be further developed to explore the efficacy of the combination therapy strategy in patients with complex *ALK* fusions, and provide more data and reference for clinical diagnosis and treatment.

Department of Jilin Provincial Key Laboratory of Molecular Diagnostics for Lung Cancer

ALK fusion typically involves when various partner genes fuse with the 5' end of *ALK*, forming 3'-*ALK* fusion products. Non-reciprocal/reciprocal translocation events, where both 3'-*ALK* and 5'-*ALK* fusions coexist but with different partners, are rare in clinical practice, making the treatment approaches and therapeutic efficacy elusive (10). In this report, we identified a rare non-reciprocal/reciprocal translocation involving *ALK-RNF144A* (A19:R9) and *HIP1-ALK* (H30:A20). The biological function and mechanisms of *RNF144A* (ring finger protein 144A) gene remain unclear, and there are no reports on its role as an *ALK* fusion partner. *HIP1* gene has been found to fuse with *ALK*, but it exhibits significant differences in sensitivity to ALK inhibitors in LUAD and squamous cell carcinoma (9,19). To our knowledge, the *ALK-RNF144A* fusion, which contains 5'-*ALK* (A19) and 3'-*RNF144A* (R9), is reported here for the first time. In this case, *ALK* retains exons 1–19 with a breakpoint at chr2 p23.2 (5' end→3' end, 1–1,057 aa), whereas *RNF144A* retains exon 9 with the breakpoint at chr2 p25.1 (5' end→3' end, 250–318 aa). *RNF144A* belongs to the ring-between-ring (RBR) E3 ubiquitin ligase family and has been shown to regulate tumor cell growth and proliferation in breast cancer (20), bladder cancer (21), and gastric cancer (9). However, its role in lung cancer has not been reported. The other variant of *ALK* rearrangement, the *HIP1-ALK* (H30:A20) fusion, contains the 5'-*HIP1* and 3'- kinase domain of *ALK*. This fusion was first identified in NSCLC in 2014 and has been shown to be sensitive to crizotinib in xenograft (PDX) models derived from squamous cell carcinoma patients (9). Additionally, a LUAD patient with a *HIP1-ALK* fusion (H21:A20) showed a PFS of 15 months upon crizotinib treatment (22). Ou *et al.* reported a patient with ADC harboring the *HIP1-ALK* (H30:A20) fusion who achieved 5 and 12 months of PFS after crizotinib and alectinib treatment, respectively (23). However, other studies have reported poorer PFS with first-line crizotinib treatment in patients with *HIP1-ALK* fusion (11,24). Li *et al.* reported a patient with LUAD harboring *HIP1-ALK* (H19:A20) fusion who was resistant to crizotinib (19). Furthermore, due to the persistent increase of *ALK-RNF144A* and *HIP1-ALK* in the patient's second biopsy after multiline therapy, ALK inhibitor was considered to re-challenge, and better survival benefits were achieved by combination therapy strategy, suggesting that the combination therapy strategy based on *ALK*-TKI may be a potential strategy for rare and complex *ALK* fusions.

Several issues regarding the diagnosis and treatment of this patient were further discussed, and further questions were raised, as follows

After treatment, lung lesions of the patient were well controlled, rather than the metastasis of both mediastinal lymph node and bone marrow, indicating the presence of tumor heterogeneity. What's the optimal treatment protocol for this situation?

Petros Christopoulos: Discordant tumor growth caused by tumor heterogeneity indicates the need for stronger treatment. In case of oligoprogression, the current systemic therapy can be continued and complemented by local treatment, e.g., with radiotherapy or surgery of the growing lesions, as we are also standard practice for oligoprogressive NSCLC under immunotherapy (25). In case of diffuse tumor growth, a systemic therapy with different mode of action or higher potency becomes necessary, for example a next-generation TKI, like the third-generation ALK inhibitor TGRX-326 used here after alectinib, or chemotherapy. Ideally, before every therapy switches in ALK+ NSCLC, a rebiopsy with next-generation sequencing (NGS) should be performed in order to identify druggable off-target resistant mechanisms and guide the selection of subsequent drugs (26).

Erin L. Schenk: After progression on first line therapy for ALK+ NSCLC, potential options include local consolidative therapy or a change to systemic therapy. Local consolidative therapy with radiation therapy or surgery can be considered for patients with 5 or fewer progressive lesions. When consolidative therapy is not a viable option for the patient, treatment selection should optimally be guided after repeat tissue biopsy with

molecular testing. Occasionally, ALK+ dependent or ALK+ independent resistance mechanisms emerge with available targeted therapies. In the absence of a targetable mechanism of resistance, lorlatinib, if not previously given, or platinum doublet chemotherapy is often the next choice outside of a clinical trial.

Takaaki Sasaki: The observed efficacy of alectinib, considering tumor heterogeneity, indicates that certain tumor regions might have developed *ALK*-resistant mutations or activated bypass cell signaling pathways, such as *EGFR* or *MET* (27,28). The next-generation sequencing analysis from the re-biopsied mediastinal lymph node did not identify any mutations in the *ALK* tyrosine kinase domain, suggesting resistance might stem from a bypass signaling mechanism. In this case, some regions exhibited progression within 4.5 months, a period shorter than the previously reported PFS with alectinib (29). When managing such progressive areas proves challenging with localized therapy, transitioning to cytotoxic chemotherapy is advised.

Brain metastasis occurred during treatment. What is the appropriate timing for this patient to receive cranial irradiation?

Petros Christopoulos: According to the experience from the ALEX, ALTA-1L and CROWN studies as well as based on retrospective data, cranial irradiation is not needed for newly diagnosed ALK+ NSCLC patients if second- or third-generation TKI are used in the first line, because it does not prolong survival (30,31). On the other hand, in subsequent lines of treatment, either with next-generation ALK inhibitors or chemotherapy, the intracranial response rate does not exceed 50% (26), and therefore brain radiotherapy should be considered, as it has been to shown to prolong OS if given concomitantly with targeted therapies showing response rates in the range of second-line ALK+ NSCLC drugs (up to 60%) in NSCLC patients with brain metastases (32).

Erin L. Schenk: If a highly CNS penetrant next generation TKI is available as standard of care or through a clinical trial, consideration can be given to trialing the new TKI if the patient is asymptomatic from the brain metastases and can undergo repeated brain imaging within a few weeks of starting the new therapy. Symptomatic, or larger lesions may require local radiation therapy or surgical resection.

Takaaki Sasaki: Radiation therapy should ideally be reserved as a last option. Following treatment with pemetrexed, carboplatin, and bevacizumab, brain metastasis has been observed. Given its superior brain penetration among *ALK*-TKIs, lorlatinib is recommended (33).

After receiving several generations of ALK-TKIs, pathological evaluation from biopsy of the left acetabular lesion showed a transformation from ADC to squamous cell carcinoma. What's the optimal treatment protocol for squamous cell carcinoma transformation?

Petros Christopoulos: A squamous transformation of ALK+ ADC is rare with very few published cases in the literature to guide therapeutic decisions. If more potent *ALK*-TKI are available, which have not been used before for this particular patient, they should definitely be considered first, as several studies show that ALK+ NSCLC with deviant histology, for example squamous or neuroendocrine lung carcinomas, retain sensitivity to ALK inhibitors if the *ALK* fusion remains detectable (34,35). If next-generation sequencing reveals any other druggable alterations, the treatment should target these, as well, otherwise standard (immuno-) chemotherapy should be considered.

Erin L. Schenk: For squamous transformation, chemotherapy with a taxane based regimen would be recommended.

Takaaki Sasaki: To date, studies have reported the efficacy of *ALK*-TKIs in treating *ALK* fusion gene-positive lung cancers with histological presentations such as squamous cell carcinoma and neuroendocrine tumors. Though the therapeutic outcomes are somewhat less favorable than those observed in ADC, they still demonstrate significant efficacy (36-39). Given that the biopsy sample continues to exhibit *HIP1-ALK*, selecting an as-yet unused *ALK*-TKI seems prudent.

Regarding tumor heterogeneity, could micro lesions by needle biopsy provide comprehensive and precise information to guide the diagnosis and treatment in advanced lung cancer?

Petros Christopoulos: Multiregion sampling has revealed considerable spatial heterogeneity with adverse prognostic implications for many lung cancers (40). Therefore, performing multiple biopsies at initial diagnosis could improve the yield of potential therapeutic targets and also reveal the presence of an additional histologic component in some mixed pulmonary neoplasms, which can be missed if a small biopsy of one site only is performed (41). However, multiregion biopsies are not practicable in the

routine setting, because they would multiply the health care costs and also increase the procedural risk of complications for the patients. Another, minimally invasive method to address spatial tumor heterogeneity is the regular use of liquid in addition to tissue biopsies, which can detect more actionable driver alterations in NSCLC and is also suitable for the longitudinal monitoring of ALK+ tumors, but remain underutilized in Europe and many other parts of the world due to the lack of formal regulatory approval and reimbursement (42,43).

Erin L. Schenk: Repeated biopsies for a patient while receiving targeted therapy at time of progression is an important component of choosing subsequent line therapies. Sampling small volumes may not provide adequate tissue yield required for molecular testing or be representative of the clonal heterogeneity in other lesions. While the opportunity to utilize multiple therapies for a patient based on molecular testing is appealing, progressive toxicity with additive therapies can be encountered.

Takaaki Sasaki: While understanding tumor heterogeneity has potential value, current knowledge does not allow for the selection of treatments solely based on its mechanisms. As a result, I contend that routine repeat biopsies in clinical practice may not be warranted. However, utilizing liquid biopsy to detect resistance mutations in the *ALK* tyrosine kinase domain could aid in the selection of the appropriate *ALK*-TKI (44).

The patient accepted 6 cycles of chemotherapy-based combination treatment. What is the next treatment strategy for the following PD?

Petros Christopoulos: I see two main further options for this patient: (I) a 4th generation ALK inhibitor, like NUV-655, either within a phase 1/2 trial (e.g., ALKOVE-1, NCT05384626) or in compassionate use (45); and (II) the quadruple immunochemotherapy according to the IMpower150 regime with carboplatin-paclitaxel-bevacizumab-atezolizumab, which has shown special activity for *EGFR/ALK*-mutated tumors after failure of TKI and is approved specifically for these patients by the European Medicines Agency (46).

Erin L. Schenk: At time of PD, biopsy of a progressive lesion and clinical trials should be considered such as those investigating antibody drug conjugates or bispecific T cell engagers.

Takaaki Sasaki: Re-administration of alectinib or combination therapy with PD-1/PD-L1 and CTLA4 inhibitors is recommended. If renal function is uncompromised, a combination of platinum agents and pemetrexed can also be considered for retreatment.

Conclusions

For the first time, we reported a novel, rare non-reciprocal/reciprocal translocation (*ALK-RNF144A* and *HIP1-ALK*) accompanied by *RB1* mutation and pathological transformation in a young female patient with advanced ADC. The patient achieved a favorable response with a combination therapy of *ALK*-TKIs, chemotherapy and anti-angiogenesis. These findings suggest re-biopsy and multiple genetic testing show important roles in determining the rationale for precise and personalized treatment and may provide a new therapeutic strategy for nonreciprocal/reciprocal *ALK* translocation NSCLC.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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