iMDT Corner

Novel HIVEP1-ALK fusion in a patient with lung adenocarcinoma demonstrating sensitivity to alectinib: a case report

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Background: Anaplastic lymphoma kinase (ALK) fusion is an important oncogenic driver in non-small cell lung cancer (NSCLC). Reports on the intergenic region (IGR) as an ALK fusion partner are rare. Here, we report the case of a patient with advanced NSCLC harboring a human immunodeficiency virus type I enhancer binding protein 1 (HIVEP1)-ALK fusion that responded effectively to alectinib.

Case Description: A 60-year-old non-smoking male was referred with a 3-month history of productive cough secondary to lung adenocarcinoma metastatic to mediastinal lymph nodes, brain, liver, and bone (T2N3M1c, stage IVB). Next-generation sequencing identified an IGR (upstream HIVEP1-) ALK fusion, and immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) results were consistent with an ALK-positive tumor. The patient was subsequently started on alectinib, with no obvious adverse reaction. After 1 month of therapy, the patient achieved significantly remission of the clinical symptoms and had led to an ongoing partial response (PR) lasting >33 months.

Conclusions: Our experience highlights the efficacy of alectinib in a patient with HIVEP1-ALK fusion positive NSCLC with multiple metastases including brain disease, and the need for multiple genetic testing methods to verify the oncogenicity of ALK fusions prior to treatment. It could provide useful guidance for the treatment of similar cases in the future.

Keywords: Non-small cell lung cancer (NSCLC); HIVEP1-ALK; alectinib; intergenic region (IGR); case report

Submitted Jan 21, 2022. Accepted for publication May 07, 2022.
doi: 10.21037/tlcr-22-288
View this article at: https://dx.doi.org/10.21037/tlcr-22-288

Introduction

Anaplastic lymphoma kinase (ALK) fusion is an important targetable oncogenic driver in non-small cell lung cancer (NSCLC). To date, over 90 ALK fusion partners have been reported (1), among which echinoderms microtubule-associated protein-like 4 gene (EML4) is the most common. By comparison, reports of the intergenic region (IGR) as an ALK fusion partner are rare. The sensitivity of these IGR-

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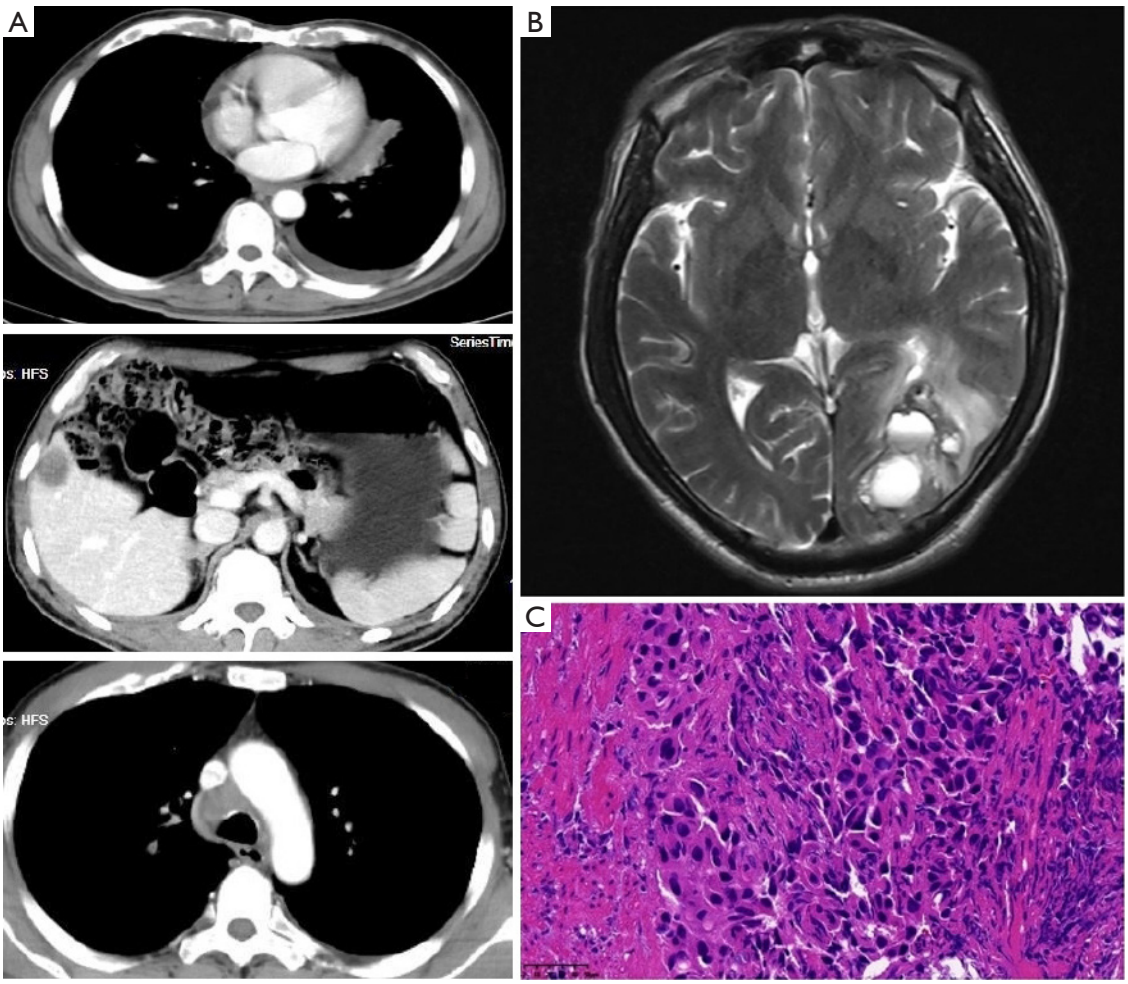

Figure 1 Imaging and pathology. (A) CT scan revealed a left lung mass, mediastinal lymph node metastases, and liver metastases; (B) MRI revealed multiple metastases in the left occipital lobe; (C) the result of hematoxylin and eosin staining (400×). CT, computed tomography; MRI, magnetic resonance imaging.

ALK fusions to ALK targeted therapies is also not clear, with one review of 28 cases reporting response to crizotinib in three patients (1). In addition, the functional significance of intergenic breakpoints is yet unknown. Here, we report the case of a patient with lung adenocarcinoma harboring an IGR (upstream HIVEP1)-ALK fusion that was sensitive to alectinib. Our report enriches the knowledge of ALK fusion sites and provides more clues for the treatment of such ALK fusion patients. We present the following article in accordance with the CARE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-288/rc).

Case presentation

In April 2019, a 60-year-old, non-smoking, Chinese male was referred to Zhejiang Cancer Hospital with a 3-month history of cough with sputum. No other clinical symptoms, including dizziness or headache, were observed. The patient had been healthy in the past, and he had no alcohol drinking habits, history of hypertension, diabetes or family history of cancer. A computed tomography (CT) scan revealed a left lung mass with metastases to mediastinal lymph nodes, bone, and liver (3.0 cm × 2.0 cm) (see *Figure 1A*). Furthermore, magnetic resonance imaging (MRI) of the brain revealed multiple metastases in the left occipital lobe (3.3 cm × 3.2 cm) (see *Figure 1B*). Bronchoscopy and hematoxylin and eosin staining confirmed the diagnosis of lung adenocarcinoma (see *Figure 1C*). Immunohistochemical (IHC) staining indicated the tumor was positive for napsin A, thyroid transcription factor 1 (TTF1), neural cell

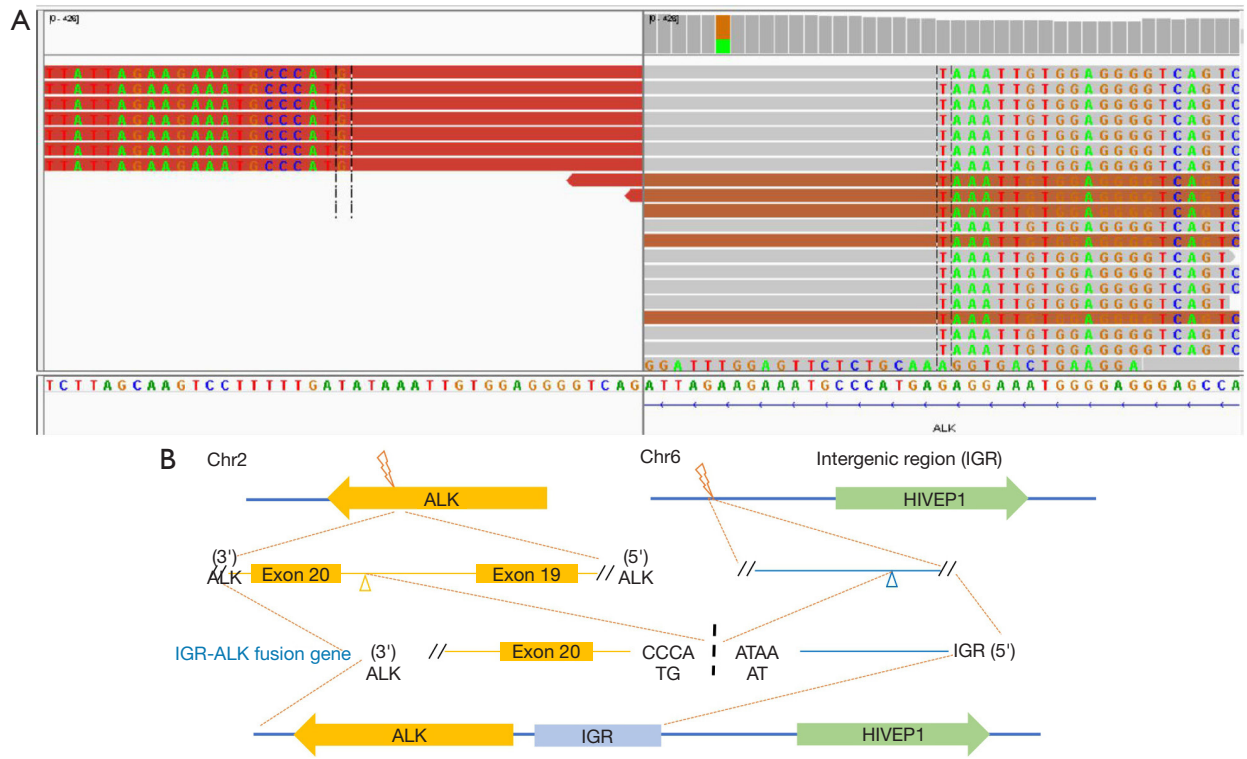

Figure 2 NGS result of ALK fusion. (A) Sequencing reads of the IGR (upstream HIVEP1) and ALK are shown by the Integrative Genomics Viewer; (B) schematic representation of the IGR (upstream HIVEP1)-ALK fusion structure. NGS, next-generation sequencing; ALK, anaplastic lymphoma kinase; IGR, intergenic region; HIVEP1, human immunodeficiency virus type I enhancer binding protein 1.

adhesion molecule (also known as CD56), synaptophysin, chromogranin A, and Ki-67 (about 30–40% positive), but negative for p40. Given the widespread radiographic findings, the patient was diagnosed with stage IVB (T2N3M1c) NSCLC.

Genome sequencing of the formalin-fixed, paraffin-embedded specimens from the bronchoscopic biopsies was conducted using next-generation sequencing (NGS). NGS showed that the patient's tumor had an IGR-ALK fusion (upstream HIVEP1:A20, 7.6% abundance in tissue) (see *Figure 2*). Next, we performed ALK IHC (Ventana-D5F3) and fluorescence in situ hybridization (FISH) tests on the tumor tissue specimens, which confirmed the ALK positivity (see *Figure 3A-3C*). IHC positivity suggested that the IGR-ALK fusion led to functional ALK expression.

Beginning on April 15, 2019, the patient was treated with alectinib 600 mg twice daily with zoledronic acid 4 mg for bone metastases. After 1 month, chest CT scans revealed that the left lung mass, the mediastinal lymph nodes, and liver metastases (1.8 cm × 1.3 cm) were reduced, and the MRI scans showed that the brain metastases had also shrunk (1.5 cm × 1.1 cm) (see *Figure 3D*). According to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1), the efficacy was judged as partial response (PR). As of January 2022, the patient is still taking alectinib with ongoing response, and no obvious drug-related adverse reactions have been observed so far.

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 Helsinki Declaration (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.

Discussion

To our knowledge, this is the first report of an IGR (upstream HIVEP1)-ALK fusion, which is a new type of rearrangement that was identified by NGS. The detected fusion consists of the IGR (upstream HIVEP1) and

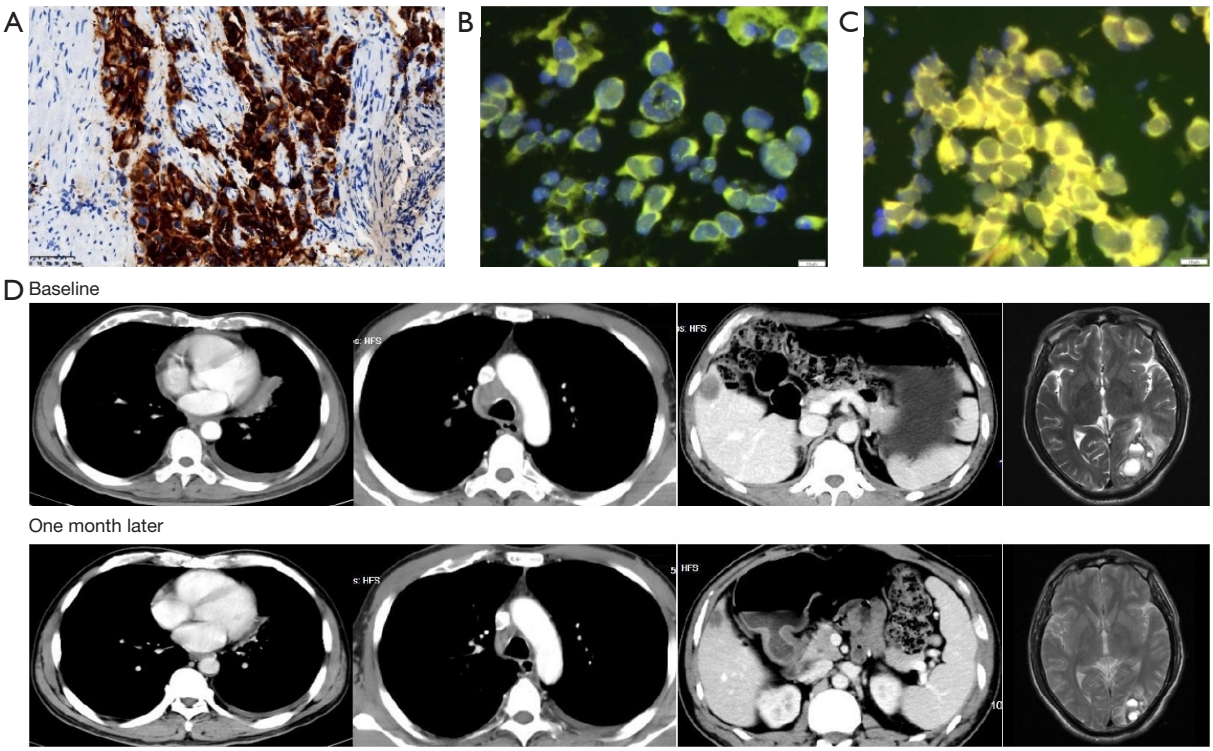

Figure 3 IHC, FISH, and dynamic imaging changes. (A) The IHC results (Ventana-D5F3) (400×); (B,C) the FISH results (1,000×); (D) dynamic imaging changes at the baseline and 1 month later. IHC, immunohistochemical; FISH, fluorescence in situ hybridization.

HIVEP1 and the 20 exon of the ALK gene.

With the development of gene detection techniques, oncogenic fusions are now more readily identifiable by multiple different methods. IHC is widely used in ALK screening and has a number of advantages, including that it is fast, economical, and simple to execute, but can be associated with false-positive results (2). On the other hand, FISH is known as the gold standard for ALK fusion gene detection because of its relatively high sensitivity and specificity, although it requires a high level of interpretation by pathologists and can still miss some cases (2). The use of the real-time polymerase chain reaction (RT-PCR) method for ALK genetic testing is controversial given that it can only detect common fusion types and may therefore fail to detect uncommon fusions.

In recent years, NGS has become more and more important in ALK genetic testing. The advantage of NGS is that it can distinguish all subtypes of ALK fusion. NGS testing can be performed at the level of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). NGS-DNA can be performed on both tissue samples and liquid biopsies; however, NGS-RNA has high requirements in relation to the sample quality, and its application for liquid biopsy samples is challenging. Additionally, NGS-DNA can provide information about regions that NGS-RNA cannot analyze, such as information about the IGR and untranslated regions (UTRs). However, NGS-RNA can assess the biological function of rare fusions by assessing the transcriptome.

In a study of 26 cases of IGRs, fusion was detected by NGS-DNA (3). Subsequent NGS-RNA confirmed expression of the fusion as transcript in 11/26 cases, of which the classic EML4-ALK fusion was found in 4 cases. In the present case, after the NGS-DNA indicated IGR-ALK fusion, IHC was conducted to confirm that the fusion gene was transcribed and translated to form a functional fusion protein. As NGS-RNA was not conducted, we do not know whether the formed fusion protein is of the classic EML4-ALK type or of the HIV1EP-ALK type. Thus, if a patient's NGS-DNA results indicate an IGR-ALK fusion, IHC or NGS-RNA should also be conducted to verify the formation of the fusion protein or transcription and exclude an inactive fusion.

In the ALEX trial, the median progression-free survival

(PFS) with first-line alectinib group was 34.8 months compared to 10.9 months with crizotinib (4). For patients who develop resistance to crizotinib, the subsequent second-line PFS of alectinib was only 9.6 months (5), therefore the first-line PFS period of the patients who received alectinib was much longer than that of those who received sequential treatment. Studies have shown that 15–35% of NSCLC patients have brain metastases at the time of initial treatment (6,7). Alectinib is not a substrate of P-glycoprotein, is not actively excreted from the intracranial environment, and can cross the blood-brain barrier, resulting in higher drug concentrations in the cerebrospinal fluid (8). In the ALEX trial, alectinib-treated patients with brain metastases at the baseline had an overall response rate of 76.6%, and the median PFS period was significantly higher than that under crizotinib (27.7 *vs.* 7.4 months, hazards ratio =0.35).

To date, most of the IGR-ALK patients reported to have been effectively treated with ALK-TKI chose to receive crizotinib as the first-line treatment. With the in-depth study of ALK rare fusions, some scholars have found that different ALK-TKIs may have different therapeutic effects on the same type of rare ALK fusion. For example, for the rare ALK fusion HIP1-ALK, primary drug resistance occurred during treatment with crizotinib, a PR was achieved after switching to alectinib treatment, and the PFS period was >9 months (9). In the study of Li *et al.*, only 3 patients with IGR-ALK fusion achieved PR after receiving crizotinib treatment. The 3 patients showed no progression before the article was published, but the duration of treatment was 6, 10, and 16 months, respectively (3). Alectinib may be a first-line option for certain rare ALK fusions that are considered to result in functional ALK expression, as suggested by either confirmatory IHC or NGS-RNA. It may be a particularly effective option for patients with rare ALK fusions when brain metastases are present given alectinib's intracranial activity. However, further study is needed before generalizing this to all rare ALK fusions.

In conclusion, this report provides the first clinical evidence of the efficacy of alectinib in treating patients with HIVEP1-ALK fusion positive lung cancer with multiple metastases including brain disease. This report can provide guidance for the management of future cases with detection of these rare alterations in NGS-DNA. Although NGS-DNA can detect novel fusion partners, validation by NGS-RNA or IHC is still important in the case of IGR-ALK fusions. We encourage clinicians around the world to report on these new fusions in order to increase available evidence for treatment of these patients.

Several issues arise in relation to the diagnosis and treatment of this patient that require further discussion

Question 1*: *When NGS-DNA discovers novel ALK fusions*, *does it need to be verified by NGS-RNA?

Expert opinion 1: Dr. Mariacarmela Santarpia

DNA-based NGS assays do not provide the information whether the fusion gene is transcribed into a protein. This could be possible with RNA-based sequencing approaches, that should be better used in the case of fusion variants, although with some limitations, such as difficulties in obtaining optimal quality and/or quantity of RNA from FFPE samples.

Expert opinion 2: Dr. Petros Christopoulos

When novel ALK fusions are detected using DNA-NGS, subsequent RNA-NGS or ALK immunohistochemistry can confirm presence of an active gene fusion that is transcribed and translated to form an oncogenic protein. In case that such an orthogonal validation of the DNA-NGS result is not possible (e.g., no more material available, technical failure, etc.), treatment with an ALK inhibitor, preferable a next-generation compound, like the alectinib used here, is warranted, because the great majority of these patients will respond ALK TKI. Special attention is warranted in the special case that another typical oncogene alteration, e.g., KRAS G12C or a classic EGFR mutation, is detected in addition to the novel ALK fusion: in this rare scenario, a mixed tumor might be present, but it is also likely that ALK is not the oncogenic driver here (i.e., that the ALK fusion is inactive), because oncogenic drivers are mutually exclusive in NSCLC normally.

Expert opinion 3: Dr. Nathaniel J. Myall

Yes, I would suggest that NGS-RNA should be used in these circumstances. From other studies that have been cited in this manuscript, it has been suggested that fusions involving intergenic breakpoints can be variable and/or complex. NGS-RNA provides more functional data about the rearrangement and can also clarify the likelihood that the given novel rearrangement leads to a functional protein overexpression. Especially when we do not have consensus data regarding the efficacy of ALK TKI therapies against

novel ALK fusions, NGS-RNA data may allow providers to better predict the likelihood of response with these therapies against any given rare ALK fusion.

Question 2: *Should alectinib be the first choice when rare fusions are found?*

Expert opinion 1: Dr. Mariacarmela Santarpia
Few data are available for alectinib in rare fusions (10). However, it is well recognized that alectinib has good central nervous system (CNS) activity and a tolerable safety profile in ALK-rearranged tumors, as also demonstrated in this patient.

Expert opinion 2: Dr. Petros Christopoulos
When rare fusions are found, a next-generation compound, mainly alectinib, or brigatinib currently, should be the treatment of choice. Additionally, it should be noted that the third-generation lorlatinib is meanwhile also available as first-line treatment in many countries and represents a further option for these cases; in the near future, lorlatinib might even displace second-generation inhibitors as the standard first-line treatment, if updates from the CROWN study show considerably better results than those of the ALEX and ALTA-1L studies. In any case, an ALK inhibitor newer than the first-generation TKI crizotinib is needed, because of the higher systemic and brain efficacy. Interestingly, retrospective data suggest that some rare ALK fusions may confer a worse prognosis than the typical EML4-ALK fusion, which is also a reason for use of more potent drugs in these patients.

Expert opinion 3: Dr. Nathaniel J. Myall
This case report shows that alectinib may be active for at least some rare fusions. However, in the absence of overall consensus/large-volume data, the choice to use an ALK inhibitor upfront should depend on a number of factors including (I) burden of metastatic disease (e.g., larger burden of disease with impending organ compromise may benefit from systemic chemotherapy first, which has the potential to be active regardless of driver mutation status); (II) knowledge of whether the rare fusion is activating or a bystander (e.g., use of confirmatory IHC/NGS-RNA testing); and (III) the patient's goals of care.

Question 3: *Does this patient require radiation therapy?*

Expert opinion 1: Dr. Mariacarmela Santarpia
It depends by the symptoms complained by the patient at the diagnosis and by radiation oncologists evaluation.

Expert opinion 2: Dr. Petros Christopoulos
No, for ALK patients with newly diagnosed asymptomatic brain metastases who receive alectinib, brigatinib or lorlatinib upfront, brain radiotherapy can safely be deferred until the time of TKI failure based on experience from the respective phase 3 clinical trials and recently published real-world evidence (11,12). This is due to the high intracranial efficacy of newer ALK inhibitors, which exceeds that of radiotherapy. According to retrospective data, a similar strategy could be pursued even for patients with larger and symptomatic lesions (13), but the evidence is weaker here, because such cases were generally excluded from prospective clinical trials.

Expert opinion 3: Dr. Nathaniel J. Myall
There is not clear need for radiation therapy given the continued excellent response to alectinib. In metastatic non-small cell lung cancer, the main indications for radiation are (I) palliation, (II) treatment of oligometastatic progression, or (III) consolidation in the setting of oligometastatic disease at diagnosis with good response to systemic therapy.

Question 4: *How should this patient be treated after disease progression?*

Expert opinion 1: Dr. Mariacarmela Santarpia
Should be performed another biopsy to study eventually novel mutations (e.g., MET alterations) or next-generation ALK TKI with CNS activity (e.g., lorlatinib) or chemotherapy.

Expert opinion 2: Dr. Petros Christopoulos
The third generation ALK inhibitor lorlatinib has the best evidence as subsequent therapy after failure of second generation ALK TKI, such as alectinib. This is largely due to its broad activity against secondary ALK resistance mutations, such as G1202R, which are detectable in up to 50% of patients failing alectinib. Of course, ideally a tissue of liquid biopsy should be performed at the time of treatment failure, because other resistance mechanisms, such as MET amplifications, are also encountered in some patients, and require special handling. In case of oligoprogression, continuation of alectinib and radiotherapy of the enlarging lesion is also reasonable, especially if the blood liquid biopsy at that time is negative, as there are some retrospective data that ALK

patients with oligoprogression and negative liquid biopsies have indolent disease and a very good prognosis (14). On the other hand, the treatment switch should not be delayed too much in those who need it, because up to 1/3 of these patients might show rapid clinical deterioration and miss any subsequent therapy (15). One very sensitive way to closely monitor ALK patients under treatment are serial liquid biopsies, which have been shown to detect emergence of resistance mutations and therapy failure several months earlier than radiologic imaging (16).

Expert opinion 3: Dr. Nathaniel J. Myall

If there is localized or oligometastatic progression, I would likely elect to continue alectinib in this patient but add local therapy (radiation) to the 1–2 sites of progression. In the case of more widespread progression, I would consider platinum-doublet chemotherapy (e.g., carboplatin/pemetrexed +/– bevacizumab) given that this is a widely accepted second-line option in ALK-positive lung cancer and given the lack of consensus regarding the efficacy of lorlatinib or other next-generation TKI therapies for rare fusions such as this one. However, a repeat biopsy and genetic sequencing at the time of progression might identify secondary/acquired ALK resistance mutations that could suggest activity with lorlatinib or another next-generation ALK TKI therapy before proceeding later with chemotherapy.

Acknowledgments

The authors appreciate the academic support from the AME Lung Cancer Collaborative Group.

Funding: This work was supported by the Zhejiang Medical and Health Science and Technology Program (No. 2022KY644 and No. 2018KY023).

Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-288/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-288/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been 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 Helsinki Declaration (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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(English Language Editor: L. Huleatt)

Cite this article as: Gu X, Wang W, Wu W, Zhang Y, Shao L, Santarpia M, Christopoulos P, Myall NJ, Shi Z, Lou G. Novel HIVEP1-ALK fusion in a patient with lung adenocarcinoma demonstrating sensitivity to alectinib: a case report. Transl Lung Cancer Res 2022;11(5):902-909. doi: 10.21037/tlcr-22-288