A Rare Fusion of *CLIP1* and *ALK* in a Case of Non–Small-Cell Lung Cancer With Neuroendocrine Features

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Clinical Practice Points

- We report the case of a nonsmoker woman with a lung carcinoma with neuroendocrine features harboring a new anaplastic lymphoma kinase (*ALK*) fusion variant involving the cytoskeleton-associated proteins-Gly domain containing linker protein 1 (*CLIP1*) gene.
- Lung carcinomas with neuroendocrine features are a rare subtype of non–small-cell lung cancer (NSCLC) and a scarce rate of *ALK* fusions have been reported in the literature.
- However, case reports suggest that ALK inhibitors could be effective in this histological subset, emphasizing that ALK testing should include NSCLC with neuroendocrine features.
- Several *ALK* fusion variants have been identified but their effects on clinicopathological features and clinical responses to treatment have not yet been determined, and even less so for non–microtubule-associated protein-like 4–ALK (*EML4-ALK*) fusions, and fusions occurring in rare histologic subtypes of lung cancer.
- The use of next-generation sequencing-based technologies, which allow the simultaneous analysis of multiple biomarkers, and also the characterization of known and of new fusion variants, should therefore be favored, because they will contribute to a better understanding of the clinicopathologic features of *ALK* rearrangements in lung cancer.

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Introduction

Anaplastic lymphoma kinase (*ALK*) rearrangements are detected in 5% of nonsquamous non–small-cell lung cancer (NSCLC) and are effectively treated with ALK inhibitors.[1] This oncogenic event is uncommon in other lung cancer subtypes.

Several tools can be used to detect the presence of gene fusions in a clinical setting, and up to now, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) have been the most widely used for the diagnosis of *ALK* rearrangements. FISH uses fluorescent probes that bind specifically to the breakpoint region in the *ALK* gene. This technique is sensitive and specific but requires specialized and expensive equipment and also expertise. IHC is easy to use and various monoclonal ALK antibodies are available, allowing the detection of ALK fusion proteins.[2] Recently, the US Food and Drug Administration approved an IHC kit (Ventana ALK D5F3 CDx Assay from Ventana Medical Systems, Tucson, AZ) as a companion diagnostic tool for crizotinib prescription. In this approach, FISH is only used for IHC-equivocal samples. Despite the efficiency of these diagnosis methods, some studies have shown FISH/IHC-discordant results,[3] and neither FISH nor IHC can

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identify the nature of the *ALK* fusion partner nor the fusion transcript generated. Indeed, numerous *ALK* fusion variants have been described corresponding to different gene breakpoints.[4]

Next-generation sequencing (NGS) is very well suited to clinical settings because it allows the identification of several gene abnormalities, including mutations and fusion variants in multiple samples. Different techniques exist, starting from DNA or RNA and including or not a multiplex-polymerase chain reaction (PCR) step or biotin-labeled oligonucleotide probes with variable sizes of gene panels. Detection of *ALK* fusions has been accurately and sensitively achieved with these methods.[5-7]

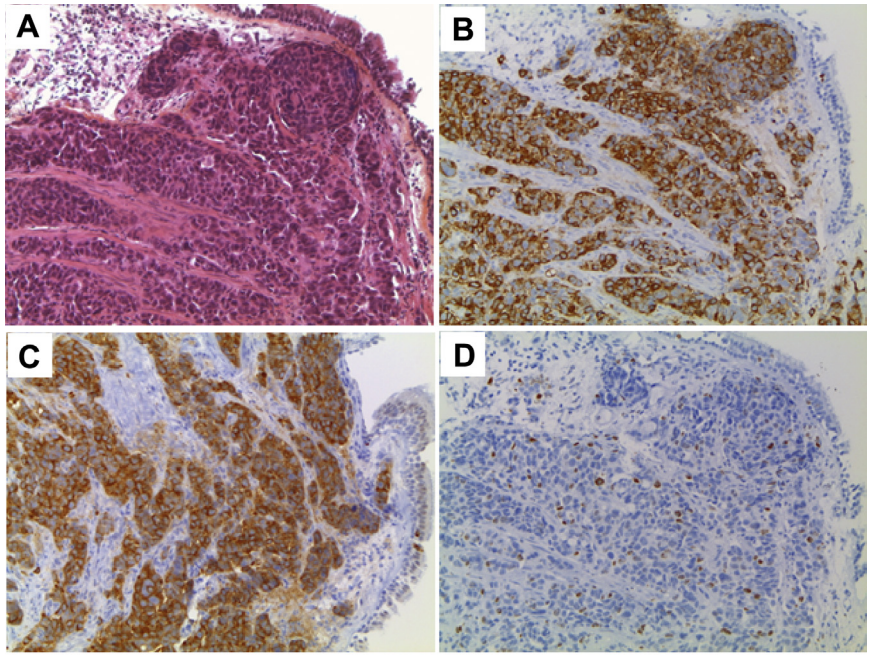
Echinoderm microtubule-associated protein-like 4 (*EML4*) is the most common partner of *ALK* in NSCLC and the most frequent variant is *EML4(13)-ALK(20)* consisting of a fusion between exon 13 of *EML4* and exon 20 of *ALK*, also called *EML4-ALK* variant 1. The effect of these *ALK* rearrangements on clinicopathological features and clinical responses to treatment remain uncertain, and even more so for *ALK* rearrangements occurring in rare histologic subtypes of lung cancer.[7-10]

We present the case of a patient with an advanced NSCLC with neuroendocrine features that harbored a rare cytoskeleton-associated proteins-Gly domain containing linker protein 1 (*CLIP1*)-*ALK* rearrangement.

Case

A 71-year-old nonsmoker woman, without comorbidity, was referred to our hospital for chronic S1 radiculopathy. Spine magnetic resonance imaging showed a diffuse nodular infiltration of

Figure 1 **Upper Panel: Light Micrographs Showing (A) Hematoxylin, Eosin, and Saffron Staining; (B) Positive Chromogranin Immunohistochemistry (IHC); (C) Positive Synaptophysin IHC; and (D) Mindbomb E3 Ubiquitin Protein Ligase 1 (MIB1) IHC. Original magnification ×200. Lower Panel: Summary of the Antibodies Used and the Staining Obtained Regarding the Immunohistochemical Characterization of the Tumor Specimen**



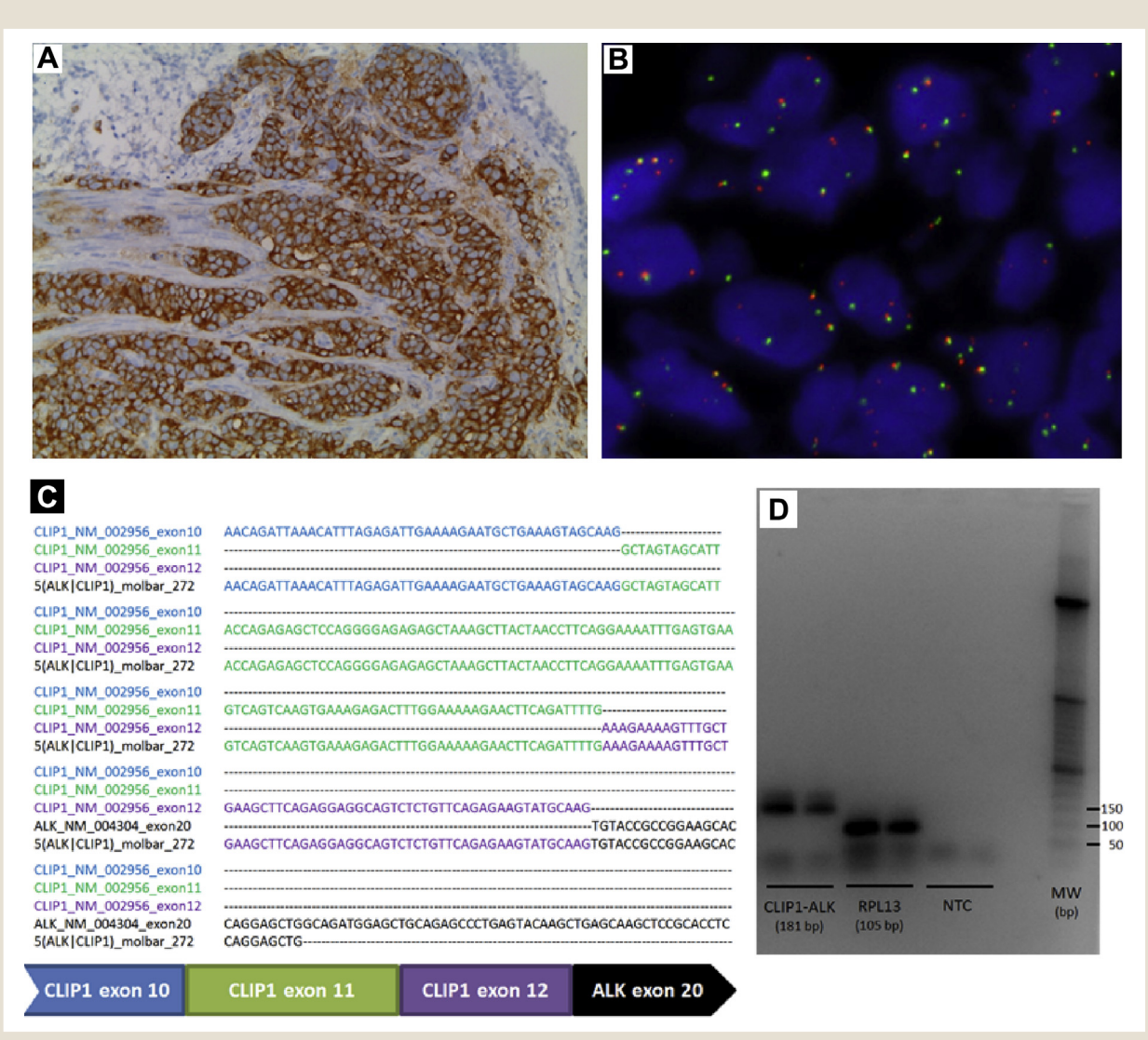
Antibody (clone)	Staining
TTF1 (clone)	Negative
P40 (clone)	Negative
Synaptophysin (clone)	Positive
Chromogranin A (clone)	Positive
CD56 (clone)	Negative
MIB1 (clone)	Positive in 15% of tumor cells

Abbreviation: TTF1 = thyroid transcription factor-1.

vertebrae. Body computed tomography imaging showed a 16-mm nodule in the left upper lobe, multiple mediastinal and left hilar lymph node enlargement, a solid nodule in the right adrenal gland, and several condensing bone lesions suggesting diffuse bone metastases. Fiberoptic bronchoscopy showed a lesion in the apical segmental bronchus of the right upper lobe. The limited tissue available from a small bronchial biopsy showed an NSCLC with neuroendocrine features. Indeed, the tumor cells were negative for TTF1 and p40 staining. Synaptophysin and chromogranin stainings were positive and CD56 was negative. Mindbomb E3 ubiquitin protein ligase 1 protein was detected in 15% of tumor cells, eliminating a small-cell lung carcinoma (Figure 1).

The tumor was negative for epidermal growth factor receptor, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, B-Raf proto-oncogene, serine/threonine kinase, and erb-b2 receptor tyrosine kinase 2 activating mutations using pyrosequencing. ALK IHC (5A4 clone) was positive in 80% of tumor cells with a staining intensity of 2+ (Figure 2A). *ALK* break-apart FISH analysis (ZytoLight SPEC *ALK* Dual Color Break-apart Probe; Clinisciences, Nanterre, France) showed multiple split 3' and 5' signals

Figure 2 **Characterization of the Anaplastic Lymphoma Kinase (ALK) Positivity of the Tumor Specimen Using (A) Immunohistochemistry (IHC) Using the 5A4 Antibody; (B) Fluorescent In Situ Hybridization (FISH) Using the ZytoVision ZytoLight SPEC *ALK* Dual Color Break-Apart Probe (Clinisciences, Nanterre, France; Rearranged *ALK* is Indicated by Separated 3' [Green] and 5' [Red] Signals); (C) the Consensus Sequence for the *CLIP1-ALK* Fusion Transcript Detected Using Anchored Multiplex Polymerase Chain Reaction (PCR) With the ArcherDx FusionPlex ALK, RET, ROS1 Kit (ArcherDx, Boulder, CO) on an IonTorrent PGM Machine and Analysis (ThermoFisher) With the Online ArcherDx Software (5.0 Version); and (D) Confirmation Using Real-Time Reverse Transcription PCR With a Preamplification Step, Followed by Agarose Gel Electrophoresis of the Presence of a *CLIP1-ALK* Fusion Transcript in the Tumor Sample. Primers Used Were: Forward Primer: Primer 2 Cytoskeleton-Associated Proteins-glycine Domain Containing Linker Protein 1 (CLIP1; Forward) and Primer ALK gene-specific primer 2 (Reverse); for Details see Supplemental Table 1 in the Online Version**



in 86% of the nuclei analyzed (Figure 2B). A 3'5' *ALK* imbalance was detected with RNA-based NGS using the Ion AmpliSeq RNA Lung Fusion Cancer Research Panel and the Ion Reporter software (ThermoFisher, Villebon-sur-Yvette, France), indicating the presence of a fusion with an unknown partner or a fusion not included in the panel (data not shown, *ALK* 3'5' imbalance = 0.1422). An anchored multiplex PCR (Archer FusionPlex ALK, RET, ROS1 v2 for Ion Torrent kit; ArcherDx, Boulder, CO) was therefore combined to the RNA sequencing to identify the *ALK* fusion partner.[5] This method allows the identification of an unknown sequence (*ALK* fusion partner) adjacent to a known sequence (*ALK* kinase domain). Briefly, after double-stranded cDNA synthesis, a first PCR is performed, using an *ALK*-specific primer and a second primer complementary to a universal adapter ligated to the fusion partner. A second nested PCR is then performed with an *ALK*-specific primer closer to the fusion point, leading to target amplicons ready for clonal amplification and sequencing. The results were analyzed with the software provided by Archer (Archer Analysis 5.0; ArcherDx) and allowed us to identify a fusion involving the *CLIP1* and *ALK* genes. More specifically, this fusion involved exon 12 of *CLIP1* and exon 20 of *ALK* (Figure 2C). The presence of the fusion between exon 12 of *CLIP1* and exon 20 of *ALK* was confirmed using real-time reverse transcription PCR using 2 sets of primers for *CLIP1* and 2 sets for *ALK* (the result obtained with 1 set of primers is shown in Figure 2D, and the primer sequences are shown in Supplemental Table 1 in the online version). The patient died before the initiation of any treatment because of disease progression.

Discussion

A fusion between intron 13 of *CLIP1* and exon 20 of *ALK* has been previously described in a cutaneous Spitz tumor,[11] a subgroup of melanocytic neoplasms, and recently, a *CLIP1-ALK* fusion was detected between exons 22 of *CLIP1* and 20 of *ALK*, in an NSCLC sample, with the same anchored multiplex kit that we used.[12] However, to our knowledge, this is the first report of an *ALK* rearrangement with *CLIP1* in a case of lung carcinoma with neuroendocrine features. Among more than 250 ALK-positive samples that were tested using RNA-based sequencing in our laboratory, with the ThermoFisher AmpliSeq and/or ArcherDx FusionPlex techniques, this is the only case harboring a fusion between *CLIP1* and *ALK*, pointing toward a rare fusion event. Similar to *EML4* and other *ALK* partner genes, *CLIP1* codes for a microtubule-associated protein, which links endocytic vesicles to microtubules.[13]

Neuroendocrine lung tumors comprise small-cell lung cancers, pulmonary carcinoids, and pulmonary large-cell neuroendocrine carcinomas. Molecular analyses of different lung neuroendocrine tumors have shown a scarce rate of *ALK* rearrangements, far lower than in lung adenocarcinomas.[14-24] Case reports suggest that anti-ALK treatments could be effective in this histological subset, especially with second-generation ALK inhibitors.[20-27] In the present case, the patient died before any anti-ALK treatment could be initiated because of clinical worsening due to cancer progression.

Numerous *ALK* fusion transcripts have been described, corresponding to different gene breakpoints in different *ALK* fusion partners. The clinical and biological effects of these different *ALK* variants are uncertain, and even more so in neuroendocrine tumors of the lung. In adenocarcinomas, clinical studies suggest a better crizotinib sensitivity for *EML4-ALK* variants 1 and 2 compared with *EML4-ALK* variant 3, but to date no statistically significant results have been obtained on the response to crizotinib of non-*EML4-ALK* variants.[7-10] Clearly, more data are needed on the link between histological, clinical, and biological characteristics (including the nature of the *ALK* fusion variant) in relation to response to treatment to the various anti-ALK therapies available.

Conclusion

In conclusion, ALK testing should not be restricted to lung adenocarcinomas, but should also include NSCLC with neuroendocrine features. Moreover, because other targetable alterations have been described in NSCLC with neuroendocrine features, such as neurotrophic tyrosine receptor kinaserearrangements or mesenchymal-epithelial transition proto-oncogene, receptor tyrosine kinase exon 14 mutations,[28,29] multiplex testing using NGS should be performed in this histological subset.

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Supplemental Data

Supplemental Table 1 List of Primers for CLIP1-ALK Fusion Detection Using Reverse Transcription Polymerase Chain Reaction

Forward Primer	Reverse Primer	Amplicon Size, bp
Primer 1 CLIP1 5'-TTACCAGAGAGCTCCAGGGG-3'	Primer ALK GSTP2 5'-CAGCTCCTGGTGCTTCCGGCG-3'	191
Primer 2 CLIP1 5'-GCTCCAGGGGAGAGAGCTAA-3'	Primer ALK GSTP2 5'-CAGCTCCTGGTGCTTCCGGCG-3'	181
Primer 1 CLIP1 5'-TTACCAGAGAGCTCCAGGGG-3'	Primer 2 ALK 5'-AGTATGCAAGTGTACCGCCG-3'	175
Primer 2 CLIP1 5'-GCTCCAGGGGAGAGAGCTAA-3'	Primer 2 ALK 5'-AGTATGCAAGTGTACCGCCG-3'	165

Abbreviations: ALK = anaplastic lymphoma kinase; bp = base pair; CLIP1 = CAP-Gly domain containing linker protein 1; GSP2 = gene-specific primer 2.