CANCER BIOLOGY & THERAPY
2018, VOL. 19, NO. 11, 962–966
https://doi.org/10.1080/15384047.2018.1480282

Taylor & Francis
Taylor & Francis Group

BEDSIDE TO BENCH REPORT

OPEN ACCESS Check for updates

CMTR1-ALK: an *ALK* fusion in a patient with no response to ALK inhibitor crizotinib

Xue Du[a*], Yun Shao[a*], Hongjun Gao[b], Xueli Zhang[a], Han Zhang[a], Yi Ban[a], Haifeng Qin[b], and Yanhong Tai[a]

[a]Department of Pathology, Hospital of Chinese PLA, Cancer Center of People's Liberation Army of China, Beijing, China; [b]Department of Lung Cancer, Hospital of Chinese PLA, Cancer Center of People's Liberation Army of China, Beijing, China

ABSTRACT

The targeted treatment of advanced non-small cell lung cancer (NSCLC) harboring genomic rearrangement of *ALK* is a paradigm for personalized oncology. More than 15 different ALK fusion partners have been discovered in NSCLC patients. Most of these *ALK* fusions responded well to the ALK inhibitor crizotinib. Crizotinib is an oral MET/ALK inhibitor used as first-line therapy in the treatment of advanced NSCLC harboring ALK rearrangement. An understanding of the mechanisms by which tumors harbor primary drug resistance or acquired resistance to targeted therapies is critical for predicting which patients will respond to a specific therapy and for the identification of additional targetable pathways to maximize clinical benefits. Cap methyltransferase 1(*CMTR1*) also known as hMTr1, which is translate a human cap1 2′-o-ribose methyltransferase. Here, we report the newly found *ALK* fusion, *CMTR1-ALK*, in a patient who has no response to the *ALK* inhibitor crizotinib. The results remind us that detecting *ALK* status is important, but that determining the *ALK* fusion type and function may be more important for patient.

ARTICLE HISTORY
Received 28 December 2017
Revised 14 May 2018
Accepted 20 May 2018

KEYWORDS
Anaplastic lymphoma kinase (*ALK*); *CMTR1*; ALK-tyrosine kinase inhibitor (TKI); *ALK*-rearranged NSCLC; fusion partner gene; crizotinib

Introduction

Non-small-cell lung cancer (NSCLC) accounts for approximately 80–85% of lung cancer deaths and is a leading cause of cancer-related mortality for both men and women worldwid.[1–5] Anaplastic lymphoma kinase (ALK) rearrangements have been identified in 5–6% of NACLC.[6] The targeted treatment of advanced NSCLC harboring genomic rearrangement of ALK is a paradigm in personalized oncology.[7]

The Echinoderm microtubule-associated protein-like 4 (*EML4-ALK*) fusion gene is found in 3–5% of NSCLC. *EML4-ALK* fusion in NSCLC occurs in at least 23 variants. All known variants have a breakpoint in intron 19 of *ALK*, but a variety of introns in *EML4* experience rearrangement, which results in inclusion of differing fragments of the N-terminal region of *EML*.[8] Hybrid-capture-based genomic sequencing that targets intron 19 of ALK can capture all possible breakpoint events involving this intron and unequivocally identify the partner gene. *In vitro* modeling of *EML4-ALK* demonstrates that it is an oncogenic driver that is sensitive to multiple ALK kinase inhibitors such as the approved drug crizotinib, consistent with *in vivo* studie.[9] Clinical trials have established *ALK* inhibitors as standard treatment for patients with advanced ALK-rearranged NSCLC, with crizotinib response rates of approximately 74% and median progression-free survival (PFS) of 10.9 months, compared with approximately 45% and 7.0 months, with pemetrexed plus either cisplatin or carboplatin, respectivel.[6]

Detection of ALK rearrangements in patients with NSCLC has recently become a routine pathological diagnosis. There are three major conventional diagnostics for ALK fusion: fluorescence *in situ* hybridization (FISH), real time reverse-transcriptase polymerase chain reaction (RT-PCR), and immunohistochemistry (IHC). Next generation sequencing has displayed impressive capability to detect FISH-negative and novel fusion type ALK-rearranged NSCL[10] and has developed into a clinically practical alternative for diagnosis of ALK rearrangements in addition to FISH, IHC, and RT-PCR based techniques.

Besides *EML4*, there are other partner genes that can be fused with *ALK*. Currently, more than 15 different ALK fusion partners have been discovered in NSCLC including *EML4, KIF5B, KLC1*, and *TFG*. Most of these *ALK* fusions in NSCLC patients responded well to the ALK inhibitor crizotinib. Recently, we reported a novel *ALK* fusion partner gene *CMTR1* in an NSCLC patient who has no response to the ALK inhibitor crizotinib. *CMTR1* also known as hMTr1, which is translate a human cap1 2′-o-ribose methyltransferas.[11] We analyzed the *CMTR1-ALK* fusion gene sequence and determined that it is a null fusion type. This result suggests that while the detection of *ALK* status is important, confirming the fusion gene production may be more important.

Case presentation

A 75-year-old Chinese man with a 1-pack-year smoking history of 50 years and a history of early-stage NSCLC (adenocarcinoma of lung T1N0M0) diagnosed 7 years before the patient presented with mediastinal adenopathy with probable metastatic disease within multiple lymph nodes in the left side of his neck. The patient had a lung resection on Apr. 2010.

CONTACT Yanhong Tai taiyanhong29@163.com; Haifeng Qin hifo@263.net No. 8 DongDaJie, Fengtai District, Beijing 100071, China
*These authors contributed equally to this work and should be considered co-first author.

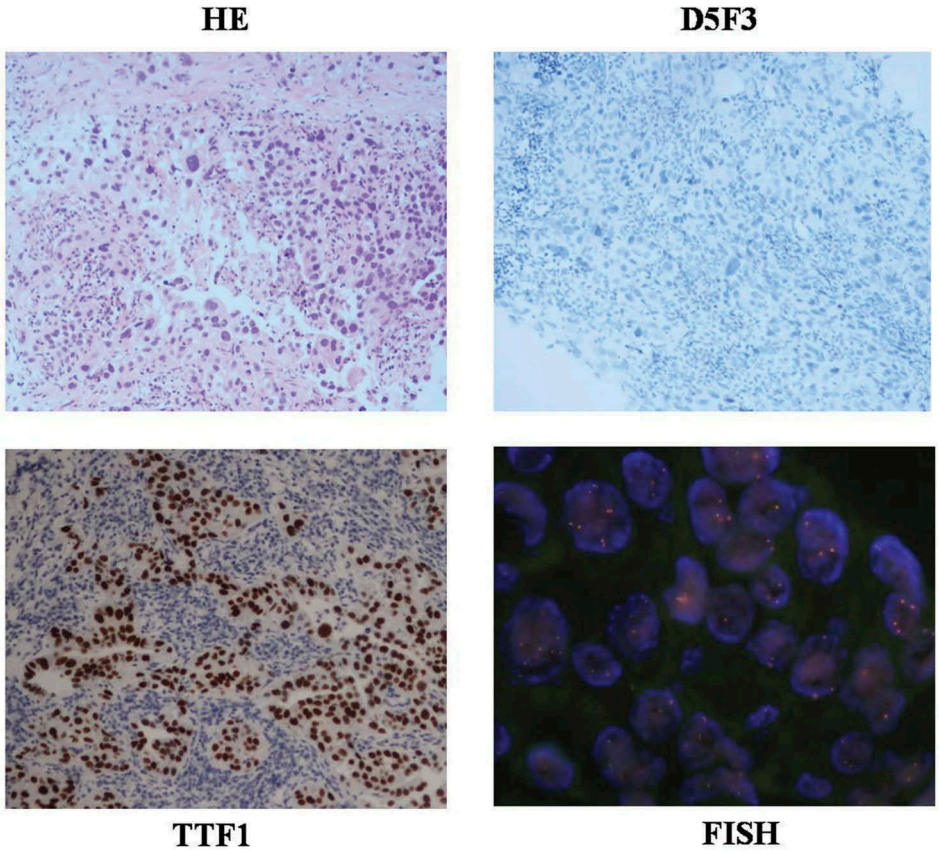

Figure 1. Hematoxylin and eosin(HE) staining, TTF1 staining, Ventana IHC(D5F3), and FISH staining slides from patient.

After surgery, the disease was under control until July 2014 when a lung lesion was found by computed tomography scan. The patient had signs and symptoms of dysphagia, choking, and hoarseness in September 2014. He took 5 cycles of radiation treatment (Dt 32.5 gry) starting in October 2014 (Figure S1). Needle aspiration biopsy of the cervical lymph node in July 2016 revealed that the tumor was a metastatic, poorly differentiated carcinoma (Figure 1). This sample was identified as TTF1 (Figure 1) and was napsin A expression-positive by IHC staining. Ventana IHC (D5F3) analyses of ALK protein expression were negative (Figure 1).

In order to take molecular targeted therapeutic drugs, the lymph node FFPE specimen was subjected to qRT-PCR, FISH, and genomic profiling using a clinical grade NGS assay (Illumina Hiseq 4000 NGS platforms). Results showed that this specimen was PCR-positive, FISH-negative, and NGS-positive (Table 1). NGS result revealed that this tumor specimen harbored a previously unknown *CMTR1-ALK* fusion variant (Figure 2) involving rearrangement and fusion of exon 1 to 2 of *CMTR1* to exon 20 to 29 of *ALK* (C2; A20). This fusion comprises only 3% of the sequencing runs where tumor purity in the biopsy sample was estimated to be 40% (Table 1).

Based on these results, treatment was initiated with 250 mg crizotinib per day starting in September 2016. The disease still developed 1 month after drug treatment showing that this patient has no response to crizotinib (Figure S1). Then the patient dropped out of the crizotinib treatment and was treated with pemetrexed in November 2016, which delayed disease progression. The disease has remained under control for approximately 13 months at this time.

Table 1. Pathologic characteristics and molecular test results of the patient.

Sample Types	LB	ALK FISH	2%	NGS	CMTR1-ALK	*EGFR/KRAS* utations	WT/WT
TTF1 IHC	+	ALK IHC	-	NGS ALK(%)	3%	*BRAF* V600 EMutations	WT
Naspin A IHC	+	ALK RTPCR	+	Tumor Content	40%	*ROS1* Fusion	WT

Discussion

In recent years, genetic alterations that are responsible for initiation and maintenance of the malignant phenotype have been identified in several cancers including NSCLC. Drugs targeting these alterations, often named "driver" genetic alterations, can provide significant clinical benefit. One of the genetically defined subsets of NSCLC is the *ALK* positive subset. In recent years, major therapeutic advances have occurred in this subset of NSCLC.

In 2007, Soda and colleagues first identified the EML4-ALK oncogenic fusion gen.[12] It is now recognized that there are several different variants of *EML4-ALK* translocation that are dependent on variations in the length of the *EML4* gene involved in the fusion. In addition, in less than 5% of *ALK* + NSCLCs, the fusion partner is not *EML4* and instead is one of more than 15 genes such as *KIF5B, TFG, KLC1, TPR, HIP1, BIRC6*, and *BCL11*.[11,13–18]

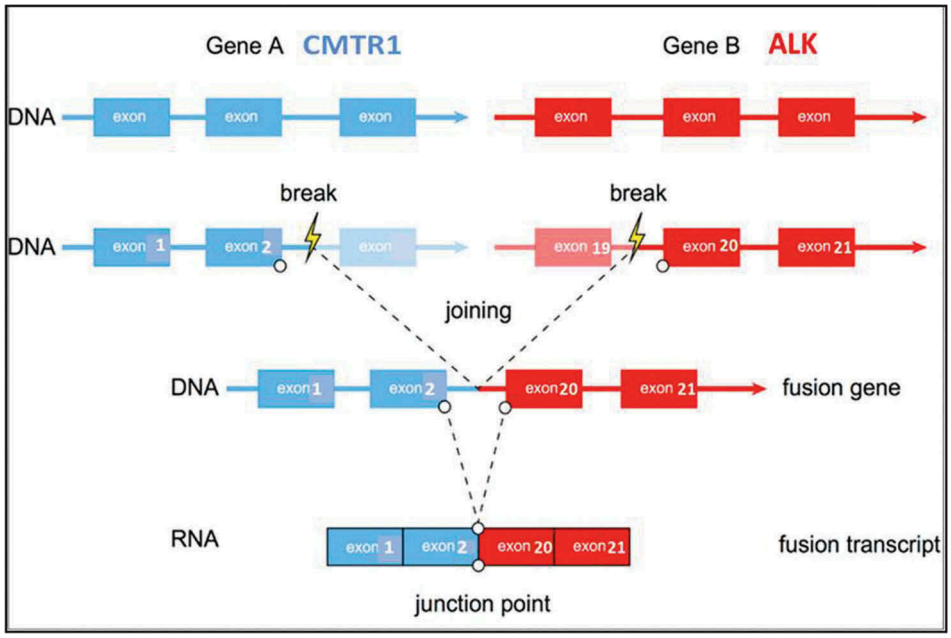

Figure 2. Schematic structure of the genomic DNA and RNA sequence.

Advances in the management of *ALK*+ NSCLC started with the recognition that crizotinib can provide clinical benefit in these patients. Recently, Yoshida et al. reported the variability of crizotinib activity in tumors with different *ALK* variant.[19] The variants are defined by the size of the *EML4* gene involved in the *ALK* fusion. In a relatively small series, Yoshida and colleagues demonstrated that the median PFS was 11 months in variant 1, the most common variant, but was only 4.2 months in patients with tumors that were not variant 1. These data suggest that better understanding of the biological factors that influence the activity of crizotinib may allow a more personalized approach to treating *ALK*+ NSCLC patient.[20]

For this patient, we first used RT-PCR to detect the *ALK* gene and found that he was ALK-positive. After one month of taking crizotinib, the patient was found to have primary drug resistance. To analyze the cause of primary drug-resistance, we used three other methods to detect the *ALK*-status. The results revealed that the patient was *ALK*-positive on the DNA level (PCR and NGS) but negative on the protein level (IHC-D5F3). Additionally, the "gold standard" FISH assay result was *ALK*-negative. The positive result from PCR was confusing, because the commercial PCR kit we used does not include the fusion type of this patient (Table S1). In future research, we will explore the underlying mechanism for this result using different methods.

The NGS result revealed a novel *CMTR1-ALK* fusion gene in NSCLC, which we reported in another paper (in preparation). In that study we analyzed the *CMTR1-ALK* fusion gene using online software PRABI (http://doua.prabi.fr/software/cap3), which indicated that the coiled-coil structure of CMTR1-ALK protein has a weakened dipolymerization capacity which partly causes reduced ALK protein kinase activity.

To confirm our speculation, we simulated the structure of this novel fusion protein. Based on NGS, this tumor specimen harbored a new *CMTR1-ALK* fusion variant involving rearrangement and fusion of exon 1 to 2 of *CMTR1* to exon 20 to 29 of *ALK* (C2; A20). The breakpoint of the fusion gene is in intron 2 of the *CMTR1* genomic DNA. During the process of building the fusion protein structure, we analyzed the *CMTR1* mRNA sequence and found that exons 1 and 2 of *CMTR1* contain 386 nucleotides, which is not a multiple of 3 required for a codon. This means that in exons 1 and 2 of the *CMTR1* gene replication and expression are linked together with follow-up exons. Once they fuse with *ALK*, the last 2 nucleotides of *CMTR1* will occupy 1 nucleotide of *ALK* (Figure 3).

The normal structure of *ALK* (Figure 4,[21] together with its fusion pattern, may function as a cell surface receptor for specific ligands that regulate the proliferation or differentiation of neural cell.[22] In this case, the *CMTR1-ALK* fusion gene caused an ALK gene frameshift mutation, which means that the fusion gene cannot translate kinase-active *ALK* fusion protein, explaining the IHC *ALK*-negative result.

According to China Food and Drug Administration (CFDA), when *ALK* fusion status is positive by any of the three platforms – FISH, IHC (Ventana), or RT-PCR – there is evidence for using targeted drug therapy in that patient. Despite the excellent efficacy of crizotinib in most *ALK*-positive lung cancers, heterogeneous responses have been reported and the reason for this variability is unknow.[9,23,24] The mechanism of primary resistance is complicated and deserves more detailed exploratio.[25] According to previous studies, there are three major mechanisms of resistance to targeted drug therapy: genetic alteration in the target; activation of bypass tracks or phenotypic change in the tumor such as development of epithelial mesenchymal transition; or limited penetration of "sanctuary" sites such as the central

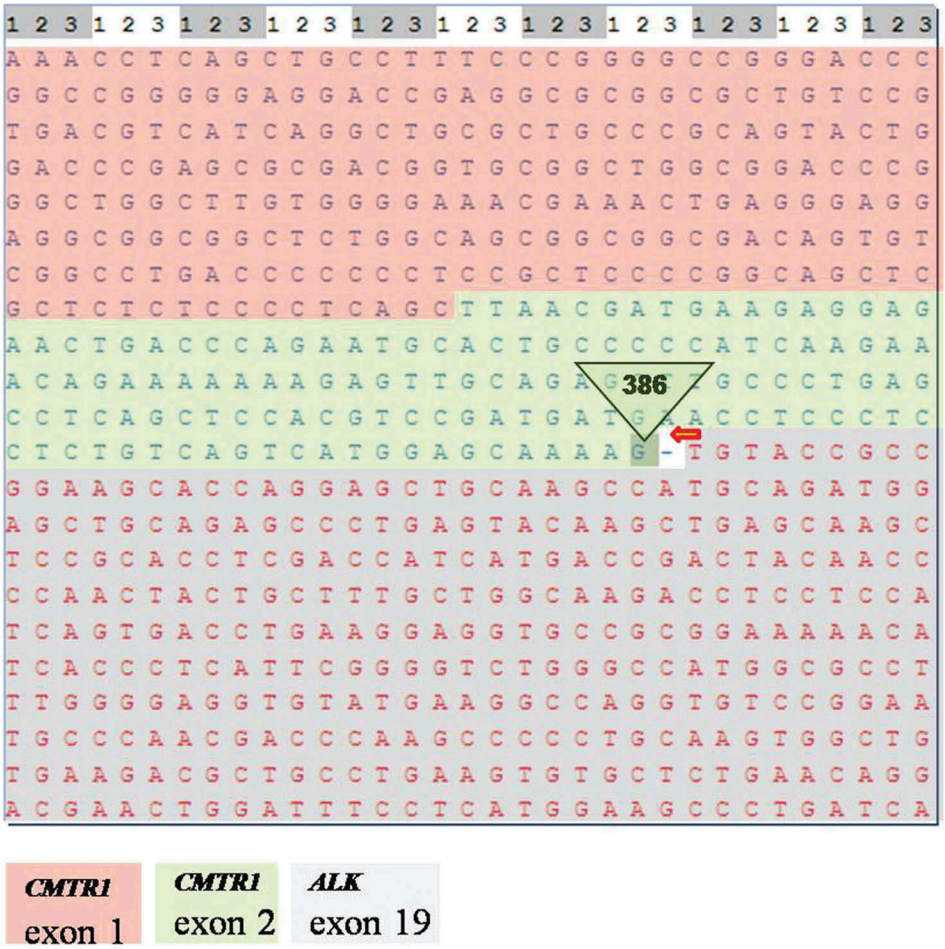

Figure 3. mRNA sequence of CMTR1-ALK fusion gene.

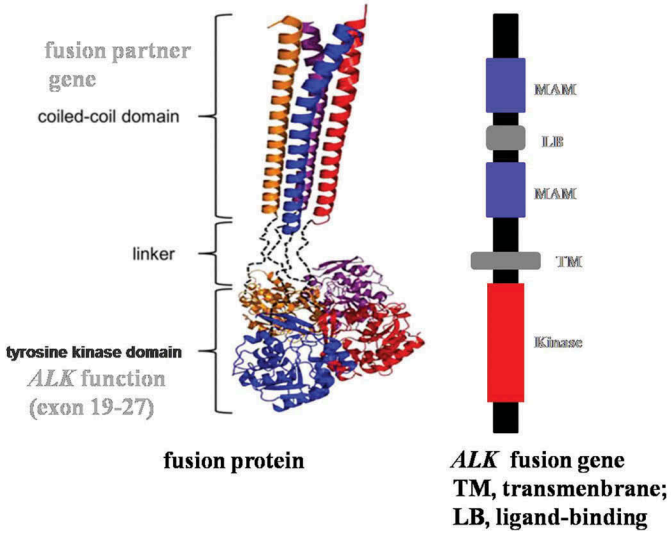

Figure 4. Schematic illustration of ALK and the general fusion strategy.

nervous syste.[20,26] All three mechanisms of resistance have been identified in patients treated with crizotini.[20,27,28] We are the first to report intrinsic resistance to crizotinib caused by *ALK* gene frameshift mutation. In this case, the *ALK* fusion status was positive, but the patient had primary resistance to *ALK* inhibitor. By NGS and analysis of the fusion gene sequence, we found that the *ALK* fusion type is a null fusion which cannot translate "driven" protein that cause cancer incidence. This result shows that when the detection of *ALK* status is positive in a patient with drug resistance, we should pay more attention to the underlying mechanism.

Conflicts of interest

The authors have no conflicts of interest to declare.

Funding

This research was supported by National Natural Science Foundation of China. The grant number is [81372834];

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. Cancer J Clinicians. 2015;65(2):87–108. doi:10.3322/caac.21262.
2. Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev. 2010;19(8):1893–1907. doi:10.1158/1055-9965.epi-10-0437.

3. Wen M, Wang X, Sun Y, Xia J, Fan L, Xing H, Zhang Z, Li X. Detection of EML4-ALK fusion gene and features associated with EGFR mutations in Chinese patients with non-small-cell lung cancer. OncoTargets Ther. 2016:1989. doi:10.2147/ott.s100303.
4. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. Cancer J Clinicians. 2013;63(1):11–30. doi:10.3322/caac.21166.
5. Shaw AT, Engelman JA. ALK in lung cancer: past, present, and future. J Clin Oncol. 2013;31(8):1105–1111. doi:10.1200/jco.2012.44.5353.
6. Devarakonda S, Morgensztern D, Govindan R. Genomic alterations in lung adenocarcinoma. Lancet Oncol. 2015;16(7):E342–E351. doi:10.1016/S1470-2045(15)00077-7.
7. Shaw AT, Hsu PP, Awad MM, Engelman JA. Tyrosine kinase gene rearrangements in epithelial malignancies. Nat Rev Cancer. 2013;13(11):772–787. doi:10.1038/nrc3612.
8. Heuckmann JM, Balke-Want H, Malchers F, Peifer M, Sos ML, Koker M, Meder L, Lovly CM, Heukamp LC, Pao W, et al. Differential protein stability and ALK inhibitor sensitivity of EML4-ALK fusion variants. Clin Cancer Res. 2012;18(17):4682–4690. doi:10.1158/1078-0432.ccr-11-3260.
9. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 2013;368(25):2385–2394. doi:10.1056/NEJMoa1214886.
10. Ali SM, Hensing T, Schrock AB, Allen J, Sanford E, Gowen K, Kulkarni A, He J, Suh JH, Lipson D, et al. Comprehensive genomic profiling identifies a subset of crizotinib-responsive ALK-rearranged non-small cell lung cancer not detected by fluorescence in situ hybridization. Oncologist. 2016;21(6):762–770. doi:10.1634/theoncologist.2015-0497.
11. Takeuchi K, Choi YL, Togashi Y, Soda M, Hatano S, Inamura K, Takada S, Ueno T, Yamashita Y, Satoh Y, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res. 2009;15(9):3143–3149. doi:10.1158/1078-0432.ccr-08-3248.
12. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara SI, Watanabe H, Kurashina K, Hatanaka H, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007;448(7153):561–U3. doi:10.1038/nature05945.
13. Wong DWS, Leung ELH, Wong SKM, Tin VPC, Sihoe ADL, Cheng LC, Au JSK, Chung LP, Wong MP. A novel KIF5B-ALK variant in nonsmall cell lung cancer. Cancer. 2011;117(12):2709–2718. doi:10.1002/cncr.25843.
14. Togashi Y, Soda M, Sakata S, Sugawara E, Hatano S, Asaka R, Nakajima T, Mano H, Takeuchi K, Lo AWI. KLC1-ALK: A novel fusion in lung cancer identified using a formalin-fixed paraffin-embedded tissue only. Plos One. 2012;7(2):e31323. doi:10.1371/journal.pone.0031323.
15. Choi YL, Lira ME, Hong M, Kim RN, Choi SJ, Song JY, Pandy K, Mann DL, Stahl JA, Peckham HE, et al. A novel fusion of TPR and ALK in lung adenocarcinoma. J Thoracic Oncol. 2014;9(4):563–566. doi:10.1097/jto.0000000000000093.
16. Fang DD, Zhang B, Gu QY, Lira M, Xu Q, Sun HY, Qian MX, Sheng WQ, Ozeck M, Wang ZX, et al. HIP1-ALK, a novel ALK fusion variant that responds to crizotinib. J Thoracic Oncol. 2014;9(3):285–294. doi:10.1097/jto.0000000000000087.
17. Shan L, Jiang PD, Xu F, Zhang WL, Guo L, Wu J, Zeng YX, Jiao YC, Ying JM. BIRC6-ALK, a novel fusion gene in ALK break-apart FISH-negative lung adenocarcinoma, responds to crizotinib. J Thoracic Oncol. 2015;10(6):E37–E39. doi:10.1097/jto.0000000000000467.
18. Tian Q, Deng WJ, Li ZW. Identification of a novel crizotinib-sensitive BCL11A-ALK gene fusion in a nonsmall cell lung cancer patient. Eur Respir J. 2017;49(4) doi:10.1183/13993003.02149-2016.
19. Ruano-Ravina A, Provencio-Pulla M. Differential crizotinib response duration among ALK fusion variants in ALK-positive non-small-cell lung cancer. Transl Cancer Res. 2017; 6:S54–S56. doi:10.21037/tcr.2017.02.26.
20. Gadgeel SM. Sequencing of ALK inhibitors in ALK plus non-small cell lung cancer. Curr Treat Options Oncol. 2017;18(6):12. doi:10.1007/s11864-017-0479-8.
21. Diener M, Kopka B, Pohl M, Jaeger K-E, Krauss U. Fusion of a coiled-coil domain facilitates the high-level production of catalytically active enzyme inclusion bodies. ChemCatChem. 2016;8(1):142–152. doi:10.1002/cctc.201501001.
22. Mano H. ALKoma: a cancer subtype with a shared target. Cancer Discovery. 2012;2(6):495–502. doi:10.1158/2159-8290.cd-12-0009.
23. Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, Felip E, Cappuzzo F, Paolini J, Usari T, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer (vol 371, pg 2167, 2014). N Engl J Med. 2015;373(16):1582. doi:10.1056/NEJMx150034.
24. Ou SHI, Ahn JS, De Petris L, Govindan R, Yang JCH, Hughes B, Lena H, Moro-Sibilot D, Bearz A, Ramirez SV, et al. Alectinib in crizotinib-refractory ALK-rearranged non-small-cell lung cancer: a phase II global study. J Clin Oncol. 2016;34(7):661-+. doi:10.1200/jco.2015.63.9443.
25. Toyokawa G, Seto T. ALK inhibitors: what is the best way to treat patients with ALK(+) non-small-cell lung cancer? Clin Lung Cancer. 2014;15(5):313–319. doi:10.1016/j.cllc.2014.05.001.
26. Camidge DR, Pao W, Sequist LV. Acquired resistance to TKIs in solid tumours: learning from lung cancer. Nat Rev Clin Oncol. 2014;11(8):473–481. doi:10.1038/nrclinonc.2014.104.
27. Katayama R, Shaw AT, Khan TM, Mino-Kenudson M, Solomon BJ, Halmos B, Jessop NA, Wain JC, Yeo AT, Benes C, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. Sci Transl Med. 2012;4(120):120ra17–120ra17. doi:10.1126/scitranslmed.3003316.
28. Doebele RC, Pilling AB, Aisner DL, Kutateladze TG, Le AT, Weickhardt AJ, Kondo KL, Linderman DJ, Heasley LE, Franklin WA, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res. 2012;18(5):1472–1482. doi:10.1158/1078-0432.ccr-11-2906.