Lung Cancer 160 (2021) 32–35

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Identification of a EML4-ALK exon 19 fusion variant in lung adenocarcinoma and alectinib resistance

Di Liu [a,b,c], Xinyan Xu [a,b,c], Junmiao Wen [a,b,c], Chi Zhang [d,*], Min Fan [a,b,c,*]

[a] Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China
[b] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, People's Republic of China
[c] Shanghai Key Laboratory of Radiation Oncology, Shanghai, People's Republic of China
[d] School of Biological Sciences, University of Nebraska, Lincoln, NE, USA

ARTICLE INFO

Keywords:
Lung cancer
ALK variants
ALK TKIs
Case report

ABSTRACT

Alectinib, a highly selective inhibitor of anaplastic lymphoma kinase (ALK), has shown a high response rate and long progression-free survival in primary treatment of ALK-positive non-small-cell lung cancer (NSCLC). De novo resistance or refractory subtype is rare event. Herein, we identify the first case with serial next-generation sequencing (NGS) results that harboured a rare echinoderm microtubule associated protein like 4 gene (*EML4*) -*ALK* (breaking site at exon 19) fusion in a lung adenocarcinoma (LUAD) patient who acquired alectinib resistance rapidly (less than 3 months), followed by multi-drug resistance and short survival time.

1. Case report

A 28-year-old female patient (a never-smoker) without family history and history of prior malignancies, radiation and chemotherapy, was admitted to Fudan University Shanghai Cancer Center in January 2020, for left lower limb pain. Magnetic resonance imaging (MRI) showed L3/4 intraspinal tumor in the posterior lumbar spine (Fig. 1A), and then lumbar spinal tumor resection and internal fixation were performed. The pathological diagnosis indicated metastatic poorly differentiated lung adenocarcinoma (LUAD). Positron emission tomography/computed tomography (PET-CT) found a primary tumor (3.2 cm) in the right upper lobe lung with mediastinal lymphadenopathy, multiple bones (T10, L3-5, sacrum, pelvis and ribs), and bilateral lung metastases with pleural effusion (Fig. 1A). An anaplastic lymphoma kinase (ALK) immunohistochemical testing (Ventana Medical System) led to a diagnosis of advanced ALK-positive LUAD (cT4N2M1c, stage IVc; Fig. 1B and C). Alectinib (600 mg/day) was administered from February 11, 2020. As the result, the pain was relieved and the patient's performance status improved. Meanwhile, Foundation Medicine (FM) FoundationOne CDx test, a NGS based diagnosis test, of the patient's spinal tumor tissue sample identified *EML4-ALK* (breaking site at exon 19) fusion (Fig. 2A), *SMARCA4-CARM1* fusion, and *TP53* R196* mutation (Table 1). The patient did not come back for a follow-up in the next three months due to the COVID-19 pandemic.

In May 2020, the patient returned to the clinics with right buttock pain. PET/CT showed the primary tumor enlargement (4 cm), left sacroiliac joint metastasis shrinkage, and right sacral and anterosuperior iliac metastases progression with T1 new lesion. The patient had disease progression, and then crizotinib (500 mg/day) was administered followed by sequential pelvis metastasis radiotherapy (DT: 3000 cGy/12 fraction) and the pain slightly relieved.

In July 2020, the patient felt dyspnea and CT showed massive right pleural effusion (Fig. 1A). Drainage of pleural effusion was performed, and cancer cells were identified in the fluid. The pleural effusion samples were subjected to FM for a FoundationOne CDx test. Before receiving the report, bevacizumab combined with pemetrexed and cisplatin chemotherapy regimen was administered for one cycle on August 6, 2020. The pain was not relieved and grade III neutropenia and leukopenia happened, then alectinib (600 mg/day) was taken from August 14. On August 27, the FoundationOne CDx test report showed that, besides *EML4-ALK* fusion, *SMARCA4-CARM1* fusion, and *TP53* mutation, more mutations, including *MET* Y1230H, Y1230N, D1228N mutation, and *PIK3CB*, *MET*, *EPHB1*, *MYC* amplification, appeared (Table 1). Almost at the same time, the patient developed increased dyspnea, CT showed right pleural metastases enlargement with pleural effusion regrown and left pulmonary metastases progression (Fig. 1A). Cabozantinib (40 mg/day) was taken, followed by drainage of malignant pleural effusion and one more FoundationOne CDx test. The test revealed similar genetic

* Corresponding authors at: Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China (M. Fan).
E-mail addresses: czhang5@unl.edu (C. Zhang), fanmin_fuscc@aliyun.com (M. Fan).

https://doi.org/10.1016/j.lungcan.2021.07.020
Received 14 April 2021; Received in revised form 26 July 2021; Accepted 30 July 2021
Available online 6 August 2021

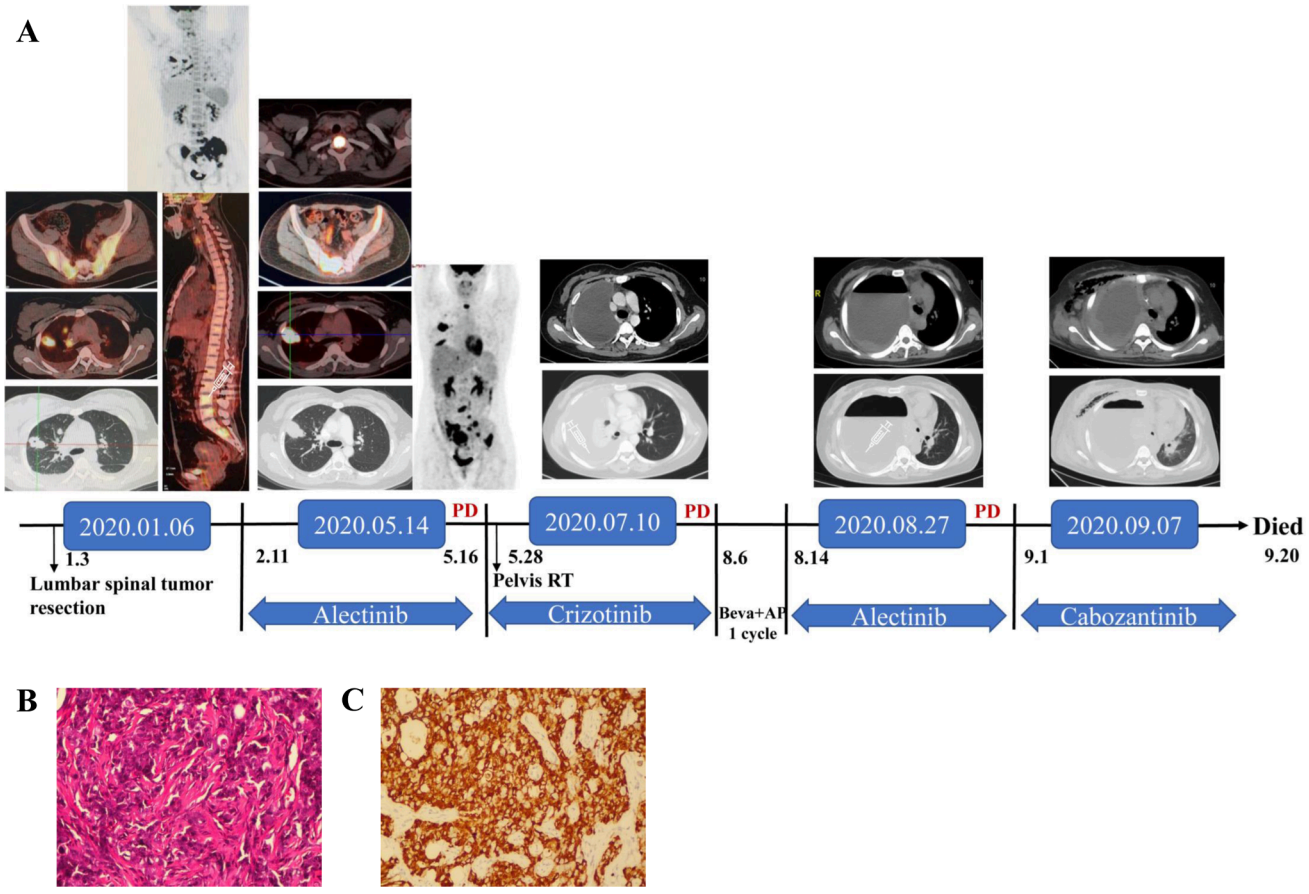

Fig. 1. Patient clinical course and pathological findings to the metastatic bone tumor tissue sample. (A) The clinical course of the diagnosis and treatment of the patient. (B) Hematoxylin and eosin staining, original magnification ×200. (C) ALK rearrangement detected by immunochemistry (Ventana Medical Systems, Tucson, AZ), original magnification ×200. PD, progressive disease; RT: radiotherapy; Beva, bevacizumab; AP, pemetrexed and cisplatin; ALK, anaplastic lymphoma kinase gene.

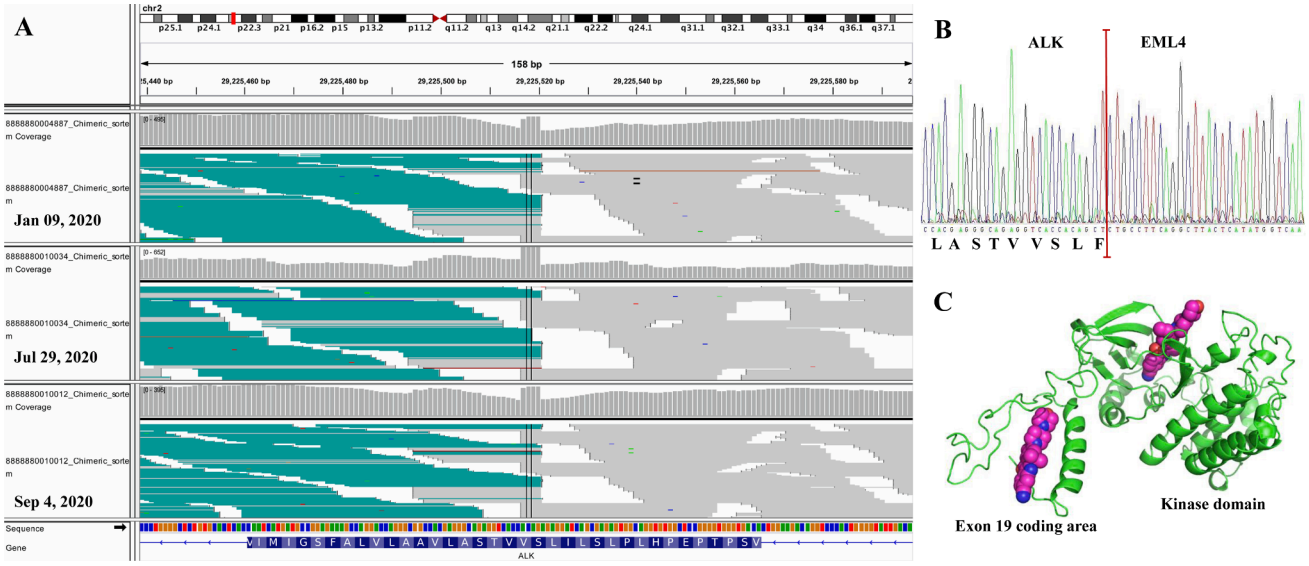

Fig. 2. The next-generation sequencing findings and the Sanger sequencing validation of the *EML4-ALK* gene fusions in the case. (A) Sequencing chimeric reads of *ALK* detected at three times of tissue samples are shown by the Integrative Genomic Viewer (IGV). (B) Sanger sequencing of the fusion boundary of the *ALK* exon 19 and *EML4* intron 13 in the pleural effusion samples. (C) Modeled protein complex structure of ALK protein and alectinib molecules (purple in sphere model). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparison of NGS mutation profiling between baseline and after treatment.

Mutations	AF/CN (Jan 09, 2020[b])	AF/CN (Jul 29, 2020[c])	AF/CN (Sep 4, 2020[c])
EML4-ALK (E13; A19[a])	11.3%	14.9%	19.6%
TP53 R196*	64.7%	26.7%	62.9%
SMARCA4-CARM1 (C1; S25[a])	40.2%	9.9%	28.4%
TMB (Mutations/Mb)	3	6	4
Microsatellite status	MSS	MSS	MSS
MET Y1230H		3.1%	–
MET D1228N		2.5%	1.7%
MET Y1230C		–	1.1%
PIK3CB amplification		CN = 10	CN = 17
MET amplification		CN = 14	CN = 18
EPHB1 amplification		CN = 10	CN = 17
MYC amplification		CN = 9	–

The percentages represent the mutant allele fraction.
EML4, echinoderm microtubule associated protein like 4 gene; *ALK*, anaplastic lymphoma kinase gene; AF, allele fraction; CN, copy number; Jan, January; Jul, July; Sep, September; *SMARCA4*, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 gene; *CARM1*, coactivator associated arginine methyltransferase 1 gene; TMB, tumor mutation burden; MSS, microsatellite stability; *MET,* mesenchymal epithelial transition factor receptor gene; *PIK3CB*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta gene; *EPHB1*, EPH receptor B1 gene; *MYC*, v-myc avian myelocytomatosis viral oncogene homolog gene.
[a] Breakpoint in the exon.
[b] Bone metastases before treatments.
[c] Pleural effusions after treatments.
[*] Describe a stop codon.

variations as July 29 (Table 1). The patient died of tumor progression 14 days thereafter (Fig. 1A).

2. Discussion

ALK positive non-small cell lung cancer (NSCLC) is a heterogeneous disease, and identifying *ALK* gene rearrangements is important as ALK fusion variants [1] may affect clinical efficacy. With the increasing adoption of the deep sequencing methods for testing molecular profiling of ALK positive NSCLC, more *ALK* fusion partners and fusion forms have been reported in recent years [2]. Most *ALK* variants were sensitive to ALK tyrosine kinase inhibitors (TKIs), and de novo resistance or refractory subtype is uncommon. In the present case, we found a rare *EML4-ALK* (breaking site at exon 19) fusion variant which correlated with rapidly acquired alectinib resistance and multi-drug refractory.

We validated *EML4-ALK* fusion by Sanger sequencing after DNA amplification with polymerase chain reaction (PCR) to the pleural effusion samples (Fig. 2B and Supplement Data 1 for the primer sequence and the *EML4-ALK* DNA sequence). It is plausible that the extremely rapidly acquiring resistance to alectinib of this patient was associated with the special structure of the novel *ALK*-rearrangement. Compared with the *EML4-ALK* V1 variant, the additional DNA sequence in exon 19 of *ALK* coding partial ALK transmembrane helix domain, which contains several hydrophilic amino acids. A protein complex structure of ALK and two alectinib molecules, modeled by I-TASSER [3] and SwissDock [4], showed the potential binding site of alectinib molecule on the exon 19 coding region of *ALK* (Fig. 2C and Supplement Data 2). The potential TKIs binging sites caused by the additional part could compete with the original TKIs binging sites in ALK tyrosine kinase domain. It is discovered that EML4-ALK fusions can form de novo membraneless cytoplasmic protein granules which can activate RAS in a lipid membrane-independent manner and higher-order protein assembly is critical for oncogenic RAS/ MAPK signalling [5]. In our discovered *EML4-ALK* variant, ALK has about 12 more strong hydrophilic amino acids than other variants. In principle, these strong hydrophilic amino acids can significantly increase EML4-ALK to assemble granules by hydrophobic interactions. This is also potential reason to explain the new *EML4-ALK* fusion variant cause the case worse.

It has been found that *ALK/TP53* co-mutated patients have inferior progression free survival (PFS) compared with *TP53* wild-type patients treated with alectinib (median PFS, 11.7 months vs. not reached, P = 0.0008) [6,7]. For this case, the patient experienced progression less than three months after alectinib administration, which is well below the median PFS of 11.7 months. This data seemed to suggest that the identified *EML4-ALK* (breaking site at exon 19) fusion variant was correlated with poor response to ALK TKIs and worse outcome independent of *TP53* status. Future studies are needed.

The *TP53* R196* mutation, an early stop codon, led to almost complete loss of TP53 protein function, such as securing genomic stability, DNA repair and apoptosis. Studies revealed that the co-occurrence of early *TP53* mutations in ALK positive NSCLC can lead to chromosomal instability, including amplification of known cancer genes [8]. In this case, *MET*, *MYC*, *PIK3CB*, and *EPHB1* genes' amplifications were detected in the pleural effusion samples on July 29 (Table 1). *MET* amplifications were shown to be one of the acquired mechanisms of ALK TKIs resistance [9,10]. Two months after crizotinib administered, more mutations, including *MET* Y1230H, Y1230C and D1228N mutations, were acquired as well. Previously, mutations in *MET* tyrosine kinase domain mediates crizotinib resistance has been reported [9,11].

To the best of our knowledge, this is the first time the rare *EML4-ALK* (breaking site at exon 19) fusion was reported. The presence of this rare *EML4-ALK* fusion identified high-risk case with earlier treatment failure and a need for more aggressive surveillance and treatment strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Supported by Fund for Young Doctor from Shanghai Anticancer Association (SACA-CY19C12), Clinical Research Plan of SHDC (SHDC2020CR3025B), CSCO-Leading Cancer Research Fund (Y-2019AZZD-0561), CSCO-MSD Cancer Research Fund (YMSD2020-0336). We thank the American Journal Experts (https://www.aje.com/) for editing this manuscript.

Ethical Statement

Informed consent was obtained from the patient. All procedures performed in studies involving human participants were in accordance with the institutional review board and the independent ethics committee of Fudan University Shanghai Cancer Center (FUSCC) and with the Helsinki Declaration (as revised in 2013).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2021.07.020.

References

[1] J.J. Lin, V.W. Zhu, S. Yoda, B.Y. Yeap, A.B. Schrock, I. Dagogo-Jack, N.A. Jessop, G. Y. Jiang, L.P. Le, K. Gowen, P.J. Stephens, J.S. Ross, S.M. Ali, V.A. Miller, M. L. Johnson, C.M. Lovly, A.N. Hata, J.F. Gainor, A.J. Iafrate, A.T. Shaw, S.-H. Ou, Impact of EML4-ALK variant on resistance mechanisms and clinical outcomes in ALK-positive lung cancer, J. Clin. Oncol. 36 (2018) 1199–1206, https://doi.org/10.1200/JCO.2017.76.2294.
[2] S.-H.-I. Ou, V.W. Zhu, M. Nagasaka, Catalog of 5' fusion partners in ALK-positive NSCLC Circa 2020, JTO Clin. Res. Rep. 1 (2020), 100015, https://doi.org/10.1016/j.jtocrr.2020.100015.

[3] J. Yang, R. Yan, A. Roy, D. Xu, J. Poisson, Y. Zhang, The I-TASSER Suite: protein structure and function prediction, Nat. Methods 12 (2015) 7–8, https://doi.org/10.1038/nmeth.3213.
[4] A. Grosdidier, V. Zoete, O. Michielin, SwissDock, a protein-small molecule docking web service based on EADock DSS, Nucleic Acids Res. 39 (2011) W270–7, https://doi.org/10.1093/nar/gkr366.
[5] A. Tulpule, J. Guan, D.S. Neel, H.R. Allegakoen, Y.P. Lin, D. Brown, Y.-T. Chou, A. Heslin, N. Chatterjee, S. Perati, S. Menon, T.A. Nguyen, J. Debnath, A. D. Ramirez, X. Shi, B. Yang, S. Feng, S. Makhija, B.o. Huang, T.G. Bivona, Kinase-mediated RAS signaling via membraneless cytoplasmic protein granules, Cell 184 (2021) 2649–2664.e18, https://doi.org/10.1016/j.cell.2021.03.031.
[6] A. Tanimoto, S. Matsumoto, S. Takeuchi, S. Arai, K. Fukuda, A. Nishiyama, K. Yoh, T. Ikeda, N. Furuya, K. Nishino, Y. Ohe, K. Goto, S. Yano, Proteasome inhibition overcomes ALK-TKI resistance in ALK-rearranged/TP53-mutant NSCLC via Noxa expression, Clin. Cancer Res. 53 (2020) 1410–1421, https://doi.org/10.1158/1078-0432.ccr-20-2853.
[7] S.S. Zhang, M. Nagasaka, V.W. Zhu, S.-H.-I. Ou, Going beneath the tip of the iceberg. Identifying and understanding EML4-ALK variants and TP53 mutations to optimize treatment of ALK fusion positive (ALK+) NSCLC, Lung Cancer 158 (2021) 126–136, https://doi.org/10.1016/j.lungcan.2021.06.012.
[8] C. Alidousty, T. Baar, L.G. Martelotto, C. Heydt, S. Wagener, J. Fassunke, N. Duerbaum, A.H. Scheel, S. Frank, B. Holz, E. Binot, A. Kron, S. Merkelbach-Bruse, M.A. Ihle, J. Wolf, R. Buettner, A.M. Schultheis, Genetic instability and recurrent MYC amplification in ALK-translocated NSCLC: a central role of TP53 mutations, J. Pathol. 246 (2018) 67–76, https://doi.org/10.1002/path.5110.
[9] G. Recondo, J. Che, P.A. Jänne, M.M. Awad, Targeting MET dysregulation in cancer, Cancer Discov. 10 (2020) 922–934, https://doi.org/10.1158/2159-8290.CD-19-1446.
[10] I. Dagogo-Jack, S. Yoda, J.K. Lennerz, A. Langenbucher, J.J. Lin, M.M. Rooney, K. Prutisto-Chang, A. Oh, N.A. Adams, B.Y. Yeap, E. Chin, A. Do, H.D. Marble, S. E. Stevens, S.R. Digumarthy, A. Saxena, R.J. Nagy, C.H. Benes, C.G. Azzoli, M. S. Lawrence, J.F. Gainor, A.T. Shaw, A.N. Hata, MET alterations are a recurring and actionable resistance mechanism in ALK-positive lung cancer, Clin. Cancer Res. 26 (2020) 2535–2545, https://doi.org/10.1158/1078-0432.CCR-19-3906.
[11] A.B. Schrock, A. Lai, S.M. Ali, V.A. Miller, L.E. Raez, Mutation of MET Y1230 as an acquired mechanism of crizotinib resistance in NSCLC with MET exon 14 skipping, J. Thorac. Oncol. 12 (2017) e89–e90, https://doi.org/10.1016/j.jtho.2017.02.017.