

CASE REPORT

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Activity of EGFR-tyrosine kinase and ALK inhibitors for *EML4-ALK*-rearranged non-small-cell lung cancer harbored coexisting *EGFR* mutation

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Abstract

Background: The *EML4-ALK* (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) fusion oncogene represents a novel molecular target in a small subset of non-small-cell lung cancers (NSCLCs). The *EML4-ALK* fusion gene occurs generally in NSCLC without mutations in epidermal growth factor receptor (*EGFR*) and *KRAS*.

Case presentation: We report that a case of *EML4-ALK*-positive NSCLC with *EGFR* mutation had a response of stable disease to both an EGFR tyrosine kinase inhibitor (EGFR-TKI) and ALK inhibitor.

Conclusions: We described the first clinical report of a patient with *EML4-ALK*-positive NSCLC with *EGFR* mutation that had a response of stable disease to both single-agent EGFR-TKI and ALK inhibitor. *EML4-ALK* translocation may be associated with resistance to EGFR-TKI, and EGFR signaling may contribute to resistance to ALK inhibitor in *EML4-ALK*-positive NSCLC.

Keywords: *EML4-ALK*, *EGFR* mutation, Lung cancer

Background

The *EML4-ALK* (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) fusion oncogene was recently identified as a novel genetic alteration in non-small-cell lung cancer (NSCLC) [1]. *EML4-ALK* fusions have been detected in 2 to 7% of NSCLC patients. Patients harboring *ALK* rearrangements tend to be never and light smokers, have a history of adenocarcinoma, and be younger in age [1-6]. In general, the *EML4-ALK* fusion oncogene existed exclusively in NSCLC patients without the epidermal growth factor receptor (*EGFR*) gene mutation [1,7,8].

ALK inhibitors such as crizotinib are clinically effective in NSCLC patients harboring *ALK* rearrangements [9]. Crizotinib produced a high response rate and prolonged

median progression-free survival among patients with *ALK*-positive NSCLC [9]. Crizotinib was recently approved by the US Food and Drug Administration and Japanese Ministry of Health, Labour and Welfare for the treatment of patients with advanced, *ALK*-rearranged NSCLC.

In this paper, we report a patient with NSCLC with concomitant *ALK* rearrangement and *EGFR* mutation that had a response of stable disease to both an EGFR tyrosine kinase inhibitor (EGFR-TKI) and ALK inhibitor.

Case presentation

In December 2009, a 55-year-old female who had never smoked was noted to have left lung opacity on a routine chest X-ray. No significant previous medical history was reported. Computed tomography (CT) scan of the chest revealed a 1.5 × 1.5 cm nodular lesion in the left upper lobe and hilar lymph node metastasis. Transthoracic needle biopsy histology revealed adenocarcinoma, and the histopathological subtype of the specimen was

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papillary adenocarcinoma with signet-ring cell components (Figure 1A-1C). The specimen was positive for periodic acid–Schiff (PAS) (Figure 1C). On immunohistochemical staining, the tumor cells were positive for thyroid transcription factor-1 (TTF-1) (Figure 1D). Laboratory findings were within normal range, except for the carcinoembryonic antigen (CEA) level of 158.0 ng/mL (normal range, 0 to 4.3 ng/mL) in the serum. She had multiple dorsal vertebra metastases (cT1N1M1b, stage IV).

Analysis for *EGFR* gene mutation was performed using a cytological specimen by means of the peptide nucleic acid–locked nucleic acid (PNA-LNA) polymerase-chain-reaction (PCR) clamp method as described previously [10,11]. The specimen showed a deletion in exon 19 (L747-A750del T751S). We collected mRNA from the same tumor specimens using Pinpoint Slide RNA Isolation System in order to clarify whether there was *EML4-ALK* (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) fusion gene in each tumor. Reverse transcription polymerase-chain-reaction (RT-PCR) followed by direct sequencing confirmed the presence of *EML4-ALK* variant 2 [1] (Figure 2). In addition, *EML4-ALK* was identified by using fluorescent in situ hybridization (FISH) for *ALK* rearrangements (Figure 3B) and was confirmed by immunohistochemistry for ALK expression in tumor [2] (Figure 3A).

A platinum doublet was chosen as first line therapy according to existing treatment protocol in 2009. Four

cycles of combination chemotherapy comprising cisplatin and pemetrexed was administered at 3-week intervals. She was judged as having a stable disease. After 7 months, spinal magnetic resonance imaging (MRI) revealed progression of the dorsal vertebra lesions. Therefore, EGFR-TKI was chosen as a 2nd-line therapy. She received gefitinib therapy at 250 mg/day administered orally for 2 months. CT imaging of the chest showed that the pulmonary nodule was not growing after gefitinib therapy, and the tumor marker levels had not changed. However, spinal MRI demonstrated growing dorsal vertebra metastases 2 months after the start of gefitinib therapy. The carcinoembryonic antigen (CEA) level increased from 117 ng/ml to 250 ng/ml. Therefore, the patient was judged as having progressive disease. After local radiation therapy with a total of 30 Gy for dorsal metastases, a second EGFR-TKI was chosen given the stable primary disease. She received another EGFR-TKI, erlotinib (150 mg/day), as 3rd-line therapy. After being progression-free for 3 months, spinal MRI revealed a growing thoracic vertebra metastasis. She received 4th-line treatment with 2 cycles of docetaxel (DTX). However, her disease progressed 6 months later. Finally, she received a targeted inhibitor of ALK. The patient initially had SD associated with a temporary decrease in the CEA level from 743 ng/ml to 520 ng/ml, but her disease progressed after 4 months of therapy. The ALK inhibitor treatment was

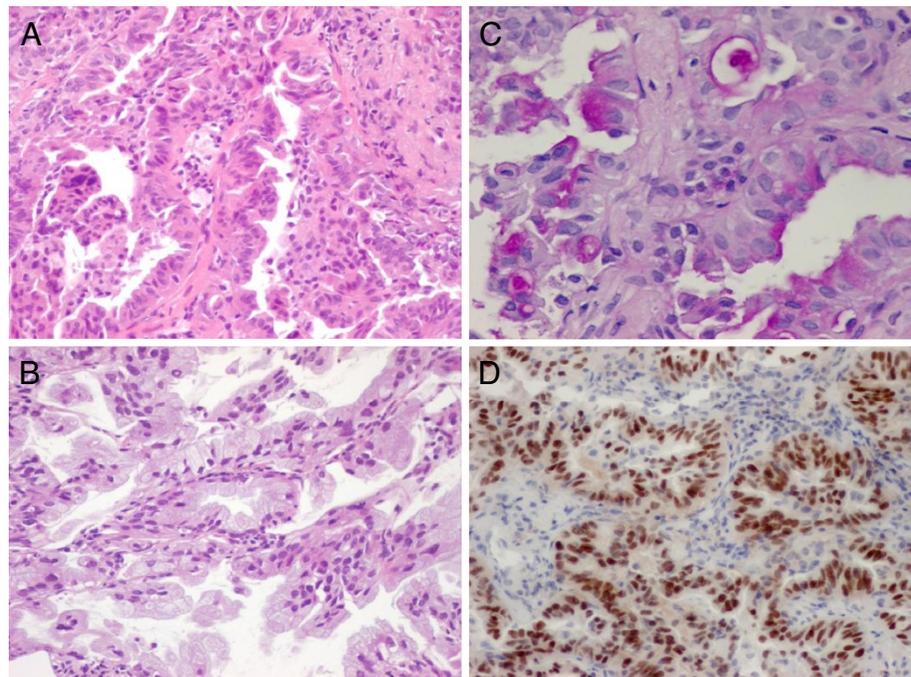


Figure 1 Histology of the primary tumour. (A) and (B) shows a papillary adenocarcinoma (hematoxylin and eosin 200× magnification), (C) a mucin stain shows positive for both signet-ring and papillary morphology (PAS, 400× magnification). (D) immunohistochemical analysis of lung adenocarcinoma specimens with *EML4-ALK* fusion using a monoclonal anti-TTF-1 antibody (200× magnification).

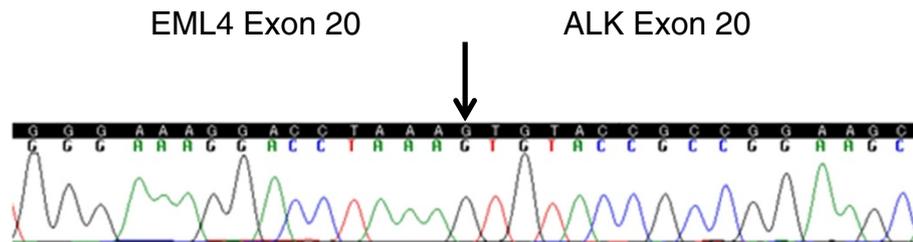


Figure 2 The sequence of the junction between *EML4* exon 20 and *ALK* exon 20.

ceased and full supportive care was given. All lines of therapy were well tolerated.

Discussion

We presented a patient with NSCLC with concomitant *ALK* rearrangement and *EGFR* mutation that had a response of stable disease to both *EGFR*-TKI and *ALK* inhibitors. The presence of *EML4-ALK* generally seems to be mutually exclusive of the presence of *EGFR* or *KRAS* mutations in NSCLC [1,7,8]. Previous reports showed twelve cases of *EML4-ALK*-positive lung cancer with *EGFR* mutation [3,12-17]. Only one patient with harboring *ALK* translocation and *EGFR* mutation was treated by *ALK* inhibitor has been reported [17]. Lee et al.

reported two *ALK*-positive and *EGFR*-mutant NSCLC patient who did not respond to *EGFR*-TKI but achieved a durable partial response to *ALK* inhibitor [17]. The present patient was a woman with no history of smoking. Her pathological diagnosis was papillary adenocarcinoma with a signet-ring cell component, which was consistent with the previously reported characteristics of *EML4-ALK*-positive lung adenocarcinoma except for the *EGFR* mutation status [1-6]. It was reported that *EGFR*-TKI therapy among patients with advanced NSCLC and *EGFR* mutations revealed a response rate of more than 60% and progression-free survival of 9 to 14 months [11,18,19]. In addition, recent reports showed that *ALK* inhibition in NSCLC patients with the *ALK* rearrangement resulted in tumor shrinkage or stable disease in most patients [9]. Unfortunately, *EGFR*-TKI treatment was not effective in the tumor regression nor tumor marker level of present patient (disease might be controlled), but treatment with an *ALK* inhibitor resulted in SD with decreasing tumor markers. Therefore, this case showed that *ALK* rearrangement might be superior to *EGFR* mutation for the driver mutation.

It was reported that *EML4-ALK* fusion was associated with resistance to *EGFR*-TKIs [20]. Patients with NSCLC in the *EML4-ALK* cohort and the wild type cohort showed similar response rates to platinum-based combination chemotherapy and no difference in overall survival [20]. Whereas *EGFR* mutations confer sensitivity to *EGFR*-TKIs, *EML4-ALK* is strongly associated with resistance to *EGFR*-TKIs. In a previous case of concomitant *EGFR* mutation and *ALK* translocation, the patient presented the most durable response to an *EGFR*-TKI and was a case demonstrating no *EML4-ALK* expression by immunohistochemistry with an *EML4-ALK* rearrangement characterized by an isolated 3_ FISH signal [12]. Our patient presented a concurrent *EML4-ALK* rearrangement and *ALK* expression by immunohistochemistry; however, *EGFR*-TKI was not effective.

Among patients with both *EML4-ALK* rearrangement and *EGFR* mutation, *in vitro* studies showed that *EGFR* signaling can contribute to *ALK* inhibitor resistance in *EML4-ALK* NSCLC [14]. In addition, these findings suggested that a cancer cell line that harbors a concurrent

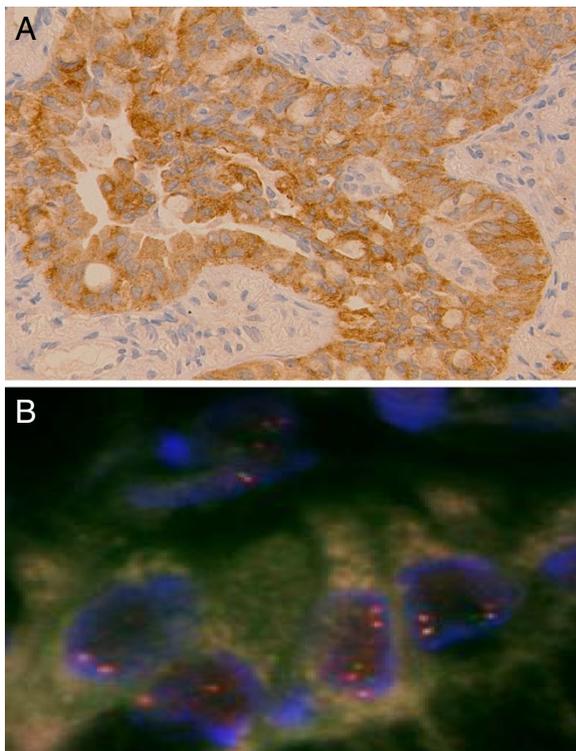


Figure 3 Diagnosis of an *EML4-ALK*-positive non-small cell lung cancer. (A) Immunostaining for *ALK* protein expression in tumor cells. (B) The results of a break-apart FISH assay of tumor cells from a patient with rearrangement of the gene encoding *ALK*.

ALK rearrangement and an *EGFR* mutation would be expected to be resistant to both single agent *ALK* and *EGFR* inhibitors [14]. We suggest that the combination of both *ALK* and *EGFR* inhibitors as early-line treatment may represent an effective therapy for this subset of NSCLC patients.

Conclusions

This is the first clinical report of a patient with *EML4-ALK*-positive NSCLC with *EGFR* mutation that had a response of stable disease to both single-agent *EGFR*-TKI and *ALK* inhibitor. The *EML4-ALK* fusion gene defines a new molecular subset of NSCLCs with distinct clinical and pathologic features. NSCLCs with *ALK* rearrangement are highly sensitive to *ALK* inhibition. However, *EGFR* signaling may contribute to *ALK* inhibitor resistance in *EML4-ALK* NSCLC. Therefore, we suggest that this provides a translational opportunity whereby laboratory studies should be undertaken to understand the biological link between *ALK* rearrangement and *EGFR* mutation, with a view to establishing whether there is preclinical justification for using combination therapy for NSCLC with concomitant *ALK* rearrangement and *EGFR* mutation.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Abbreviations

EML4: Echinoderm microtubule-associated protein-like 4; *ALK*: Anaplastic lymphoma kinase; NSCLC: Non-small cell lung cancer; *EGFR*: Epidermal growth factor receptor; TKI: Tyrosine kinase inhibitor; CT: Computed tomography; PAS: periodic acid–Schiff; TTF-1: Thyroid transcription factor-1; PNA-LNA: Peptide nucleic acid–locked nucleic acid; PCR: Polymerase chain reaction technique; FISH: Fluorescent in situ hybridization; SD: Stable disease; MRI: Magnetic resonance imaging (MRI); CEA: Carcinoembryonic antigen; RT-PCR: Reverse transcription polymerase chain reaction.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AM prepared the manuscript and the literature search; RN and MS reviewed and edited the manuscript; HM and AG corrected and revised the manuscript; KS, KK, SK, YM, MS and TS treated and observed the patient; MK and ST performed the histopathological, immunohistochemical examinations; and AY, KH, KT, NY and YI reviewed the manuscript. All authors read and approved of the final manuscript.

Acknowledgements

The authors wish to thank Mari Masuda for technical support in the sequence alignment.

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Received: 17 January 2013 Accepted: 22 May 2013

Published: 29 May 2013

References

1. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, 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):561–566.
2. Takeuchi K, Choi YL, Soda M, Inamura K, Togashi Y, Hatano S, Enomoto M, Takada S, Yamashita Y, Satoh Y, et al: Multiplex reverse transcription-PCR screening for *EML4-ALK* fusion transcripts. *Clin Can Res* 2008, **14**(20):6618–6624.
3. Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, Choi HG, Kim J, Chiang D, Thomas R, et al: *EML4-ALK* fusion gene and efficacy of an *ALK* kinase inhibitor in lung cancer. *Clin Can Res* 2008, **14**(13):4275–4283.
4. Mano H: Non-solid oncogenes in solid tumors: *EML4-ALK* fusion genes in lung cancer. *Cancer Sci* 2008, **99**(12):2349–2355.
5. Wong DW, Leung EL, So KK, Tam IY, Sihoe AD, Cheng LC, Ho KK, Au JS, Chung LP, Pik Wong M: The *EML4-ALK* fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type *EGFR* and *KRAS*. *Cancer* 2009, **115**(8):1723–1733.
6. Perner S, Wagner PL, Demichelis F, Mehra R, Lafargue CJ, Moss BJ, Arbogast S, Soltermann A, Weder W, Giordano TJ, et al: *EML4-ALK* fusion lung cancer: a rare acquired event. *Neoplasia* 2008, **10**(3):298–302.
7. Inamura K, Takeuchi K, Togashi Y, Nomura K, Ninomiya H, Okui M, Satoh Y, Okumura S, Nakagawa K, Soda M, et al: *EML4-ALK* fusion is linked to histological characteristics in a subset of lung cancers. *J Thorac Oncol* 2008, **3**(1):13–17.
8. Shinmura K, Kageyama S, Tao H, Bunai T, Suzuki M, Kamo T, Takamochi K, Suzuki K, Tanahashi M, Niwa H, et al: *EML4-ALK* fusion transcripts, but no *NPM*-, *TPM3*-, *CLTC*-, *AT1C*-, or *TFG-ALK* fusion transcripts, in non-small cell lung carcinomas. *Lung Cancer* 2008, **61**(2):163–169.
9. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Janne PA, Costa DB, et al: Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010, **363**(18):1693–1703.
10. Nagai Y, Miyazawa H, Huqun, Tanaka T, Udagawa K, Kato M, Fukuyama S, Yokote A, Kobayashi K, Kanazawa M, et al: Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. *Cancer Res* 2005, **65**(16):7276–7282.
11. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, et al: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated *EGFR*. *N Engl J Med* 2010, **362**(25):2380–2388.
12. Popat S, Vieira de Araujo A, Min T, Swansbury J, Dainton M, Wotherspoon A, Lim E, Nicholson AG, O'Brien ME: Lung adenocarcinoma with concurrent exon 19 *EGFR* mutation and *ALK* rearrangement responding to erlotinib. *J Thorac Oncol* 2011, **6**(11):1962–1963.
13. Tiseo M, Gelsomino F, Boggiani D, Bortesi B, Bartolotti M, Bozzetti C, Sammarelli G, Thai E, Arduzzoni A: *EGFR* and *EML4-ALK* gene mutations in NSCLC: a case report of erlotinib-resistant patient with both concomitant mutations. *Lung Cancer* 2011, **71**(2):241–243.
14. Sasaki T, Koivunen J, Ogino A, Yanagita M, Nikiforow S, Zheng W, Lathan C, Marcoux JP, Du J, Okuda K, et al: A novel *ALK* secondary mutation and *EGFR* signaling cause resistance to *ALK* kinase inhibitors. *Cancer Res* 2011, **71**(18):6051–6060.
15. Tanaka H, Hayashi A, Morimoto T, Taima K, Tanaka Y, Shimada M, Kurose A, Takahashi S, Okumura K: A case of lung adenocarcinoma harboring *EGFR* mutation and *EML4-ALK* fusion gene. *BMC Cancer* 2012, **12**(1):558.
16. Kuo YW, Wu SG, Ho CC, Shih JY: Good response to gefitinib in lung adenocarcinoma harboring coexisting *EML4-ALK* fusion gene and *EGFR* mutation. *J Thorac Oncol* 2010, **5**(12):2039–2040.
17. Lee JK, Kim TM, Koh Y, Lee SH, Kim DW, Jeon YK, Chung DH, Yang SC, Kim YT, Kim YW, et al: Differential sensitivities to tyrosine kinase inhibitors in NSCLC harboring *EGFR* mutation and *ALK* translocation. *Lung Cancer* 2012, **77**(2):460–463.

18. Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, Chao TY, Nakagawa K, Chu DT, Saijo N, *et al*: **Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS).** *J Clin Oncol* 2011, **29**(21):2866–2874.
19. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, *et al*: **Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial.** *Lancet Oncol* 2010, **11**(2):121–128.
20. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, Solomon B, Stubbs H, Admane S, McDermott U, *et al*: **Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK.** *J Clin Oncol* 2009, **27**(26):4247–4253.

doi:10.1186/1471-2407-13-262

Cite this article as: Miyanaga *et al*: Activity of EGFR-tyrosine kinase and ALK inhibitors for *EML4-ALK*-rearranged non-small-cell lung cancer harbored coexisting *EGFR* mutation. *BMC Cancer* 2013 **13**:262.

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