Case Report

A Patient With Lung Adenocarcinoma With *BRAF* Gene Fusion and Response to Vemurafenib

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Clinical Practice Points

- *BRAF*, a key molecule in the MAP/ERK kinase signaling pathway, is located downstream of *EGFR* and *ROS1*. *BRAF* fusions are an emerging treatment target for melanomas and other solid tumors. The frequency of *BRAF* fusions and its targeting potential in non–small-cell lung cancer (NSCLC), which occurs rarely in Chinese populations.
- We report a case of a *TRIM24-BRAF* fusion in lung adenocarcinoma. The patient was a 60-year-old never-smoking male, who was diagnosed with lung adenocarcinoma. Biopsies were obtained, and a *TRIM24-BRAF* fusion was detected by next generation sequencing. The patient received vemurafenib therapy and was considered to have a partial response. The progression-free survival was 3.5 months.
- To our knowledge, this is the first case of a patient with lung adenocarcinoma with a *TRIM24-BRAF* fusion who received vemurafenib. *BRAF* fusions are rare driver gene alterations in NSCLC, and *BRAF* fusions may respond to targeted therapy. In the future, the routine use of multi-gene sequencing assays may aid in the discovery of rare gene mutations, and further work should explore the frequency of *BRAF* fusions in Chinese patients. We also need to explore differences in response to BRAF-tyrosine kinase inhibitors between carriers of sensitive *BRAF* mutations and *BRAF* fusions among patients with NSCLC.

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Introduction

Non–small-cell lung cancer (NSCLC) accounts for approximately 85% of lung cancers and remains a major cause of cancer-related death around the world.[1] In the past few decades, significant strides have been made in the detection of oncogenic drivers in NSCLC and the development of specific tyrosine kinase inhibitors (TKI), such as those that target epidermal growth factor receptor (*EGFR*), chromosomal rearrangements involving the anaplastic lymphomakinase gene (*ALK*), and c-ros oncogene 1 (*ROS1*).[2-4] The *BRAF* gene as a major oncogenic driver was first described in lung cancer in 2002.[5] *BRAF* encodes a RAF kinase, which signals downstream of RAS and activates the MAPK pathway.[6] Moreover, *BRAF* gene mutations are a potential therapeutic target for multiple tumor types.[6] *BRAF* mutations are present in approximately 2% to 4% of lung adenocarcinomas, and approximately one-half of these are V600E mutations.[7] Nowadays, with the development of next generation sequencing (NGS) platforms, *BRAF* fusions are easily discovered.

BRAF gene fusions represent a different mechanism of BRAF activation and have been described in several solid tumor types.[8] In 2005, Ciampi et al[9] first reported the presence of the *AKAP9-BRAF* fusion as a new mechanism for activating the MAPK signaling pathway in thyroid cancer, opening a new chapter in *BRAF* fusion research. Then, *BRAF* fusions were found in other cancers, such as gliomas, pancreatic carcinomas, NSCLC, and colorectal cancers. Ross et al[10] tested 20,000 tumors and identified 55 *BRAF* gene fusions in 12 different tumor types. They found that gene fusions

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occurred more frequently in certain histologic subtypes, information which will help guide treatment strategies for patients with these tumor subtypes. They found that the presence of *BRAF* fusions was 0.2% (8/4013) in NSCLC. The types of fusions in NSCLC included *EPS15-BRAF*, *NUP214-BRAF*, *ARMC10-BRAF*, *BTF3L4-BRAF*, *AGK-BRAF*, *GHR-BRAF*, *ZC3HAV1-BRAF*, and *TRIM24-BRAF*. In addition, a comprehensive assay, MSK-IMPACT, was used in one study to detect gene alterations from more than 10,000 patients with advanced cancer.[11] That study found 33 cases of *BRAF* fusion, including 0.38% (6/1563) in NSCLC, of which all were lung adenocarcinoma. These fusions included 2 cases of where the fusion partner was *AGK*, and 1 case each of fusion with *PJA2*, *SND1*, *MRPS33*, and *PARP12*.

However, reports describing the presence of *BRAF* fusions and the use of anti-BRAF therapies for NSCLC with *BRAF* fusion alterations have been limited to date in China. BRAF inhibitors, such as vemurafenib, in patients with the *BRAF* V600E mutation showed good response in NSCLC.[12] The sensitivity of *BRAF* fusions to TKI drugs remains unclear and controversial. Here, we report a patient with NSCLC with a *TRIM24-BRAF* fusion who showed significant clinical sensitivity to treatment with vemurafenib therapy. Though BRAF V600E had poor outcomes achieving a response to platinum-based chemotherapy, dual MAPK pathway inhibition with BRAF and MEK inhibitors, dabrafenib plus trametinib, might improve efficacy in patients with BRAF V600E-mutant NSCLC. It is a new targeted therapy with robust antitumor activity and a manageable safety profile in these patients.[13]

Case Report

A 60-year-old man who was a nonsmoker presented to our hospital with a 2-month history of cough and expectoration. A computed tomography scan revealed a mass in the superior lobe of the left lung (Figure 1A) and hilar lymph node enlargement (Figure 1B). A brain magnetic resonance imaging scan revealed an intracranial lesion (Figure 1C). Magnetic resonance imaging also showed spinal bone metastases ($T_2N_1M_1$ stage IV) (Figure 1D). A left lung mass puncture biopsy and hematoxylin and eosin staining showed the presence of adenocarcinoma cells (Figure 2). Immunohistochemistry analysis demonstrated positivity for TTF-1 and Napsin A, and negativity for cytokeratin 5/6 and P63. Tumor tissue was detected by NGS (3D Medicines, Shanghai, China), and we detected a *TRIM24-BRAF* fusion (Figure 3), *TP53* p.C275Y, *CDKN2A* p.M52R, and *RBM10* p.S797*. The genes co-altered (*TP53*, *CDKN2A*, and *RBM10*) with BRAF fusion were not

Figure 1 **A, Computed Tomography Scan Revealed a Mass in the Superior Lobe of the Left Lung. B, Hilar Lymph Node Enlargement. C, Brain Magnetic Resonance Imaging Scan Revealed an Intracranial Lesion. D, Magnetic Resonance Imaging Showed Spinal Bone Metastases**

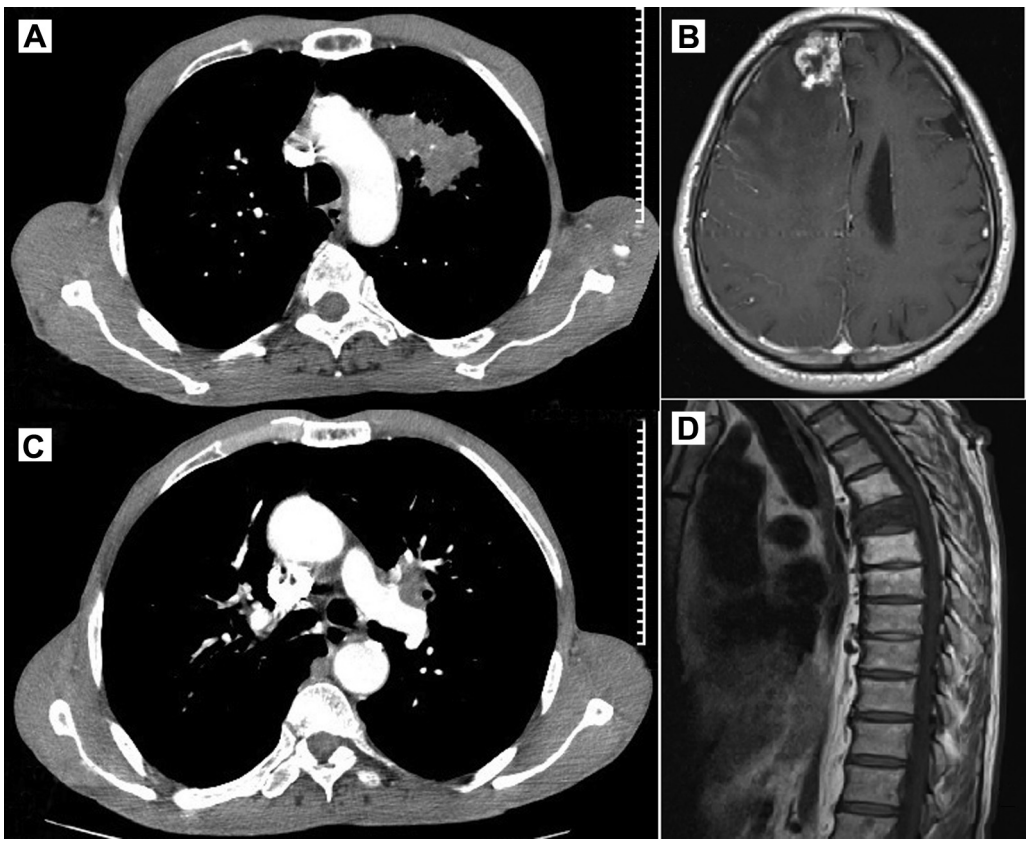
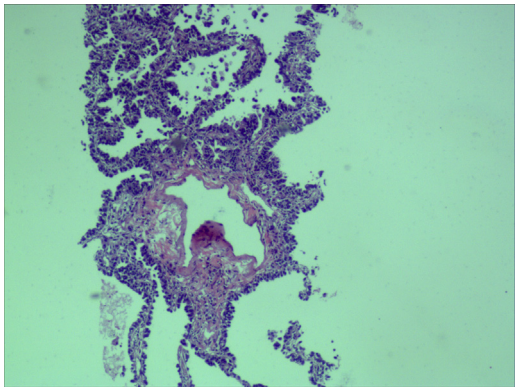


Figure 2 Left Lung Mass Puncture Biopsy and Hematoxylin and Eosin Staining Showed the Presence of Adenocarcinoma Cells



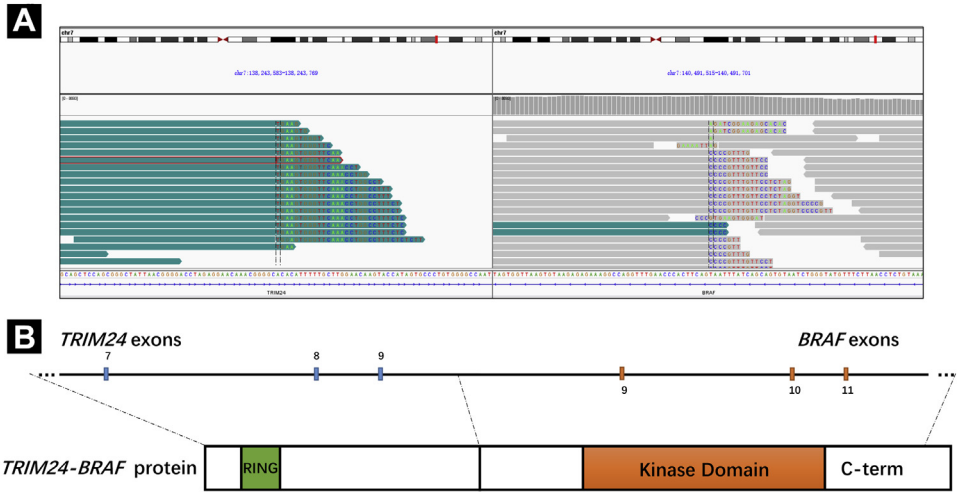
concurrent with other known drivers. The NGS assay was performed using the HiSEquation 4000 (Illumina). After the genetic diagnosis, the patient received a chemotherapy regimen of PP (pemetrexed 500 mg/m^2, Day 1; Carboplatin area under the curve [AUC] = 5, Day 1) in January 2016. He also received whole brain radiotherapy, and the total dose was 30 Gy administered in 10 fractions (3 Gy fractions once a day, 5 days a week). The response to PP was stable disease. After 6 cycles of PP chemotherapy, the disease progressed in September 2016. Therefore, the patient was switched to a chemotherapy treatment of docetaxel (75 mg/m^2, Day 1) combined with bevacizumab (5 mg/kg, Day 1) in October 2016. The efficacy was partial response. Docetaxel and bevacizumab were used as maintenance treatments until November 2017, when the patient was diagnosed with progressive disease again. The patient was unable to tolerate chemotherapy and chose to receive vemurafenib treatment. Thus, vemurafenib (960 mg orally twice daily) was administered starting in December 2017. The patient was considered to have a partial response after 6 weeks of vemurafenib treatment (Figure 4A). Unfortunately, after 3.5 months (in March 2018), the disease progressed (Figure 4B), and the patient died in August 2018. The overall survival was 31 months from the time of diagnosis to death.

Discussion

To our knowledge, our case is the first report of a patient with *TRIM24-BRAF* fusion with lung adenocarcinoma responding to vemurafenib treatment. Considering this rare condition and the good response to targeted therapy, this case should heighten clinical awareness of *BRAF* fusions in lung cancer.

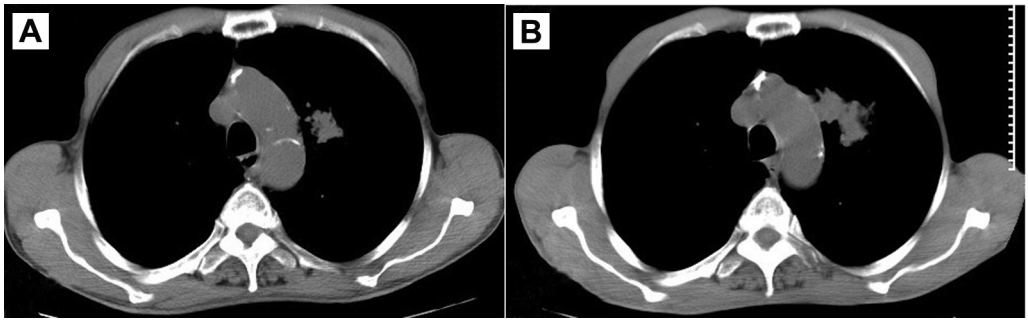
BRAF gene fusions are extremely rare and biologically distinct from *BRAF* mutation V600E. Although *BRAF* fusions are rare in advanced solid tumors, the published literature demonstrates enrichment in certain histologic subsets including pilocytic astrocytoma, Spitzoid melanoma, pancreatic acinar carcinoma, and papillary thyroid cancer.[14,15] The incidence of *BRAF* fusions is very low in NSCLC, but in the current era of precision treatment, we should be aware of *BRAF* fusions. Studies have found *BRAF* fusions as resistance mechanisms after EGFR-TKI treatment in advanced NSCLC.[16] Similarly, *BRAF* fusions occur in untreated patients with NSCLC, especially in patients with lung adenocarcinoma. In the latest recent report, Reddy et al[17] explored the frequency of *BRAF* fusions in NSCLC, and they investigated 17,128 NSCLC formalin-fixed paraffin-embedded samples sequenced by hybridization capture-based comprehensive genomic profiling. *BRAF* fusions were identified in 42/17,128 (0.2%) of the

Figure 3 *TRIM24-BRAF* Fusion is Clinically Actionable. A, The Integrative Genomics Viewer Snapshot of *TRIM24-BRAF*. Soft-Clipped Bases can Match Each Other in Reverse Complementarity. B, Schematic Representation of the *TRIM24-BRAF* Fusion Protein Domain Structure



Green = *TRIM24*; grey = *BRAF*; *BRAF* KD = kinase domain.

Figure 4 A, Partial Response After 6 Weeks of Vemurafenib Treatment. B, Disease Progressed After 3.5 Months



samples of NSCLC profiled. *BRAF* fusions are similar to other kinase fusions in that they tend to be mutually exclusive of other activating mutations in the MAPK pathway. The most frequent 5′ partners in that study were *AGK*, *DOCK4*, and *TRIM24*.[17] In addition, they showed that the genes most frequently co-altered in patients with *BRAF* fusions were *TP53* (67%), *CDKN2A* (31%), *EGFR* (29%), and *CDKN2B* (26%). Lung cancers with *BRAF* fusions tended to be adenocarcinomas, or had adenocarcinoma features, whereas none of these fusions were detected in squamous or small-cell lung cancers.[10] In our report, we found a patient with NSCLC with a *TRIM24-BRAF* fusion. Although the incidence of *BRAF* fusions in lung cancer is very low, clinicians should be aware that patients with this genetic mutation can also be treated with current therapies. Thus, the incidence of *BRAF* fusions in Chinese patients with lung cancer should be explored further.

BRAF gene mutations lead to constitutive activation of the protein's Ser/Thr kinase activity and downstream activation of the RAF/MEK/ERK pathway.[18,19] This signaling pathway is complex, is dependent on RAF dimerization, and is limited by feedback inhibition of RAS signals. The *BRAF* mutation V600E is distinct in that it functions as an activated monomer independent of RAS signaling and can be targeted with BRAF inhibitors. Sorafenib, a multikinase inhibitor that inhibits RAF, has had limited efficacy as an anticancer drug in patients with BRAF-activating point mutations.[20] Evidence supporting the treatment of tumors harboring *BRAF* fusions with therapies targeting this kinase have recently started to emerge. In a report by Ross et al,[10] a patient with Spitzoid melanoma harboring a *ZKSCAN1-BRAF* fusion responded to treatment with the MEK inhibitor trametinib. A soft tissue sarcoma featuring a *KIAA1549-BRAF* fusion reportedly responded to sorafenib in combination with bevacizumab and temsirolimus. Piotrowska et al[21] established a cell line (MGH845-1) from a core needle liver biopsy of the patient and confirmed the presence of the PCBP2-BRAF fusion gene and EGFR T790M loss. The MGH845-1 cells were sensitive to the MEK inhibitor trametinib but not to the RAF inhibitors dabrafenib or LXH245. In addition, Menzies et al[22] also showed clinical activity of trametinib in 2 heavily pre-treated patients with metastatic melanoma with BRAF fusions (one with a PPFIBP2-BRAF fusion and the other with a KIAA1549-BRAF fusion). Unfortunately, there is no information on BRAF or MEK inhibitors used in patients with BRAF fusion lung cancer. Vemurafenib is a BRAF inhibitor, and a prospective clinical trial in patients with metastatic NSCLC with *BRAF* V600E showed response rates of 42% and a median PFS of 7.3 months.[23] Thus, we used vemurafenib treatment in our patient, who showed a good response. However, the PFS was short. The patient had other treatment lines prior to the administration of vemurafenib therapy, and this might have influenced its effect.

Owing to the extremely low frequency of *BRAF* fusions in solid tumors, only case reports validating the use of agents targeting *BRAF* fusions can bring significant clinical improvement. Thus, the sensitivity of *BRAF* fusion-driven tumors to BRAF inhibitors or MEK inhibitors remains unclear. Further studies are needed to explore the biological and oncogenic implications of *BRAF* fusions, and prospective clinical studies are needed to search for a targeted strategy. In practice, next-generation DNA sequencing and comprehensive genomic profiling should be used in oncology clinics.

In the future, we should explore the frequency of *BRAF* fusions in Chinese patients. Also, we need to explore the effect of BRAF inhibitors or MEK inhibitors in patients with NSCLC with *BRAF* fusions.

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Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; 68: 7-30.
2. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; 361:947-57.
3. Shaw AT, Ou SH, Bang YJ, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med* 2014; 371:1963-71.
4. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* 2013; 368:2385-94.
5. Parker A, Stevens C, Watt S, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002; 417:949-54.
6. Wan PT, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004; 116: 855-67.

7. Paik PK, Arcila ME, Fara M, et al. Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. *J Clin Oncol* 2011; 29:2046-51.
8. Palanisamy N, Ateeq B, Kalyana-Sundaram S, et al. Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. *Nat Med* 2010; 16:793-8.
9. Ciampi R, Knauf JA, Kerler R, et al. Oncogenic AKAP9-BRAF fusion is a novel mechanism of MAPK pathway activation in thyroid cancer. *J Clin Invest* 2005; 115:94-101.
10. Ross JS, Wang K, Chmielecki J, et al. The distribution of BRAF gene fusions in solid tumors and response to targeted therapy. *Int J Cancer* 2016; 138:881-90.
11. Feldman DR, Gounder MM, Hakimi AA, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017; 23:703-13.
12. Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *J Thorac Oncol* 2015; 373:726-36.
13. Planchard D, Smit EF, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously untreated BRAF(V600E)-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol* 2017; 18:1307-16.
14. Botton T, Yeh I, Nelson T, et al. Recurrent BRAF kinase fusions in melanocytic tumors offer an opportunity for targeted therapy. *Pigment Cell Melanoma Res* 2013; 26:845-51.
15. Chmielecki J, Hutchinson KE, Frampton GM, et al. Comprehensive genomic profiling of pancreatic acinar cell carcinomas identifies recurrent RAF fusions and frequent inactivation of DNA repair genes. *Cancer Discov* 2014; 4:1398-405.
16. Schram AM, Chang MT, Jonsson P, et al. Fusions in solid tumours: diagnostic strategies, targeted therapy, and acquired resistance. *Nat Rev Clin Oncol* 2017; 14: 735-48.
17. Reddy VP, Gay LM, Elvin JA, et al. BRAF fusions in clinically advanced non-small cell lung cancer: an emerging target for anti-BRAF therapies. *J Clin Oncol* 2017; 35(15 Suppl):9072.
18. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. *Lancet Oncol* 2011; 12:175-80.
19. Hutchinson KE, Lipson D, Stephens PJ, et al. BRAF fusions define a distinct molecular subset of melanomas with potential sensitivity to MEK inhibition. *Clin Cancer Res* 2013; 19:6696-702.
20. Wilhelm S, Carter C, Lynch M, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* 2006; 5:835-44.
21. Piotrowska Z, Isozaki H, Lennerz JK, et al. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. *Cancer Discov* 2018; 8:1529-39.
22. Menzies AM, Yeh I, Botton T, et al. Clinical activity of the MEK inhibitor trametinib in metastatic melanoma containing BRAF kinase fusion. *Pigment Cell Melanoma Res* 2015; 28:607-10.
23. Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *N Engl J Med* 2015; 373:726-36.