



CASE REPORT

Chemotherapy combined with Endostar as salvage treatment for EGFR-tyrosine kinase inhibitor primary resistance in an advanced non-small cell lung cancer patient with *EGFR* L858R mutation and *ROS1* fusion: A case report

Dong Qiu^{1,2} , Yu Zhang², Ying-bo Xue², Qi Shen², Hang Li², Ping Huang³, Jian-jun Hu³ & Yong-sheng Wang¹ 

1 Department of Thoracic Oncology, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center of Biotherapy, Chengdu, China

2 Department of Oncology, Guizhou People's Hospital, Guiyang, China

3 Department of Pathology, Guizhou People's Hospital, Guiyang, China

Keywords

Chemotherapy; *EGFR*; non-small cell lung cancer; primary resistance; *ROS1*.

Correspondence

Yongsheng Wang, Department of Thoracic Oncology, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center of Biotherapy, 17 People's South Road, Chengdu, Sichuan 610041, China.

Tel: +86 189 8060 2258

Fax: +86 28 85422707

Email: wangys75@gmail.com

Received: 6 December 2018;

Accepted: 14 January 2019.

doi: 10.1111/1759-7714.13003

Thoracic Cancer **10** (2019) 1023–1028

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related morbidity and mortality both worldwide and in China.^{1,2} Improved understanding of the molecular changes that drive tumor progression has revolutionized the clinical management of NSCLC. EGFR-tyrosine kinase inhibitors (TKIs) have shown promising results in NSCLC patients with *EGFR*-positive mutations;³ however, approximately 20–30% of patients harboring *EGFR*-activating mutations demonstrate a poor response to EGFR-TKIs, which indicates a risk of primary resistance and the likelihood that alternative determining factors conferring sensitivity exist.⁴ We report a case of an advanced NSCLC

Abstract

EGFR-activating mutations have been recognized as the most important predictor of response to EGFR-tyrosine kinase inhibitors (TKIs); however, 20–30% of patients harboring *EGFR*-activating mutations show poor responses. The mechanisms of such EGFR-TKI primary resistance are still poorly understood. In our case, a non-small cell lung cancer patient developed intrinsic EGFR-TKI resistance and was then confirmed to simultaneously harbor an L858R mutation and *ROS1* rearrangement. Salvage chemotherapy plus Endostar showed enduring therapeutic effects, achieving a disease-free survival period of 24 months and overall survival of 30 months. This suggests that co-activation of different oncogenic signal pathways might be a potential mechanism of EGFR-TKI primary resistance. Chemotherapy combined with anti-angiogenesis should be considered an important salvage strategy. Further studies are warranted to verify these findings and explore the underlying mechanisms involved.

patient with intrinsic EGFR-TKI resistance who achieved disease-free survival (DFS) of 24 months and overall survival (OS) of 30 months after salvage treatment of chemotherapy combined with anti-angiogenesis.

Case report

In August 2015, a 63-year-old Chinese man with a heavy smoking history (60 cigarettes/day for approximately 40 years) was admitted to our hospital. Computed tomography (CT) showed a mass in the right lung and possible metastases in the mediastinal lymph nodes (Fig 1a). Cranial magnetic resonance imaging, abdominal CT, and bone

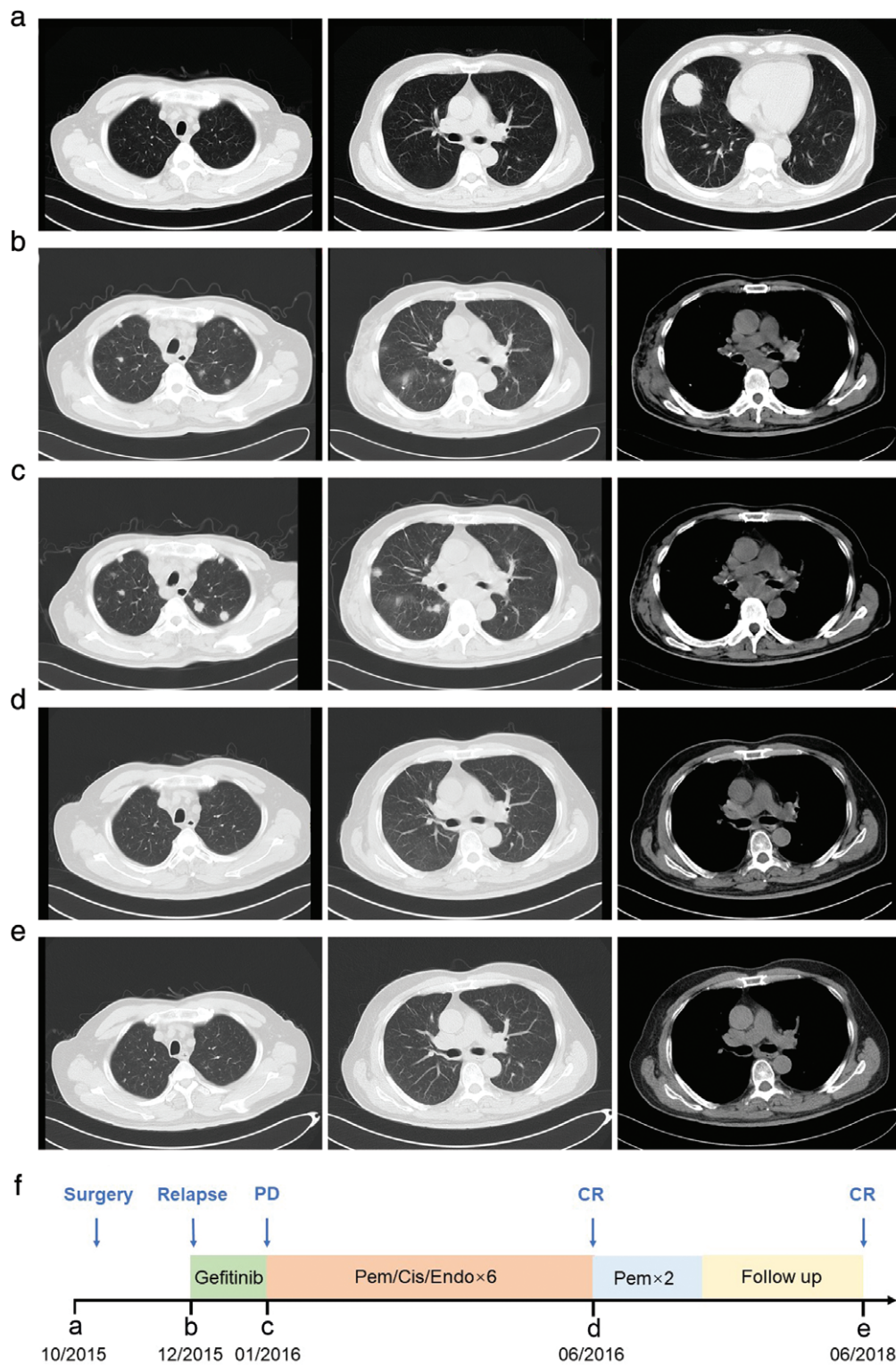


Figure 1 Thoracic computed tomography (CT) imaging shows the tumor mass and metastases after various treatments. (a) Before surgery, a mass in the right middle lung and possible lymph node metastases in the mediastinal seven region were observed on CT. (b) Two months after surgery, CT imaging showed multi-metastases in the bilateral lungs and mediastinal lymph nodes. (c) After one month of gefitinib treatment, CT imaging showed progressive disease (PD). (d) After six cycles of chemotherapy, CT imaging showed a complete response (CR). (e) CR status at the last follow-up on 5 June 2018. (f) The timeline of different treatments.

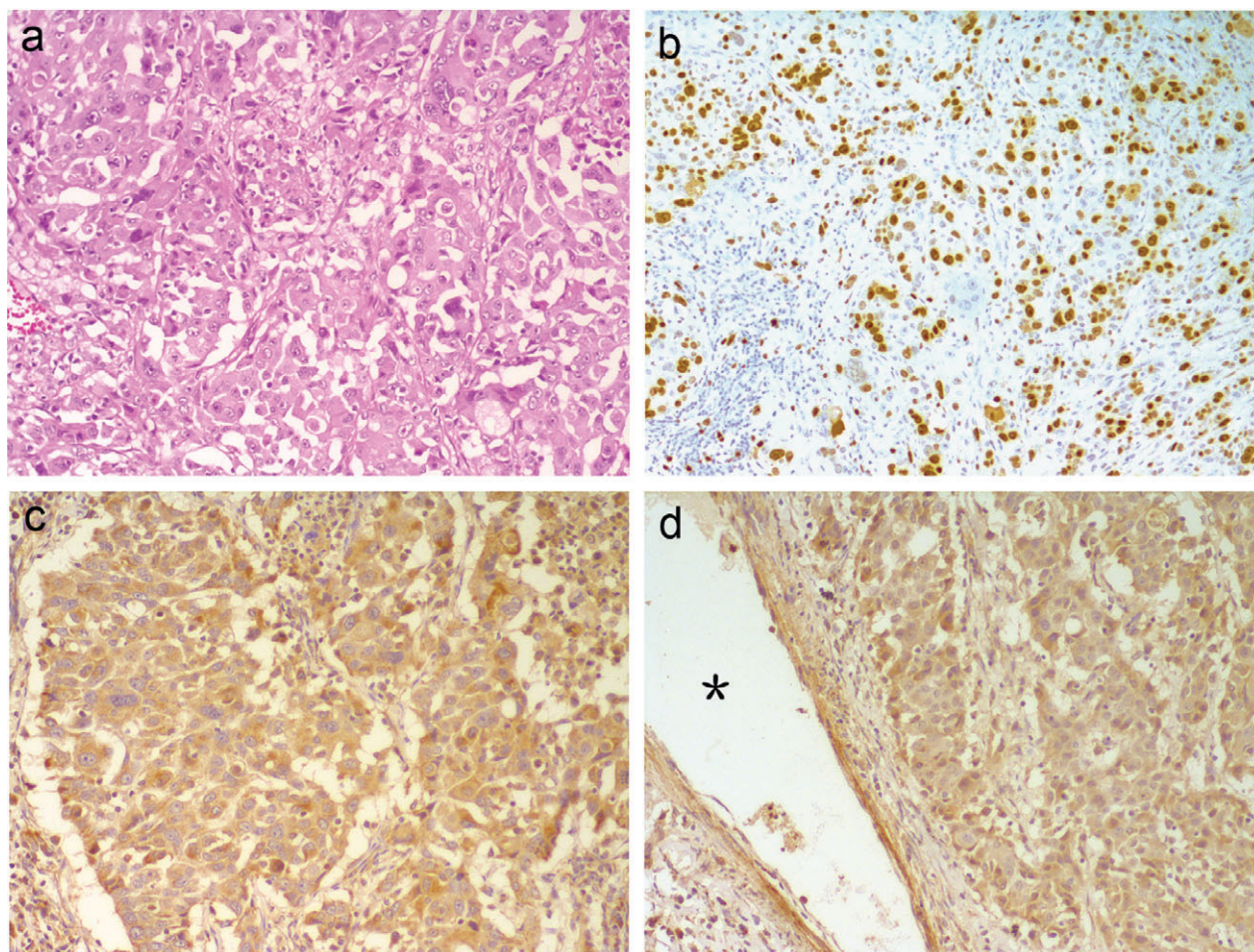


Figure 2 Representative examples of hematoxylin and eosin (HE) and immunohistochemistry findings. (a) Surgical samples confirmed adenocarcinoma with a mass predominant pattern according to typical HE morphology (200 \times). (b) Ki-67 positive expression (100 \times). (c) High vascular endothelial growth factor (VEGF) expression in tumor tissues (200 \times). (d) High VEGF receptor 2 expression in tumor cells and vascular endothelial cells (200 \times) (* shows the small vessel).

scans were found to be normal. On 22 October 2015, the patient underwent right middle lobectomy with systematic lymphadenectomy. Pathological analysis revealed a poorly differentiated pulmonary adenocarcinoma (pT2bN2M0, stage IIIa) (Fig 2a,b). An *EGFR* mutation (L858R) was reported (Fig 3a).

On 22 December 2015, a CT scan showed new multi-metastases (Fig 1b) and gefitinib was administered.^{3,4} Re-evaluation demonstrated progressive disease (according to Response Evaluation Criteria In Solid Tumors 1.1) (Fig 1c). The patient showed primary resistance to gefitinib and refused re-biopsy. Real-time (RT)-PCR (ARMS, Amoy Diagnostics, Xiamen, China) was performed a second time using the surgical samples, and of the six driver genes (*EGFR*, *ROS1*, *KRAS*, *ALK*, *BRAF* and *PIK3CA*) tested, L858R mutation was confirmed and *ROS1* fusion was found (Fig 3b-e). Fluorescence in situ hybridization

analysis (ZytoLight, ZytoVision GmbH, Bremerhaven, Germany) also indicated *ROS1* rearrangement (Fig 3f).

ROS1 inhibitors were not prescribed for this patient because they were unavailable in China in January 2016.⁵ From January to March 2016, doublet chemotherapy (pemetrexed and cisplatin) plus rh-Endostatin (Endostar) was administered for two cycles. The therapeutic evaluation was a partial response. The patient was then administered an additional four cycles with the same agents. Imaging demonstrated a complete response (CR) (Fig 1d). Pemetrexed maintenance was administered for another two cycles until September 2016. The patient then refused further maintenance therapy. At his last follow-up in June 2018, the patient had maintained a CR (Fig 1e), with DFS of 24 months and OS of 30 months (Fig 1f). The toxicities experienced were mainly grade 2 hematologic and gastrointestinal.

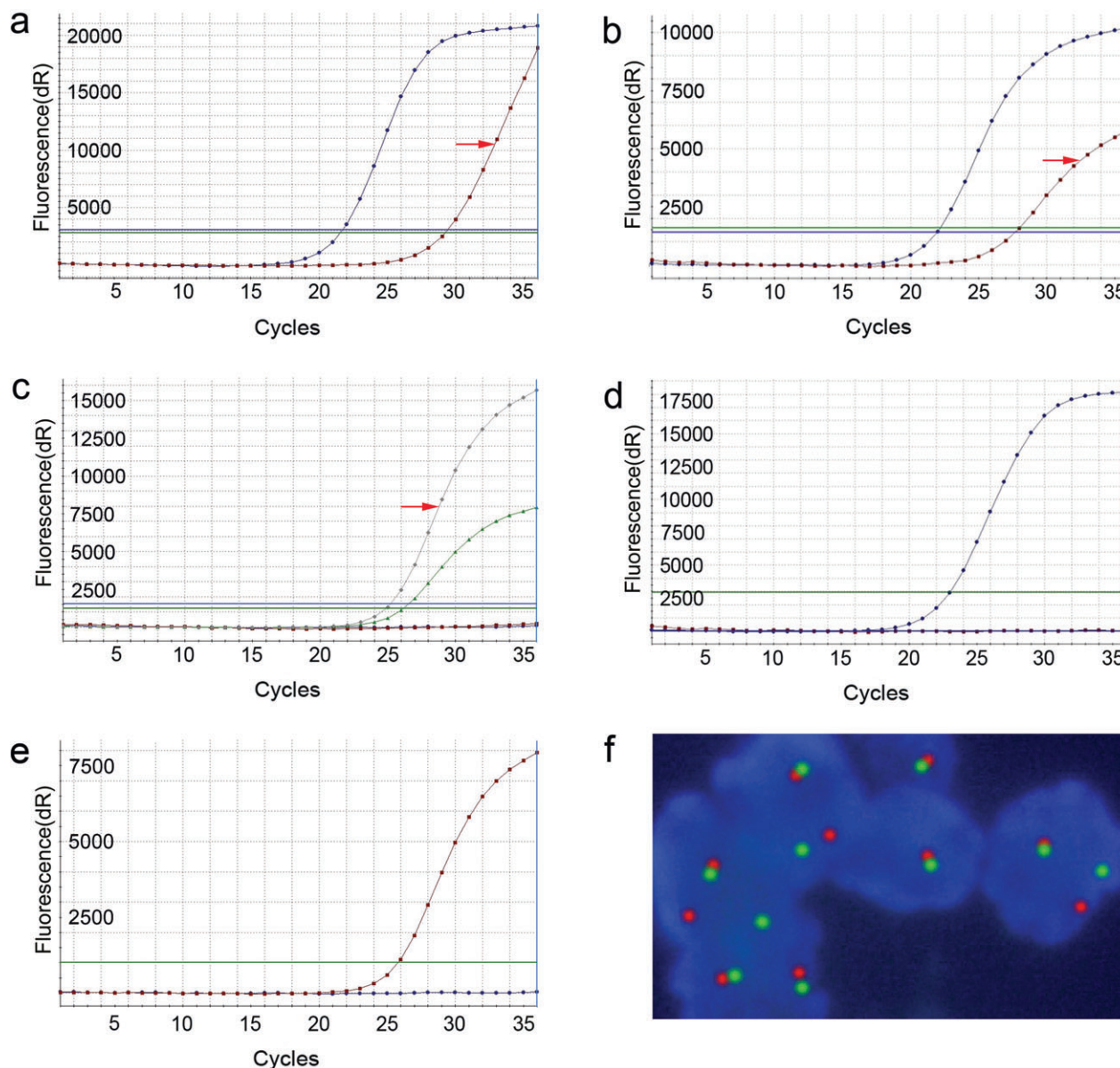


Figure 3 Detection of driver genes using surgical tumor samples by real time-PCR and fluorescence in situ hybridization (FISH). The red arrow indicates the positive curve. (a) First time detection of *EGFR* showed an exon 21 L858R mutation. (b) After gefitinib was administered, secondary analysis showed an L858R mutation and (c) *ROS1* fusion. (d) *EGFR* T790M and (e) *KRAS* were negative. (f) FISH analysis for *ROS1*. Several split signals and isolated green signals indicated *ROS1* rearrangement. The distribution of *ROS1* FISH-positive events was 27.1%.

Discussion

Only 1–2% of patients with NSCLC have *ROS1* fusion,⁶ and patients who harbor both *EGFR* mutation and *ROS1* rearrangement are extremely rare. Few investigations have focused on the combination of *EGFR* mutation and other gene-driven activation because of the low incidence. The clinical-pathological characteristics and the response to EGFR-TKIs of such patients with multiple mutations

remain controversial.⁷ To our knowledge, this is the first case to report simultaneous L858R mutation and *ROS1* rearrangement detected in a single NSCLC patient with intrinsic gefitinib resistance.

Mechanisms of EGFR-TKI resistance are known to be highly heterogeneous and are mainly focused on acquired resistance (Table 1).^{8–14} However, the mechanism of primary resistance to EGFR-TKIs is still poorly understood.

Table 1 Major mechanisms of EGFR-tyrosine kinase inhibitor resistance

Mechanism	Acquired Resistance
Modification of target gene	EGFR T790M mutation ⁸
Activation of alternative pathway	c-Met amplification ⁹ HGF ¹⁰ HER2 ¹¹ BRAF ¹²
Alteration of downstream	PTEN loss ¹³ PIK3CA mutation ¹⁴ KRAS activation ¹²
Others	Epithelial to mesenchymal transition ¹⁴ Small cell lung cancer transformation ¹⁴

Recent studies have indicated that multiple resistance factors can be induced simultaneously in a single cancer. Examples of these processes include the co-existence of ALK amplification and gatekeeper mutations causing resistance to ALK inhibitors¹⁵ MET mutation and activation of the EGFR pathway simultaneously conferring resistance to MET-TKIs¹⁶ and activation of bypass signaling resulting in resistance in lung cancer cells harboring ROS1 fusions.¹⁷ These findings, taken together with our case, suggest that co-activation of other oncogenic signal pathways might result in uncontrolled proliferation or survival in lung cancers, and then confer intrinsic EGFR-TKI resistance.

The identification of resistance mechanisms is essential to develop strategies to overcome resistance. Current approaches to combat resistance include salvage of irreversible EGFR-TKIs, and EGFR-TKIs combined with drugs targeting other activation pathways. In this case, chemotherapy combined with Endostar showed a durable and satisfactory therapeutic effect and was well tolerated with little toxicity. Chemotherapy should be strongly considered for patients with EGFR-TKI primary resistance. Moreover, angiogenesis plays a critical role in solid tumor survival and invasion. Endostar has been demonstrated to downregulate expression of vascular endothelial growth factors (VEGFs) and VEGF receptors (VEGFRs) to inhibit tumor angiogenesis and block VEGF-C signaling to inhibit tumor lymphangiogenesis.¹⁸ In our patient, VEGF and VEGFR2 were strongly expressed in the tumor tissues (Fig 2c,d), and this might be the reason why this patient showed a positive response to the anti-angiogenic agent combined with chemotherapy.

The primary resistance mechanisms to EGFR-TKIs are still not fully understood. In our report, EGFR mutation and ROS1 fusion were found to co-exist in a heavy smoking NSCLC patient. The co-activation of different signal pathways might result in EGFR-TKI primary resistance. Chemotherapy could be a potential option to combat intrinsic EGFR-TKI resistance, and Endostar might

enhance the effectiveness. However, it was not possible to evaluate the response to ROS1 inhibitors in our case because crizotinib or other inhibitors were unavailable. Further studies are required to verify these findings and to explore the underlying mechanisms.

Acknowledgment

The study was supported by the Guizhou High-level Innovative Talents Foundation (No. GZSYQCC [2016] 003).

Disclosure

No authors report any conflict of interest.

References

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017; **67**: 7–30.
- Chen WQ, Zheng RS, Baade PD et al. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115–32.
- Mok TS, Wu YL, Thongprasert S et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; **361**: 947–57.
- Costa DB, Kobayashi S, Tenen DG, Huberman MS. Pooled analysis of the prospective trials of gefitinib monotherapy for EGFR-mutant non-small cell lung cancers. *Lung Cancer* 2007; **58**: 95–103.
- Lin JJ, Shaw AT. Recent advances in targeting ROS1 in lung cancer. *J Clin Oncol* 2017; **12**: 1611–25.
- Bergethon K, Shaw AT, Ou SH et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol* 2012; **30**: 863–70.
- Ulivi P, Chiadini E, Dazzi C et al. Nonsquamous, non-small-cell lung cancer patients who carry a double mutation of EGFR, EML4-ALK or KRAS: Frequency, clinical-pathological characteristics, and response to therapy. *Clin Lung Cancer* 2016; **17**: 384–90.
- Kobayashi S, Boggon TJ, Dayaram T et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005; **352**: 786–92.
- Engelman JA, Zejnullahu K, Mitsudomi T et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007; **316**: 1039–43.
- Yano S, Takeuchi S, Nakagawa T, Yamada T. Ligand-triggered resistance to molecular targeted drugs in lung cancer: Roles of hepatocyte growth factor and epidermal growth factor receptor ligands. *Cancer Sci* 2012; **103**: 1189–94.
- Takezawa K, Pirazzoli V, Arcila ME et al. HER2 amplification: A potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second-site EGFR T790M mutation. *Cancer Discov* 2012; **2**: 922–33.

- 12 Ohashi K, Sequist LV, Arcila ME *et al.* Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS, or MEK1. *Proc Natl Acad Sci U S A* 2012; **109**: E2127–33.
- 13 Yamamoto C, Basaki Y, Kawahara A *et al.* Loss of PTEN expression by blocking nuclear translocation of EGR1 in gefitinib-resistant lung cancer cells harboring epidermal growth factor receptor-activating mutations. *Cancer Res* 2010; **70**: 8715–25.
- 14 Sequist LV, Waltman BA, Dias-Santagata D *et al.* Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011; **3**: 75ra26.
- 15 Katayama R, Khan TM, Benes C *et al.* Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. *Proc Natl Acad Sci U S A* 2011; **108**: 7535–40.
- 16 Qi J, McTigue MA, Rogers A *et al.* Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors. *Cancer Res* 2011; **71**: 1081–91.
- 17 Song A, Kim TM, Kim DW *et al.* Molecular changes associated with acquired resistance to Crizotinib in ROS1-rearranged non-small cell lung cancer. *Clin Cancer Res* 2015; **21**: 2379–87.
- 18 Fukumoto S, Morifuji M, Katakura Y, Ohishi M, Nakamura S. Endostatin inhibits lymph node metastasis by a down-regulation of the vascular endothelial growth factor C expression in tumor cells. *Clin Exp Metastasis* 2005; **22**: 31–8.