Expression and copy number gains of the RET gene in 631 early and mid stage non-small cell lung cancer cases

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Keywords
Copy number variation; early and middle stage; gene rearrangement; non-small cell lung cancer (NSCLC); RET expression.

Abstract
Background: To identify whether RET is a potential target for NSCLC treatment, we examined the status of the RET gene in 631 early and mid stage NSCLC cases from south central China.

Methods: RET expression was identified by Western blot. RET-positive expression samples were verified by immunohistochemistry. RET gene mutation, copy number variation, and rearrangement were analyzed by DNA Sanger sequencing, TaqMan copy number assays, and reverse transcription-PCR. ALK and ROS1 expression levels were tested by Western blot and EGFR mutation using Sanger sequencing.

Results: The RET-positive rate was 2.5% (16/631). RET-positive expression was related to poorer tumor differentiation (P < 0.05). In the 16 RET-positive samples, only two samples of moderately and poorly differentiated lung adenocarcinomas displayed RET rearrangement, both in RET-KIF5B fusion partners. Neither ALK nor ROS1 translocation was found. The EGFR mutation rate in RET-positive samples was significantly lower than in RET-negative samples (P < 0.05).

Conclusion: RET-positive expression in early and mid stage NSCLC cases from south central China is relatively low and is related to poorer tumor differentiation. RET gene alterations (copy number gain and rearrangement) exist in all RET-positive samples. RET-positive expression is a relatively independent factor in NSCLC patients, which indicates that the RET gene may be a novel target site for personalized treatment of NSCLC.

Introduction
Non-small cell lung cancer (NSCLC), which accounts for 80–85% of all lung cancer cases, has one of the highest cancer incidences in the world.1,2 It is usually diagnosed at an advanced stage and has a dismal prognosis.2 NSCLC is further divided into subtypes based on histology and approximately 30% of cases are squamous cell carcinoma. The remaining 70% are classified as non-squamous NSCLC, which includes adenocarcinomas, large-cell carcinomas, and less well-differentiated tumors.1,3

Several key genetic alterations have been found in lung cancer, such as EGFR mutations and ALK rearrangement.4–7 The application of EGFR-tyrosine kinase inhibitors (TKIs) has highlighted the importance of targeted therapeutic agents to appropriate patient populations with specific genetic alterations.8,9 In addition, gene rearrangements, such as ALK and ROS1 have also been identified in NSCLC; tumors with ALK and ROS1 rearrangement are responsive to ALK-TKIs.10

Although TKIs are effective for NSCLCs with corresponding gene mutations or rearrangements, the long-term efficacy is not satisfactory because of drug resistance.4,5 Recently, a new receptor tyrosine kinase gene, RET, has been identified in lung cancer, and is rearranged in 1% of lung adenocarcinoma cases.11 RET is a proto-oncogene (10q11.2) located on the long arm of chromosome 10, including...
21 exons with a total length of about 60,000 bp. The protein encoded by RET is a tyrosine kinase receptor, which binds to the ligand and stimulates intracellular phosphorylation, which in turn activates downstream signals and plays a critical role in proliferation, neuronal navigation, and differentiation. In NSCLC, the most common RET fusion pattern is with KIF5B. The first 15 exons of KIF5B containing kinesin motor and coiled-coil domains are rearranged to exons 12–20 of the RET gene, which contains the RET kinase domain. This rearrangement produces adverse activation of RET with homodimerization underlying the oncogenic potency of the gene fusion product. RET mutations are present in nearly all hereditary medullary thyroid cancer patients, and approximately 30% with RET gene copy number alteration are associated with poor outcomes. Therefore, RET copy number alteration is a vital gene alteration in malignant tumors.

In this study, we examined the status of the RET gene in 631 early and mid stage NSCLC cases from south central China to identify whether RET is a potential target for NSCLC treatment.

Methods
We identified RET expression in all samples using Western blot. We then analyzed RET gene mutation, copy number variation, and rearrangement in RET-positive expression samples using DNA Sanger sequencing, TaqMan copy number assays, and reverse transcription (RT)-PCR. ALK and ROS1 expression was detected by Western blot and EGFR mutation by exon sequencing.

Sample and clinical data of non-small cell lung cancer patients
NSCLC samples (n = 631, 466 men, 165 women; age range 21–84) and normal tissues (> 5 cm away from the tumor edge) were consecutively collected from patients by pulmonary lobectomy at the second Xiangya Hospital (Changsha, Hunan, China) from July 2008 to July 2014. All patients signed written consent and the hospital institutional review board approved the study. All patients were from south central China and were classified according to the World Health Organization classification system.

RET protein expression by Western blot
Frozen lung tumors and normal tissues (control) were minced in liquid nitrogen and resuspended in 1 × cell lysis buffer (10X #9803; Cell Signaling Technology, Danvers, MA, USA). Tissue suspension was then sonicated and cleared by centrifugation. RET protein immunoblot analysis was carried out according to the RET antibody (#3223, 1:1000; Cell Signaling Technology) following the manufacturer’s standard protocol. A high RET expression sample confirmed with RET rearrangement by sequencing and a RET-positive papillary thyroid carcinoma sample identified by our hospital pathologist were used as positive control samples in immunoblot screening analysis.

Immunohistochemical staining
Immunohistochemical staining for RET was characterized by RET antibody (#ab134100, 1:200, monoclonal antibody; Abcam, Cambridge, MA, USA). Five-micrometer tissue sections of RET positive and negative lung tumor samples obtained by Western immunoblotting and two RET-positive papillary thyroid carcinoma samples identified by our hospital pathologist as control samples were deparaffinized, rehydrated, and subjected to antigen retrieval at high temperature and high pressure. Slides were quenched in 3% H2O2 for 10 minutes, washed in diH2O, and then blocked with tris-buffered saline/0.1% Tween 20/5% goat serum. Slides were incubated overnight at 4°C with RET antibody. Immunohistochemical (IHC) detection was conducted using an UltraSensitive SP IHC Kit (Fuzhou Maixin Biotech, Fuzhou, China). All slides were exposed to an AEC Kit (Fuzhou Maixin Biotech, China) and counterstained with hematoxylin. Images (x20) were acquired using a Leica Light microscope (Leica Microsystems, Wetzlar, Germany) at high magnification and five horizons were randomly selected for immunohistochemical evaluation of RET. RET expression levels were scored from 0 to 2+ (0 for no staining, 0.5+ for weak, 1+ for moderate, and 2+ for strong immunoreactivity). The percentages of cells with positive RET staining within the cancerous region of a section were scored as follows: 0 for < 5% positive cells, 0.5+ for 5–10%, 1+ for 11–50%, and 2+ for 51–100%.

RET hot-point mutation analysis and reverse transcription-PCR of RET fusion
To analyze RET mutation, genomic DNA was extracted from frozen tumor tissues with positive RET protein expression using a Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA). Extracted DNA was analyzed by PCR, followed by direct sequencing, as previously described. Further analysis was performed on the exons that were more frequently mutated (exons 8, 10, 11, 13–16). The RET fusion variants of RET-positive expression samples were determined by RT-PCR using an RNA UltraSense One-step RT-PCR Kit (Life Technologies, Carlsbad, CA, USA) according to the product manual. The KIF5B primers used were: forward (5'-ATTAGGTG

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CAACTGTAGAACC-3\prime) and reverse 5\prime -CAGGCCCCATA-CATTGAT-3\prime.

**RET gene copy number analysis**

Genomic DNA extracted from frozen lung tumors with positive RET expression, five samples with negative RET expression, and the control samples were analyzed. Three different RET TaqMan Copy Number Assays (Hs00379542-cn, Overlaps Exon18-Intron18; Hs05123164-cn, Intron13; and Hs0237515-cn, Overlaps Exon4-Intron4) were used to detect the RET gene copy number variations, respectively, using an ABI7500 Fast Real-Time PCR System TaqMan sequence detector (Life Technologies, USA). TaqMan RNaseP Control Reagent (VIC dye) (Life Technologies, USA) was used as internal control. Multiplex PCR reactions contained: one TaqMan Genotyping Master Mix (Life Technologies, USA), one RNaseP Primer-Probe (VIC dye) Mix, one RET Primer-Probe Mix (FAM dye), and 5 ng template genomic DNA in a total volume of 20 μL. All experiments were conducted in quadruplicate. Data analysis was conducted using CopyCaller version 2.1 (Thermo Fisher Scientific, USA).

**Detection of EGFR mutation and ALK and ROS1 gene translocation**

DNA from RET-positive samples and 44 randomly selected RET-negative samples were amplified by PCR using primers to exons 18–21 of the EGFR gene: EGFRex18F(M13–21) tgaaaagggcgccagtcGAGTGGGCAATG, EGFRex 18R(M13–24) aacagtctagctgTGGAATTTTCAACACTCAG; EGFRex19F(M13–20) tgaacaagcccgctctCCACA GCCCCAGTGTG; EGFRex19R(M13–48) acggctaacattctc accagggGCCCAGTGTCTCTAAGG; EGFRex20F(M13–21) tgaaaagggcgccagtocGCTGTGAGCTCTTTG; EGFRex20R(M13–24) acagcttagctggAAGGTGTGTG TGTGCTG; GFRex21F(M13–20) gtaaaagggcgccagctTACGCAGGCCTAAGG; and EGFRex21R(M13–48) acg cggtagtaaatctcaccagggGCGGTCTACCCAGAATGTC.

PCR products were analyzed using bi-direct-sequencing.

ALK and ROS1 gene translocation analysis of RET-positive samples was conducted by ALK and ROS1 protein immunoblot analysis according to the ALK [D5F3] XP Rabbit mAb #3633, 1:2000) and ROS1 (ROSI [D4D6] Rabbit mAb #3287, 1:1000) antibodies, following the manufacturer’s standard protocol (Cell Signaling Technology, USA).

**Statistical analysis**

Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation. Relationships between RET expression and clinicopathologic variables were examined using chi-square tests and correlation analysis. Results were considered statistically significant at P < 0.05.

**Results**

**Patient characteristics**

The clinicopathologic data of 631 (466 men, 165 women) early and mid stage NSCLC patients were obtained from medical records. The mean age (± standard deviation) of the patients was 57.8 ± 9.57 (range 21–84). The tumor types were: squamous carcinomas (311), adenocarcinomas (287), adenosquamous carcinomas (21), and other NSCLCs (12) (Table 1).

**RET expression and immunohistochemical staining**

We detected RET expression using Western blot (Fig 1). As shown in Table 1, among the 631 early and mid stage...
NSCLC samples, only 16 displayed positive RET expression, at a rate of 2.5% (16/631). The RET-positive samples were verified by IHC staining. IHC results showed that nine samples were strong positive (++), five were moderate immunoreactivity positive (+), and the remaining two were weak positive (0.5+) and negative (−) (Fig 2). The results of IHC staining of lung cancer tissues corresponded to the Western blot results. Correlation analysis showed that positive RET expression was significantly related to poorer tumor differentiation (P < 0.05). There was no correlation between RET expression and age, gender, stage, Brinkman index, or histology classification (Table 1).

**RET alterations in RET-positive samples**

After detecting positive RET expression in the NSCLC samples, we investigated the potential mechanisms underlying such expression. We first analyzed gene mutation on hot-point exons in RET-positive samples using a Genomic DNA Puriﬁcation Kit (Thermo Fisher Scientiﬁc, USA). However, no RET gene mutations on hot-point exons 8, 10, 11, or 13–16 were detected in the 16 RET-positive samples. In order to explore the specific mechanism of RET expression in NSCLC, we detected RET fusion and RET copy number variants in the 16 RET-positive samples. RT-PCR rearrangement testing showed that only two samples of moderately and poorly differentiated lung adenocarcinomas displayed RET rearrangement, both in RET-KIF5B fusion partners. The IHC results were strong positive (++). We then investigated RET gene copy number alteration in exons 4 and 8 and intron 13 in the remaining 14 samples. All 14 RET-positive samples showed RET copy number gain compared to the normal tissues and the five RET-negative expression samples (Fig 3). These results showed that all RET-positive tumor samples presented rearrangement or copy number gain of the RET gene.

**ALK and ROS1 expression and EGFR mutation in RET-positive samples**

To identify an association between RET expression and ALK and ROS1 translocation and EGFR mutation, an analysis of ALK and ROS1 protein expression in the 16 RET-positive samples was conducted by immunoblotting. Neither ALK nor ROS1 translocation was found in the 16 RET-positive samples, while one EGFR mutation in exon 21 (L858R) was detected. We randomly selected 44 RET-negative expression samples for EGFR exon

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**Figure 1** RET expression in non-small cell lung cancer samples was detected by Western blot. The figure shows six RET-positive expression samples and two RET-positive expression samples with RET gene rearrangement. The RET-positive control is a thyroid cancer sample with RET-positive expression. T represents RET-positive expression in the tumor; N represents normal tissue from RET-positive patients.

**Figure 2** Immunohistochemical results of RET-positive expression. (a) RET-negative expression in non-small cell lung cancer (NSCLC); (b) RET-positive control in thyroid cancer. (c) RET-positive expression in NSCLC with RET rearrangement (++). (d-f) RET-positive expression in NSCLC (D-F+: +, +, +, 0.5+).
sequencing. As Table 2 shows, we found nine samples with EGFR exon 21 mutations (L858R) and six with EGFR exon 19 deletions. The EGFR mutation rate in RET-positive samples was significantly lower than in RET-negative samples ($P < 0.05$). These results indicate that ALK or ROS1 translocation or EGFR mutation rarely occurs in RET-positive patients. Therefore, RET gene alteration could be a potential target for NSCLC patients without such mutations.

**Discussion**

After decades of efforts to improve cancer therapy, targeted therapies and personalized medicine have become a new direction for cancer treatment.6 Targeted therapies can be directed at unique molecular or gene products of cancer cells to produce greater efficacy of cancer treatment with less toxicity. Therefore, it is vital to identify effective tumor markers as the targets for cancer treatment to improve patient survival rates and quality of life in recurrent or advanced-stage malignant tumors.

NSCLC has one of the highest incidences of cancer globally and causes the highest rate of cancer-related death. During the past decades, targeted drugs, such as EGFR and ALK TKIs have been developed and have shown good therapeutic effects.20,21 Although several genetic mutations have previously been reported, no cancer genome mutation has been observed in a large proportion of NSCLC patients. More than 40% of NSCLCs appear to be driven by unknown genetic events,22,23 therefore, it is important to identify new biomarkers that can stratify NSCLC patients and acquire a better response to targeted therapy.

The oncogenic effect of RET was first identified in papillary thyroid cancer, where diverse kinds of chromosomal translocations and inversions led to the formation of papillary thyroid cancer/RET fusion genes.24 Specific point mutations have also been reported as drivers in MEN2A and MEN2B.24 In addition, activated RET has been observed in prostate25 and pancreatic cancers26 and melanoma.27 The direct transforming impact of RET as a driver is also supported by transgenic mice studies of RET, which generated a variety of malignancies.28,29 A new RET gene fusion with KIF5B was first identified in lung cancer in 2012.15 RET proto-oncogene expression increased with KIF5B fusion. RET rearrangement at a frequency of 1–2% has been reported.29,30 Drugs targeted to the RET gene inhibit RET kinase, the expression product of RET. Taking this into account, we first tested RET expression by Western blot in 631 NSCLC samples. Only 16 samples displayed positive RET expression at a rate of 2.5% (16/631). A retrospective analysis conducted by Platt et al. yielded an RET expression rate of 11.6% (40/346) in Asians,31 which is much higher than our result. Differences in tumor staging may have caused our lower RET expression rate. Almost all of the patients in our study were at stages lower than IIB and surgery was indicated, whereas the patients in the

**Table 2** EGFR mutations in patients with RET-positive expression

| EGFR mutation | RET expression | $P$ |
|---------------|----------------|-----|
|               | Positive (n = 16) | Negative (n = 44) |
| Wild-type mutation | 15 | 29 | 0.046 |
| Exon 19 | 0 | 6 |
| Exon 21 | 1 | 9 |
Platt et al. study were at advanced stage (IIIB–IV) and were treated with vandetanib. However, 93.8% (15/16) of the samples in our study were verified positive by IHC staining, indicating that our results are reliable.

No RET mutations on hot-point exons 8, 10, 11, or 13–16 were found by direct sequencing. We further examined the gene rearrangement in RET-positive samples. RT-PCR showed that only 2 of the 16 samples showed rearrangement with KIF5B. Both samples were of moderately or poorly differentiated lung adenocarcinomas and showed strongly positive (+ +) by IHC. The remaining 14 RET-positive samples displayed copy number gain compared to the five RET-negative samples. Yang et al. reported 1.7% (2/116) RET translocation and 64% (74/116) RET copy number gain by fluorescence in situ hybridization. The reason for the lower RET translocation rate (2/631) and copy number gain in our study may be that we only examined RET gene alterations in RET-positive samples. The samples with RET-negative expression were not examined for gene alterations, and may have contained RET alterations. The differences between test methods, geography, and ethnicity may also have contributed to the differing results.

Tumors with ALK and ROS1 rearrangement are responsive to ALK-TKIs; however no ALK or ROS1 expression was found in the 16 samples in our study. Only one EGFR mutation in exon 21 (L858R) was detected. Thus, we randomly selected 44 RET-negative NSCLC samples for EGFR exon sequencing. We found nine samples with EGFR exon 21 mutations (L858R) and six with EGFR exon 19 deletions. Our EGFR mutation result of 26.7% (16/60) with both RET negative and positive samples is higher than the 19% (19/99) reported by Yang et al. However, only one (6.25%) EGFR mutation was found in the RET-positive samples. This result indicates that targeted drugs for ALK, ROS1, or EGFR mutations may not be effective for RET-positive tumors. Only drugs with activity against RET kinase could be effective to these tumors. Recently, Kodama et al. demonstrated the antitumor activity of alec tinib against RET-rearranged NSCLC. Alectinib inhibited RET kinase and the growth of RET fusion-positive cells. However, alectinib has low activity against ROS1 kinase. Although the RET expression rate is relatively low in early and mid stage NSCLC samples, ALK, ROS1, or EGFR mutations rarely occur in RET-positive patients. Targeted drugs are effective for RET-positive NSCLC.

The RET-positive expression rate is relatively low at early and mid stage NSCLC in patients from south central China. RET gene alterations (copy number gain and rearrangement) exist in all RET-positive samples. RET-positive expression is a relatively independent factor in NSCLC patients, which indicates that the RET gene may be a novel target site for personalized treatment of NSCLC.

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Disclosure

No authors report any conflict of interest.

References

1. Herbst RS. Current and future strategies for antiangiogenic agents in non-small-cell lung cancer. Clin Lung Cancer 2008; 9 (Suppl 2): S50.
2. Goldstraw P, Ball D, Jett JR et al. Non-small-cell lung cancer. Lancet 2011; 378: 1727–40.
3. D’Addario G, Felip E, ESMOWG. Non-small-cell lung cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 2009; 20 ((Suppl 4)): 68–70.
4. Lynch TJ, Bell DW, Sordella R et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129–39.
5. Camidge DR, Bang YJ, Kwak EL et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: Updated results from a phase 1 study. Lancet Oncol 2012; 13: 1011–9.
6. Shaw AT, Yeap BY, Solomon BJ et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: A retrospective analysis. Lancet Oncol 2011; 12: 1004–12.
7. Pao W, Miller V, Zakowski M et al. EGFR receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004; 101: 13306–11.
8. Zhu YJ, Zhang HB, Liu YH et al. Association of mutant EGFR L858R and exon 19 concentration in circulating cell-free DNA using droplet digital PCR with response to EGFR-TKIs in NSCLC. Oncol Lett 2017; 14: 2573–9.
9. Tan CS, Cho BC, Soo RA. Treatment options for EGFR mutant NSCLC with CNS involvement-Can patients BLOOM with the use of next generation EGFR TKIs? Lung Cancer 2017; 108: 29–37.
10. Berghen K, Shaw AT, Ou SH et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012; 30: 863–70.
11. Li F, Feng Y, Fang R et al. Identification of RET gene fusion by exon array analyses in “pan-negative” lung cancer from never smokers. Cell Res 2012; 22: 928–31.
12. Takahashi M, Ritz J, Cooper GM. Activation of a novel human transforming gene, ret, by DNA rearrangement. Cell 1985; 42: 581–8.
13 Goto K, Kawahara I, Kuniyasu H, Takaki M. A protein tyrosine kinase receptor, c-RET signaling pathway contributes to the enteric neurogenesis induced by a 5-HT4 receptor agonist at an anastomosis after transection of the gut in rodents. J Physiol Sci 2015; 65: 377–83.

14 Veiga-Fernandes H, Coles MC, Foster KE et al. Tyrosine kinase receptor RET is a key regulator of Peyer’s patch organogenesis. Nature 2007; 446: 547–51.

15 Ju YS, Lee WC, Shin JY et al. A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res 2012; 22: 436–45.

16 Kohno T, Ichikawa H, Totoki Y et al. KIF5B-RET fusions in lung adenocarcinoma. Nat Med 2012; 18: 375–7.

17 Ciampi R, Romei C, Cosci B et al. Chromosome 10 and RET gene copy number alterations in hereditary and sporadic medullary thyroid carcinoma. Mol Cell Endocrinol 2012; 348: 176–82.

18 Elisei R, Cosci B, Romei C et al. Identification of a novel point mutation in the RET gene (Ala883Thr), which is associated with medullary thyroid carcinoma phenotype only in homozygous condition. J Clin Endocrinol Metab 2004; 89: 5823–7.

19 Zhou Y, Zhao Y, Cui B et al. RET proto-oncogene mutations are restricted to codons 634 and 918 in mainland Chinese families with MEN2A and MEN2B. Clin Endocrinol (Oxf) 2007; 67: 570–6.

20 Zhu Q, Hu H, Weng DS et al. Pooled safety analyses of ALK-TKI inhibitor in ALK-positive NSCLC. BMC Cancer 2017; 17: 412.

21 Kwon BS, Park JH, Kim WS et al. Predictive factors for switched EGFR-TKI retreatment in patients with EGFR-mutant non-small cell lung cancer. Tuberc Respir Dis (Seoul) 2017; 80: 187–93.

22 Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol 2011; 12: 173–80.

23 Olivo-Marston SE, Mechanic LE, Mollerup S et al. Serum estrogen and tumor-positive estrogen receptor-alpha are strong prognostic classifiers of non-small-cell lung cancer survival in both men and women. Carcinogenesis 2010; 31: 1778–86.

24 Alberti L, Carniti C, Miranda C, Roccati E, Pierotti MA. RET and NTRK1 proto-oncogenes in human diseases. J Cell Physiol 2003; 195: 168–86.

25 Dawson DM, Lawrence EG, MacLennan GT et al. Altered expression of RET proto-oncogene product in prostatic intraepithelial neoplasia and prostate cancer. J Natl Cancer Inst 1998; 90: 519–23.

26 Zeng Q, Cheng Y, Zhu Q et al. The relationship between overexpression of glial cell-derived neurotrophic factor and its RET receptor with progression and prognosis of human pancreatic cancer. J Int Med Res 2008; 36: 656–64.

27 Ohshima Y, Yajima I, Takeda K et al. c-RET molecule in malignant melanoma from oncogenic RET-carrying transgenic mice and human cell lines. PLoS One 2010; 5: e10279.

28 Portella G, Salvatore D, Botti G et al. Development of mammary and cutaneous gland tumors in transgenic mice carrying the RET/PTC1 oncogene. Oncogene 1996; 13: 2021–6.

29 Kawai K, Iwashita T, Murakami H et al. Tissue-specific carcinogenesis in transgenic mice expressing the RET proto-oncogene with a multiple endocrine neoplasia type 2A mutation. Cancer Res 2000; 60: 5254–60.

30 Sabari JK, Siau ED, Drilon A. Targeting RET-rearranged lung cancers with multi-kinase inhibitors. Oncoscience 2017; 4 (3–4): 23–4.

31 Platt A, Morten J, Ji Q et al. A retrospective analysis of RET translocation, gene copy number gain and expression in NSCLC patients treated with vandetanib in four randomized phase III studies. BMC Cancer 2015; 15: 171.

32 Yang HS, Horten B. Gain of copy number and amplification of the RET gene in lung cancer. Exp Mol Pathol 2014; 97: 465–9.

33 Kodama T, Tsukaguchi T, Satoh Y et al. Alectinib shows potent antitumor activity against RET-rearranged non-small cell lung cancer. Mol Cancer Ther 2014; 13: 2910–8.

34 Gadgeel SM, Gandhi L, Riely GJ et al. Safety and activity of alectinib against systemic disease and brain metastases in patients with crizotinib-resistant ALK-rearranged non-small-cell lung cancer (AF-002G): Results from the dose-finding portion of a phase 1/2 study. Lancet Oncol 2014; 15: 1119–28.