Acknowledgements

The authors thank all the collaborators in the NCC and the LC-SCRUM/LURET studies.

Financial & competing interests disclosure

This work was supported in part by the Program for Promotion of Fundamental Studies in Health Sciences from the National Institute of Biomedical Innovation (NIBIO); Grants-in-Aid from the Ministry of Health, Labor, and Welfare for the Third-term Comprehensive 10-year Strategy for Cancer Control and for Research on New Drug and Medical Device Development and from the Ministry of Education, Culture, Sports, Science, and Technology of Japan for Scientific Research on Innovative Areas (22131006), and the National Cancer Center Research and Development Fund. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest

1 Tran B, Dancey JE, Kamel-Reid S *et al.* Cancer genomics. technology, discovery, and translation. *J. Clin. Oncol.* 30(6), 647–660 (2012).

2 Oxnard GR, Binder A, Janne PA. New targetable oncogenes in non-small-cell lung cancer. *J. Clin. Oncol.* 31(8), 1097–1104 (2013).

3 Maemondo M, Inoue A, Kobayashi K *et al.* Gefitinib or chemotherapy for non-small-cell lung cancer with mutated *EGFR*. *N. Engl. J. Med.* 362(25), 2380–2388 (2010).

4 Oizumi S, Kobayashi K, Inoue A *et al.* Quality of life with gefitinib in patients with *EGFR*-mutated non-small cell lung cancer. quality of life analysis of North East Japan Study Group 002 Trial. *Oncologist* 17(6), 863–870 (2012).

5 Shaw AT, Engelman JA. ALK in lung cancer: past, present, and future. *J. Clin. Oncol.* 31(8), 1105–1111 (2013).

6 Mano H. ALKoma: a cancer subtype with a shared target. *Cancer Discov.* 2(6), 495–502 (2012).

7 Sakamoto H, Tsukaguchi T, Hiroshima S *et al.* CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. *Cancer Cell* 19(5), 679–690 (2011).

8 Takeuchi K, Soda M, Togashi Y *et al. RET, ROS1* and *ALK* fusions in lung cancer. *Nat. Med.* 18(3), 378–381 (2012).

• **Along with [9,24], this is one of the trio papers, on *RET*-fusion discovery published in the same issue of *Nature Medicine*.**

9 Kohno T, Ichikawa H, Totoki Y *et al. KIF5B-RET* fusions in lung adenocarcinoma. *Nat. Med.* 18(3), 375–377 (2012).

• **Along with [8,24], this is one of the trio papers on *RET*-fusion discovery published in the same issue of *Nature Medicine*.**

10 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.* 22(5), 928–931 (2012).

• **Fifth study of *RET*-fusion discovery.**

11 Wang R, Hu H, Pan Y *et al. RET* fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. *J. Clin. Oncol.* 30(35), 4352–4359 (2012).

12 Kohno T, Tsuta K, Tsuchihara K, Nakaoku T, Yoh K, Goto K. *RET* fusion gene: translation to personalized lung cancer therapy. *Cancer Sci.* 104(11), 1396–1400 (2013).

13 Yoshida A, Kohno T, Tsuta K *et al. ROS1*-rearranged lung cancer. a clinicopathologic and molecular study of 15 surgical cases. *Am. J. Surg. Pathol.* 37(4), 554–562 (2013).

14 Davies KD, Le AT, Theodoro MF *et al.* Identifying and targeting *ROS1* gene fusions in non-small cell lung cancer. *Clin. Cancer Res.* 18(17), 4570–4579 (2012).

15 Mazieres J, Peters S, Lepage B *et al.* Lung cancer that harbors an *HER2* mutation: epidemiologic characteristics and therapeutic perspectives. *J. Clin. Oncol.* 31(16), 1997–2003 (2013).

16 Pao W, Hutchinson KE. Chipping away at the lung cancer genome. *Nat. Med.* 18(3), 349–351 (2012).

17 Kim HR, Lim SM, Kim HJ *et al.* The frequency and impact of *ROS1* rearrangement on clinical outcomes in never smokers with lung adenocarcinoma. *Ann. Oncol.* 24(9), 2364–2370 (2013).

18 Cai W, Li X, Su C *et al. ROS1* fusions in Chinese patients with non-small-cell lung cancer. *Ann. Oncol.* 24(7), 1822–1827 (2013).

19 Chao BH, Briesewitz R, Villalona-Calero MA. *RET* fusion genes in non-small-cell lung cancer. *J. Clin. Oncol.* 30(35), 4439–4441 (2012).

20 Xu J, He J, Yang H *et al.* Somatic mutation analysis of *EGFR, KRAS, BRAF* and *PIK3CA* in 861 patients with non-small cell lung cancer. *Cancer Biomark.* 10(2), 63–69 (2011).

21 Pao W, Girard N. New driver mutations in non-small-cell lung cancer. *Lancet Oncol.* 12(2), 175–180 (2011).

22 Paik PK, Arcila ME, Fara M *et al.* Clinical characteristics of patients with lung adenocarcinomas harboring *BRAF* mutations. *J. Clin. Oncol.* 29(15), 2046–2051 (2011).

23 Marchetti A, Felicioni L, Malatesta S *et al.* Clinical features and outcome of patients with non-small-cell lung cancer harboring *BRAF* mutations. *J. Clin. Oncol.* 29(26), 3574–3579 (2011).

24 Lipson D, Capelletti M, Yelensky R *et al.* Identification of new *ALK* and *RET* gene fusions from colorectal and lung cancer biopsies. *Nat. Med.* 18(3), 382–384 (2012).

• **Along with [8,9], this is one of the trio papers on *RET*-fusion discovery published in the same issue of *Nature Medicine*.**

25 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.* 22(3), 436–445 (2012).

• **Fourth study of *RET*-fusion discovery.**

26 Cai W, Su C, Li X *et al. KIF5B-RET* fusions in Chinese patients with non-small cell lung cancer. *Cancer* 119(8), 1486–1494 (2013).

27 Suehara Y, Arcila M, Wang L *et al.* Identification of *KIF5B-RET* and *GOPC-ROS1* fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions. *Clin. Cancer Res.* 18(24), 6599–6608 (2012).

28 Drilon A, Wang L, Hasanovic A *et al.* Response to cabozantinib in patients with *RET* fusion-positive lung adenocarcinomas. *Cancer Discov.* 3(6), 630–635 (2013).

• **US FDA-approved RET tyrosine kinase inhibitor showed a positive therapeutic**

response in three patients with *RET* fusion-positive lung adenocarcinoma (LADC).

29 Kim SC, Jung Y, Park J *et al.* A high-dimensional, deep-sequencing study of lung adenocarcinoma in female never-smokers. *PLoS ONE* 8(2), e55596 (2013).

30 Yokota K, Sasaki H, Okuda K *et al.* *KIF5B/RET* fusion gene in surgically-treated adenocarcinoma of the lung. *Oncol. Rep.* 28(4), 1187–1192 (2012).

31 Suzuki M, Makinoshima H, Matsumoto S *et al.* Identification of a lung adenocarcinoma cell line with *CCDC6–RET* fusion gene and the effect of RET inhibitors *invitro* and *in vivo*. *Cancer Sci.* 104(7), 896-903 (2013).

32 Matsubara D, Kanai Y, Ishikawa S *et al.* Identification of *CCDC6–RET* fusion in the human lung adenocarcinoma cell line, LC-2/ad. *J. Thorac. Oncol.* 7(12), 1872–1876 (2012).

33 Tsuta K, Kohno T, Yoshida A *et al.* *RET*-rearranged non-small-cell lung carcinoma: a clinicopathological and molecular analysis. *Br. J. Cancer* doi: 10.1038/bjc.2014.36 (2014) (Epub ahead of print).

34 Gild ML, Bullock M, Robinson BG, Clifton-Bligh R. Multikinase inhibitors: a new option for the treatment of thyroid cancer. *Nat. Rev. Endocrinol.* 7(10), 617–624 (2011).

35 Cabozantinib in patients with RET fusion-positive advanced non-small cell lung cancer. http://clinicaltrials.gov/show/NCT01639508

36 Gautschi O, Zander T, Keller FA *et al.* A patient with lung adenocarcinoma and *RET*-fusion treated with vandetanib. *J. Thorac. Oncol.* 8(5), e43–e44 (2013).

• **FDA-approved RET tyrosine kinase inhibitor showed a positive therapeutic response in a patient with *RET*-fusion-positive LADC.**

37 A Phase II, open-label single-arm study to evaluate the efficacy and safety of vandetanib in patients with RET fusion-positive unresectable locally advanced or metastatic non-small cell lung cancer. https://upload.umin.ac.jp

38 Vandetanib in advanced NSCLC with RET rearrangement. http://clinicaltrials.gov/show/NCT01823068

39 Study of the safety and activity of lenvatinib (E7080) in subjects with KIF5B–RET-positive adenocarcinoma of the lung. http://clinicaltrials.gov/show/NCT01877083

40 Ponatinib in advanced NSCLC w/ RET translocations. http://clinicaltrials.gov/ct2/show/NCT01813734

41 ClinicalTrials.gov. http://clinicaltrials.gov

42 Rikova K, Guo A, Zeng Q *et al.* Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* 131(6), 1190–1203 (2007).

43 Awad MM, Katayama R, Mctigue M *et al.* Acquired resistance to crizotinib from a mutation in *CD74–ROS1*. *N. Engl. J. Med.* 369(12), 1172–1173 (2013).

44 Bos M, Gardizi M, Schildhaus HU *et al.* Complete metabolic response in a patient with repeatedly relapsed non-small cell lung cancer harboring *ROS1* gene rearrangement after treatment with crizotinib. *Lung Cancer* 81(1), 142–143 (2013).

45 Bergethon K, Shaw AT, Ou SH *et al.* *ROS1* rearrangements define a unique molecular class of lung cancers. *J. Clin. Oncol.* 30(8), 863–870 (2012).

46 Stephens P, Hunter C, Bignell G *et al.* Lung cancer. intragenic ERBB2 kinase mutations in tumours. *Nature* 431(7008), 525–526 (2004).

47 Shigematsu H, Takahashi T, Nomura M *et al.* Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res.* 65(5), 1642–1646 (2005).

48 Greulich H, Kaplan B, Mertins P *et al.* Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of *ERBB2*. *Proc. Natl Acad. Sci. USA* 109(36), 14476–14481 (2012).

49 Brose MS, Volpe P, Feldman M *et al.* *BRAF* and *RAS* mutations in human lung cancer and melanoma. *Cancer Res.* 62(23), 6997–7000 (2002).

50 Davies H, Bignell GR, Cox C *et al.* Mutations of the *BRAF* gene in human cancer. *Nature* 417(6892), 949–954 (2002).

51 Naoki K, Chen TH, Richards WG, Sugarbaker DJ, Meyerson M. Missense mutations of the *BRAF* gene in human lung adenocarcinoma. *Cancer Res.* 62(23), 7001–7003 (2002).

52 Kinno T, Tsuta K, Shiraishi K *et al.* Clinicopathological features of nonsmall cell lung carcinomas with *BRAF* mutations. *Ann. Oncol.* 25(1), 138–142 (2014).

53 Gautschi O, Pauli C, Strobel K *et al.* A patient with *BRAF* V600E lung adenocarcinoma responding to vemurafenib. *J. Thorac. Oncol.* 7(10), e23–e24 (2012).

54 Peters S, Michielin O, Zimmermann S. Dramatic response induced by vemurafenib in a *BRAF* V600E-mutated lung adenocarcinoma. *J. Clin. Oncol.* 31(20), e341–e344 (2013).

55 Planchard D, Mazieres J, Riely GJ *et al.* Interim results of Phase II study BRF113928 of dabrafenib in *BRAF* V600E mutation-positive non-small cell lung cancer (NSCLC) patients. *J. Clin. Oncol.* 31(Suppl.), Abstract 8009 (2013).

56 Nakano H, Yamamoto F, Neville C, Evans D, Mizuno T, Perucho M. Isolation of transforming sequences of two human lung carcinomas: structural and functional analysis of the activated c-K-ras oncogenes. *Proc. Natl Acad. Sci. USA* 81(1), 71–75 (1984).

57 Santos E, Martin-Zanca D, Reddy EP, Pierotti MA, Della Porta G, Barbacid M. Malignant activation of a *K-ras* oncogene in lung carcinoma but not in normal tissue of the same patient. *Science* 223(4637), 661–664 (1984).

58 Taya Y, Hosogai K, Hirohashi S *et al.* A novel combination of K-ras and myc amplification accompanied by point mutational activation of *K-ras* in a human lung cancer. *EMBO J.* 3(12), 2943–2946 (1984).

59 Yuasa Y, Gol RA, Chang A *et al.* Mechanism of activation of an *N-ras* oncogene of SW-1271 human lung carcinoma cells. *Proc. Natl Acad. Sci. USA* 81(12), 3670–3674 (1984).

60 Roberts PJ, Stinchcombe TE. *KRAS* mutation: should we test for it, and does it matter? *J. Clin. Oncol.* 31(8), 1112–1121 (2013).

61 Seo JS, Ju YS, Lee WC *et al.* The transcriptional landscape and mutational profile of lung adenocarcinoma. *Genome Res.* 22(11), 2109–2119 (2012).

62 Imielinski M. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell* 150, 1107–1120 (2012).

63 Dai B, Yoo SY, Bartholomeusz G *et al.* KEAP1-dependent synthetic lethality induced by AKT and TXNRD1 inhibitors in lung cancer. *Cancer Res.* 73(17), 5532–5543 (2013).

64 Oike T, Ogiwara H, Tominaga Y *et al.* A synthetic lethality-based strategy to treat cancers harboring a genetic deficiency in the chromatin remodeling factor BRG1. *Cancer Res.* 73(17), 5508–5518 (2013).

• **Proposes a novel therapeutic strategy of LADC with SWI/SNF chromatin remodeling gene deficiency.**

65 Wilson BG, Helming KC, Wang X *et al.* Residual complexes containing *SMARCA2*

(BRM) underlie the oncogenic drive of *SMARCA4* (BRG1) mutation. *Mol. Cell. Biol.* 34(6), 1136–1144 (2014).

66 Hoffman GR, Rahal R, Buxton F *et al.* Functional epigenetics approach identifies BRM/SMARCA2 as a critical synthetic lethal target in BRG1-deficient cancers. *Proc. Natl Acad. Sci. USA* 111(8), 3128–3133 (2014).

67 Helming KC, Wang X, Wilson BG *et al.* ARID1B is a specific vulnerability in *ARID1A*-mutant cancers. *Nat. Med.* 20(3), 251–254 (2014).

68 Chan DA, Giaccia AJ. Harnessing synthetic lethal interactions in anticancer drug discovery. *Nat. Rev. Drug Discov.* 10(5), 351–364 (2011).

69 Mendelsohn J. Personalizing oncology: perspectives and prospects. *J. Clin. Oncol.* 31(15), 1904–1911 (2013).

70 Yu HA, Arcila ME, Rekhtman N *et al.* Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with *EGFR*-mutant lung cancers. *Clin. Cancer Res.* 19(8), 2240–2247 (2013).

FULL PAPER

BJC

British Journal of Cancer (2014) 110, 1571–1578 | doi: 10.1038/bjc.2014.36

Keywords: *RET* gene rearrangement; lung carcinoma; adenocarcinoma; fluorescence *in situ* hybridisation; immunohistochemistry

RET-rearranged non-small-cell lung carcinoma: a clinicopathological and molecular analysis

K Tsuta*[,1], T Kohno[2,3], A Yoshida[1], Y Shimada[2], H Asamura[4], K Furuta[1] and R Kushima[1]

[1]*Division of Pathology and Clinical Laboratories, National Cancer Center Hospital, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan;* [2]*Division of Genome Biology, National Cancer Center Research Institute, Tokyo, Japan;* [3]*Division of Translational Research, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Tokyo, Japan and* [4]*Division of Thoracic Surgery, National Cancer Center Hospital, Tokyo, Japan*

Background: To elucidate clinicopathological characteristics of non-small-cell lung carcinoma (NSCLC) cases carrying *RET* rearrangements causing oncogenic fusions to identify responders to therapy with RET tyrosine kinase inhibitors.

Methods: We investigated 1874 patients with carcinomas, including 1620 adenocarcinomas (ADCs), 203 squamous cell carcinomas (SCCs), 8 large cell carcinomas, and 43 sarcomatoid carcinomas (SACs). Fluorescence *in situ* hybridisation (FISH) and/or reverse transcription–PCR (RT–PCR) were performed to detect *RET* gene rearrangement.

Results: In all, 22 cases (1.2%) showed *RET* rearrangements; all cases were of ADC histology. Of the 22 patients, 19 possessed *KIF5B–RET* fusion genes, whereas 3 possessed *CCDC6–RET* fusion genes. The *RET*-rearranged tumours were significantly more common in younger patients ($P=0.038$) and tended to occur in patients with no history of smoking ($P=0.051$). In addition, *RET* rearrangements were not associated with gender, occupational history (particularly radioactive exposure), tumour size, lymph node status, tumour stage, or patient survival. The predominant growth pattern in *RET*-rearranged ADCs was lepidic in 6 cases, papillary in 9 cases, acinar in 2 cases, micropapillary in 1 case, and solid in 4 cases. Cells with cytoplasmic mucin production were at least focally present in 12 of the 22 (54.5%) *RET*-rearranged ADC cases. Among the 21 analysed *RET*-rearranged tumours, RET immunopositivity was observed in 15 cases (71.4%), and was significantly associated with *RET* rearrangement ($P<0.001$).

Conclusions: The *RET* rearrangements were observed in 1.2% of NSCLCs. All cases of *RET* rearrangement were ADCs. The *RET* rearrangements were more likely to be observed in younger patients. Although cytoplasmic mucin production was at least focally present in 54.5% of *RET*-rearranged ADCs, specific histological features were not detected.

After the discovery of crucial 'driver' oncogenic mutations in the *epidermal growth factor receptor* (*EGFR*) gene, the EGFR tyrosine kinase inhibitor (TKI) was found to improve survival in non-small-cell lung carcinoma (NSCLC) patients possessing an *EGFR* mutation (Lynch *et al*, 2004; Paez *et al*, 2004; Pao *et al*, 2004). A genomic alteration involving the transforming fusion gene joining the *echinoderm microtubule-associated protein-like 4* gene (*EML4*) and the *anaplastic lymphoma kinase* gene (*ALK*) was identified in 3–13% of patients with NSCLC (Soda *et al*, 2007; Koivunen *et al*, 2008; Yoshida *et al*, 2011). A dramatic response has been observed in patients with *ALK* rearrangements under treatment with an ALK TKI crizotinib (PF-02341066) during a recent clinical trial (Kwak *et al*, 2010).

The *rearranged during transfection* (*RET*) proto-oncogene encodes a receptor tyrosine kinase for members of the glial cell line-derived neurotrophic factor family of extracellular signalling molecules (Knowles *et al*, 2006). This proto-oncogene is involved in the growth and differentiation of neural crest-derived tissues

*Correspondence: Dr K Tsuta; E-mail: ktsuta@ncc.go.jp

Received 20 November 2013; revised 20 December 2013; accepted 7 January 2014; published online 6 February 2014

(Pachnis *et al*, 1993). Chromosomal rearrangements that generate a fusion gene consisting of the juxtaposition of the C-terminal region of the RET protein with the N-terminal portion of another protein can also lead to constitutive activation of the RET kinase. The *RET* gene rearrangements, as represented by papillary thyroid carcinoma (PTC), were most often observed as *coiled-coil domain containing 6* (*CCDC6*)*–RET* (PTC1) (Grieco *et al*, 1990) and *nuclear receptor coactivator 4* (*NCOA4*)*–RET* (PTC3) fusion genes (Santoro *et al*, 1994).

Several investigators, including the authors of the present study, have simultaneously reported on a novel fusion gene comprising parts of the *kinesin family member 5B gene* (*KIF5B*) and the *RET* gene in lung carcinoma (Ju *et al*, 2012; Kohno *et al*, 2012; Lipson *et al*, 2012; Takeuchi *et al*, 2012). Subsequently, other fusion partners of the *RET* genes *CCDC6*, *NCOA4*, and *tripartite motif-containing 33* (*TRIM33*) were identified in NSCLCs (Wang *et al*, 2012; Drilon *et al*, 2013). These fusion transcripts were detected in 0.6–10% of pulmonary adenocarcinomas (ADCs).

Notably, NSCLC cases that are positive for RET fusions have shown responses against existing RET TKIs, including cabozantinib and vandetanib (Drilon *et al*, 2013; Gautschi *et al*, 2013). Therefore, it is important to understand the clinicopathological characteristics of patients with *RET* fusion-positive NSCLCs for improved selection of patients who are likely to benefit from anti-RET therapy. In this study, we analysed *RET* fusions by fluorescence *in situ* hybridisation (FISH) combined with reverse transcription–PCR (RT–PCR) and RNA sequencing data from a large cohort ($n = 1874$) and investigated distinct clinicopathological characteristics of RET fusion-positive cases.

MATERIALS AND METHODS

Case selection. The institutional review board of our hospital approved the study (2010-0077). The specimens used in this study were isolated from 1927 patients who underwent lung resection for ADC, squamous cell carcinoma (SCC), large cell carcinoma (LCC), or sarcomatoid carcinoma (SAC) at the National Cancer Center Hospital (Tokyo, Japan). We collected each patient's age, gender, smoking history, outcome, maximum tumour size (in cm), and pathologic stage (in p-stage). Staging was based on the tumour–necrosis–metastasis (TNM) classification (7th edition; Goldstraw, 2009). Among patients with *RET* rearrangements, we recorded the patients' occupational histories with particular reference to radioactive exposure.

Histological analysis. Histological diagnoses were based on the most recent World Health Organisation classification (Travis *et al*, 2004). Among all ADC cases, the predominant histological patterns were classified based on the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) (Travis *et al*, 2011). In addition, in order to determine whether *RET*-rearranged lung cancer has any of the specific histological features described in prior reports (Wang *et al*, 2012), we performed a detailed histological analysis as follows: (1) the prevalent cytological feature (i.e., Clara/type II pneumocyte, columnar cell, or polygonal cell); (2) the presence of intracytoplasmic mucin production and mucinous cribriform pattern; and (3) the presence of intranuclear inclusion among 22 cases of *RET*-rearranged lung cancer.

Immunohistochemistry. For RET immunohistochemical staining, heat-induced epitope retrieval with Target Retrieval Solution (Dako Corporation, Carpinteria, CA, USA) was performed. The slides were subsequently incubated with primary antibodies against RET (EPR2871; 1 : 250 dilution; Epitomics, Burlingame, CA, USA). Immunoreactions were detected using EnVision-FLEX and LINKER (Dako). Immunopositive cases were defined as those showing cytoplasmic and/or membranous staining in $\geqslant 10\%$ of cells. We then divided the immunopositive cases into membrane staining-only and cytoplasmic-staining cases (irrespective of any membranous staining).

For the *RET*-rearranged NSCLCs, we performed immunohistochemical analysis to exclude metastatic thyroid carcinoma (see Supplementary Table 1).

FISH analysis for RET rearrangements. First, we performed using a dual-colour break-apart probe for the *RET* gene (Supplementary Table 2; Chromosome Science Labo, Inc., Sapporo, Japan). Among *RET* gene break-apart probe-positive cases, we next performed using break-apart probes for both *KIF5B* and *CCDC6*.

A total of 50 non-overlapping tumour cells with hybridisation signals examined for each case were captured using the Metafer Slide Scanning Platform (MetaSystems, Altlussheim, Germany). The signal in each cell was categorised into one of the following seven patterns: (1) fused 3′/5′ only; (2) fused 3′/5′ and both isolated 3′ and 5′ (split); (3) both isolated 3′ and 5′ (split) only; (4) fused 3′/5′ and isolated 5′; (5) fused 3′/5′ and isolated 3′; (6) isolated 5′ only; and (7) isolated 3′ only. A split signal was defined by 5′ and 3′ probes observed at a distance of greater than one-fold the signal size. A FISH-positive case was defined as $\geqslant 20\%$ of tumour cells having any split signals or any isolated 3′ (red) signals. The threshold for the *RET* gene was determined in 27 cases, yielding both FISH and previously reported RNA sequence data (Kohno *et al*, 2012) (Supplementary Figure 1).

RT–PCR analysis. Total RNA (500 ng) was reverse-transcribed onto cDNA using Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). Complementary DNA (corresponding to 10 ng total RNA) was subjected to multiplex PCR amplification using KAPA Taq DNA Polymerase (KAPA Biosystems, Woburn, MA, USA) and four primers (detection set in Supplementary Table 3). This PCR enabled the detection of all *KIF5B/CCDC6-RET* fusion variants identified to date. The reactions were conducted in a thermal cycler under the following conditions: 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 2 min, with a final extension cycle for 10 min at 72 °C. The housekeeping gene encoding glyceraldehyde-3-phosphate dehydrogenase was amplified to estimate the efficiency of cDNA synthesis. The PCR products were subjected to agarose gel electrophoresis. When visible bands were detected, the cDNA samples were further subjected to validation PCR (validation set in Supplementary Table 3). When visible bands were detected, the PCR products were subjected to Sanger sequencing in both directions by using the BigDye Terminator kit (Invitrogen) and an ABI 3130xl DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The PCR primers used in the present study are shown in Supplementary Table 3.

We defined the cases with *RET* rearrangement that were RT–PCR-positive or *RET* break-apart probe-positive as well as cases that were *KIF5B* break-apart probe-positive or *CCDC6* break-apart probe-positive in the absence of RT–PCR data.

Analysis of EGFR mutational status and ALK rearrangement. We detected two common *EGFR* mutations (deletions in exon 19 (DEL) and a point mutation at codon 858 in exon 21 (L858R)) by using high-resolution melting analysis (Fukui *et al*, 2008). The *ALK* rearrangement was analysed by immunohistochemistry, RT–PCR, and/or FISH assay (Yoshida *et al*, 2011).

Statistical analysis. Statistical analysis was performed using SPSS Statistics 21 software (IBM Corporation, Somers, NY, USA). Student's *t*-test was used to analyse continuous variables and χ^2 tests were used to analyse categorical variables. Overall survival (OS) curves were calculated using the Kaplan–Meier method. Curves were compared using the log-rank test. Univariate survival analysis was performed using a log-rank test. Statistical significance was set at $P < 0.05$.

RESULTS

Clinicopathological background. Among the 1927 cases investigated, 53 cases were excluded because they lacked RT–PCR data and the FISH analysis failed. Therefore, the final cohort included 1874 cases: 1620 ADCs (56 *in situ*, 41 microinvasive, 366 lepidic-predominant, 179 acinar-predominant, 577 papillary-predominant, 101 micropapillary-predominant, 236 solid-predominant, and 64 invasive mucinous), 203 SCCs, 8 LCCs, and 43 SACs. Among the 1620 ADC cases, 830 cases consisted of consecutively resected cases from 1998 to 2002 (Supplementary Figure 2).

Background clinicopathological data are displayed in Table 1. Because lymph node status was recorded in 1860 cases (99.3%), pathological staging was performed in 1860 cases. The mean follow-up time for all 1874 patients was 62.3 months (range, 0.1–163 months), with 1292 patients still alive at follow-up.

***RET* FISH and RT–PCR.** Among the 1874 cases investigated, 1823 cases yielded break-apart FISH data, 477 cases yielded RT–PCR data, and 456 cases yielded both FISH and RT–PCR data.

Table 1. Clinicopathologic factors of *RET*-rearranged lung carcinomas

	Total	*RET* rearrangement		
	1874	Negative (%)	Positive (%)	*P*-value
Age (year)				
Median	63.1	63.2	57.5	0.038
Range	23–89	23–89	28–78	
Gender				
Female	809	798 (43.1)	11 (50)	0.524
Male	1065	1054 (56.9)	11 (50)	
Smoking				
Never	867	852 (46.1)	15 (68.2)	0.051
Former/current	1007	1000 (53.9)	7 (31.8)	
Tumour size (cm)				
Median	3.0	3.0	2.8	0.598
Range	0.4–17.5	0.4–17.5	1.4–8.0	
N status				
Negative	1377	1362 (74.1)	15 (68.2)	0.624
Positive	483	475 (25.9)	7 (31.8)	
p-Stage				
I+II	1496	1480 (80.5)	16 (72.7)	0.189
III+IV	364	358 (19.5)	6 (27.3)	
Histology				
ADC	1620	1598 (86.3)	22	0.322
SQC	203	203 (11.0)	0	
LCC	8	8 (0.4)	0	
SAC	43	43 (2.3)	0	
***RET* immunostaining**				
Negative		1527 (86.1)	7 (33.3)	<0.001
Positive		247 (13.9)	14 (66.7)	

Abbreviations: ADC = adenocarcinoma; LCC = large cell carcinoma; *RET* = *rearranged during transfection*; SAC = sarcomatoid carcinoma; SQC = squamous cell carcinoma.

Fifty (2.7%) cases were *RET* break-apart probe positive cases (Figure 1). The RT–PCR analysis of 29 of the 50 FISH-positive cases for which RNAs were available verified that 14 (44.8%) cases possessed the *KIF5B–RET* fusion and 2 possessed the *CCDC6–RET* fusion. The most prevalent variant of *KIF5B–RET* was variant K15;R12 (10/14; 71.4%), whereas the other variants were observed in 1 case each (7.1% each; Supplementary Table 4). On the other hand, RT–PCR results were negative for all 406 FISH-negative cases. Based on these RT–PCR data, the split signal sensitivity and specificity was 100% and 44.8%, respectively. The average FISH split signal for RT–PCR-positive and -negative cases was 40.9% (range, 22–72) and 7.4% (range, 0–40), respectively ($P<0.001$). Among the 50 *RET* break-apart probe-positive cases, 13 out of the 40 analysed cases were confirmed to be *KIF5B* break-apart probe-positive cases and 3 out of the 46 analysed cases were confirmed to be *CCDC6* break-apart probe-positive cases. In conjunction with RT–PCR data, 19 cases with *KIF5B–RET* rearrangement and 3 cases with *CCDC6–RET* rearrangement were detected (Supplementary Table 4).

Clinical characteristics of patients with *RET*-rearranged NSCLCs. Based on the aforementioned FISH and RT–PCR data, 22 of the 1874 cases (1.2%) were considered to be *RET* rearrangements. The clinical characteristics of patients with *RET*-rearranged NSCLCs are displayed in Table 1 and Supplementary Table 4. Among 22 cases with *RET* rearrangements, 6 cases were reported previously (case nos. 2, 4, 7, 12, 14, and 15) (Kohno *et al*, 2012). All *RET*-rearranged cases were ADCs. When analysing consecutively resected ADC cases alone, *RET* rearrangements were observed in 7 of 830 ADC cases (0.8%). Of the 22 *RET*-rearranged cases, 19 (86%) possessed *KIF5B–RET* and 3 (14%) had *CCDC6–RET* fusions.

The *RET*-rearranged tumours were significantly more common in younger patients ($P=0.026$) and tended to occur in patients with no history of smoking ($P=0.051$). The *RET* rearrangements were not associated with gender, smoking status, tumour size, tumour stage, or lymph node status. Clinical records revealed that there were no patient histories of occupational exposure to radioactivity (Supplementary Table 5).

Among the 1874 cases examined, *EGFR* mutation was observed in 663 of the 1585 analysed cases (42.7%), and *ALK* rearrangement was observed in 55 of the 1860 analysed cases (3.0%; Table 1). All cases were detected exclusively with additional driver genetic changes (i.e., *RET*, *EGFR*, and *ALK*).

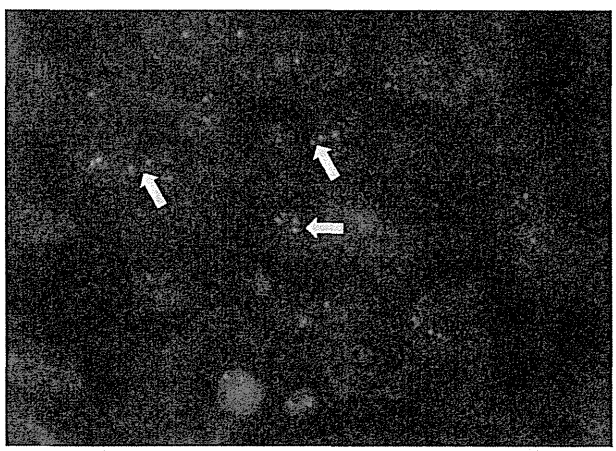


Figure 1. Representative image of fluorescence *in situ* hybridisation using a break-apart probe for *RET*-rearranged carcinoma. White arrows indicate split red–green signals. A full colour version of this figure is available at the *British Journal of Cancer* journal online.

Histological and immunohistochemical characteristics of patients with *RET*-rearranged NSCLCs. Histological findings are summarised in Table 2. The predominant growth pattern was lepidic in 6 cases, papillary in 9 cases, acinar in 2 cases, micropapillary in 1 case, and solid in 4 cases (Figures 2A and B). Focal lepidic, papillary, acinar, micropapillary, and solid patterns were observed in 16, 20, 16, 10, and 7 cases, respectively.

The predominant cell type was Clara/type II in 13 cases, columnar in 4 cases, and polygonal in 5 cases. Cells with cytoplasmic mucin production were at least focally present in 12 of the 22 (54.5%) *RET*-rearranged ADC cases. The presence of signet-ring (Figure 2C) or mucinous cribriform patterns were only observed in 27.3% and 13.6% of cases, respectively. The nuclear inclusion was at least focally present in 9 of the 22 (40.9%) *RET*-rearranged ADC cases (Figure 2D).

RET protein expression by immunohistochemistry. The RET expression was observed in 261 of the 1795 (14.5%) NSCLCs evaluated (Figure 3). The RET-immunopositive cases were significantly associated with histological subtype ($P<0.001$); 255 of the 1543 ADCs (16.5%), 1 of the 201 SCCs (0.5%), none of the 8 LCCs, and 5 of the 43 SACs (11.6%). The RET-immunopositive tumours were more commonly associated with younger patients ($P=0.006$), patients with no history of smoking ($P=0.007$), lymph node metastasis ($P<0.001$), and higher pathological stages ($P=0.001$) compared with RET-immunonegative tumours.

Among *RET*-rearranged cases, RET immunopositivity was observed in 14 of the 21 cases (66.7%) analysed with immunohistochemistry. Although RET immunoreactivity was significantly associated with *RET* rearrangement ($P<0.001$), its test performance was poor with 66.7% sensitivity and 86.1% specificity (Table 1).

Upon classification of the staining pattern of 261 RET-immunopositive cases, we observed that 158 cases (60.5%) displayed cytoplasmic staining, irrespective of membrane staining, and 103 cases (39.5%) displayed only membrane staining. All RET-immunopositive cases with *RET*-rearrangement displayed cytoplasmic staining. Among the 151 cytoplasmic staining cases, the detection rate for *RET*-rearranged cases increased from 5.4 to 9.9%.

Survival analysis. We investigated the existence of an association between patient OS and *RET* gene rearrangement. Follow-up data were available for all 1874 patients for a median of 62.3 months (range, 0.1 – 162.8 months). The *RET* gene rearrangement was not associated with OS in any of the cases analysed, among all cases ($P=0.456$; Figure 4A), among ADC-only cases, ($P=0.611$, Figure 4B), or among consecutively resected ADC cases ($P=251$, Figure 4C).

DISCUSSION

We observed that ~1.2% of NSCLC cases harboured *RET* rearrangements, all of which were ADCs, whereas neither SCCs,

Table 2. Pathological, cytological, and immunohistological features for *RET*-rearranged cases

		Histologic pattern						Cytological features					Immunohistochemical results					
No	Fusion partner	Predominant	LEP	PAP	ACI	MPC	SOL	Cell type	Mucin production	Nuclear inclusion	SRC	M-Crib	RET	Staining pattern	*TTF-1*	Napsin A	*PAX8*	Thyroglobulin
1	KIF5B	MPC	2	3	1	4	0	Type II	+	–	–	–	Pos	C	Pos	Neg	Neg	Neg
2	KIF5B	LEP	8	2	0	0	0	Type II	–	+	–	–	Pos	C	Pos	Pos	Neg	Neg
3	KIF5B	ACI	0	1	6	0	3	Polygonal	+	–	–	–	Pos	C	Pos	Pos	Neg	Neg
4	KIF5B	LEP	5	3	1	1	0	Type II	–	+	–	–	Pos	C	Pos	Pos	Neg	Neg
5	KIF5B	PAP	3	6	0	1	0	Type II	–	+	–	–	Pos	C	Pos	Pos	Neg	Neg
6	KIF5B	PAP	0	4	2	4	0	Columnar	+	–	–	–	Pos	C	Pos	Pos	Neg	Neg
7	KIF5B	ACI	3	2	5	0	0	Type II	+	–	+	–	Pos	C	Pos	Pos	Neg	Neg
8	KIF5B	PAP	1	4	3	0	2	Columnar	–	–	–	–	Neg	–	Pos	Pos	Neg	Neg
9	KIF5B	PAP	1	4	2	3	0	Columnar	+	–	+	+	Neg	–	Pos	Pos	Neg	Neg
10	KIF5B	LEP	8	2	0	0	0	Type II	–	+	–	–	Neg	–	Pos	Pos	Neg	Neg
11	KIF5B	PAP	2	6	1	1	0	Type II	–	+	–	–	Pos	C	Pos	Pos	Neg	Neg
12	KIF5B	PAP	3	6	0	0	0	Type II	–	+	–	–	Pos	C	Pos	Pos	Neg	Neg
13	KIF5B	PAP	4	5	0	0	0	Type II	–	–	–	–	Neg	–	Pos	Pos	Neg	Neg
14	KIF5B	LEP	5	4	0	1	0	Type II	+	+	–	–	Pos	C	Pos	Pos	Neg	Neg
15	KIF5B	PAP	0	4	3	3	0	Type II	+	–	+	+	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
16	KIF5B	SOL	0	2	3	0	5	Polygonal	+	–	–	–	Pos	C	Pos	Pos	Neg	Neg
17	KIF5B	SOL	1	2	1	1	5	Polygonal	+	–	–	–	Pos	C	Pos	Pos	Neg	Neg
18	KIF5B	PAP	2	3	2	2	1	Polygonal	+	–	+	–	Pos	C	Pos	Neg	Neg	Neg
19	KIF5B	LEP	8	0	2	0	0	Type II	–	+	–	–	Neg	–	Pos	Pos	Neg	Neg
20	CCDC6	LEP	5	4	1	0	0	Type II	–	+	–	–	Neg		Pos	Pos	Neg	Neg
21	CCDC6	SOL	0	0	1	0	9	Polygonal	+	–	+	–	Pos	C	Pos	Pos	Neg	Neg
22	CCDC6	SOL	0	1	4	0	5	Columnar	+	–	+	+	Pos	C	Pos	Pos	Neg	Neg

Abbreviations: ACI = acinar; C = cytoplasmic; CCDC6 = coiled-coil domain containing 6; KIF5B = kinesin family member 5B; LEP = lepidic; M = membranous; M-Crib = mucinous cribriform, MPC = micropapillary; #N/A = not assessed; Neg = negative; PAP = papillary; *PAX8* = paired box gene 8; Pos = positive; RET = rearranged during transfection; SOL = solid; SRC = signet-ring cell; *TTF-1* = thyroid transcription factor-1. Case numbers 2, 4, 7, 12, 14, and 15 were reported previously and have been highlighted in bold.

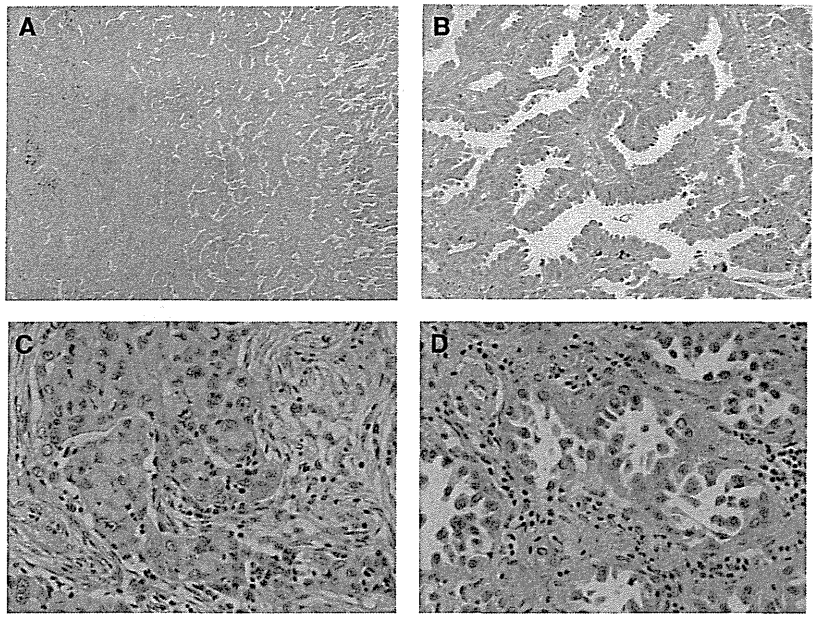


Figure 2. Representative images of *RET*-rearranged adenocarcinoma of the lung. (**A** and **B**) Many *RET*-rearranged adenocarcinomas displayed a papillary growth pattern (**A**: low magnification, and **B**: high magnification). (**C**) Solid signet-ring cell pattern was observed in a minority of *RET*-rearranged adenocarcinoma (original magnification × 200). (**D**) Some tumour cells displayed homogeneously eosinophilic-to-pale inclusions in the nuclei (original magnification × 200).

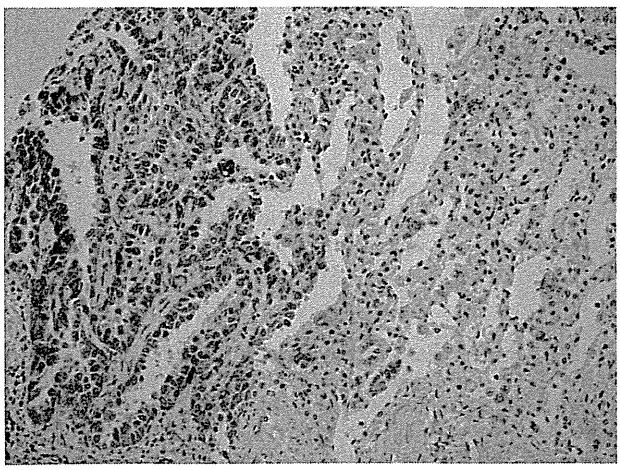


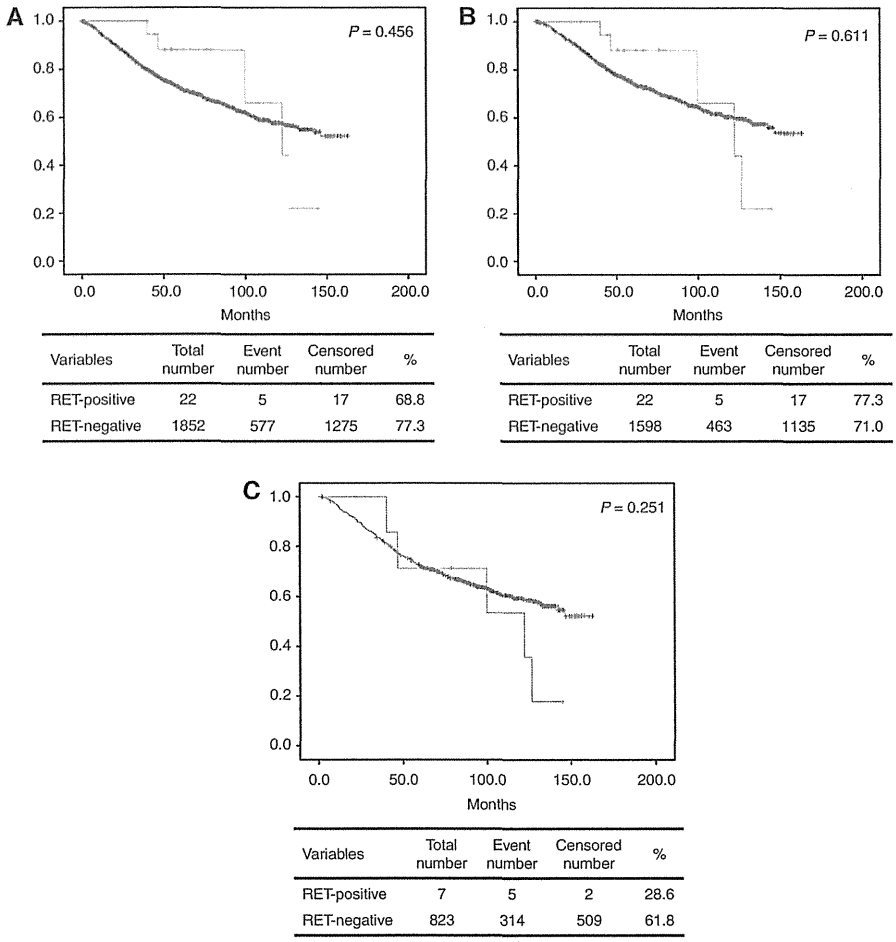
Figure 3. Representative images of RET-immunostaining positivity in a *RET*-rearranged adenocarcinoma. Diffuse, fine granular cytoplasmic staining was observed in the adenocarcinoma component, as shown in the left part of the figure, whereas negative signals were observed in nontumourous areas, as shown in the right part of the figure (original magnification × 400).

SACs, nor LCCs harboured this rearrangement. The prevalence of *RET* rearrangements (1.2%) in our cohort was in line with the range of results reported previously (0.6–10%) (Ju *et al*, 2012; Kohno *et al*, 2012; Lipson *et al*, 2012; Suehara *et al*, 2012; Takeuchi *et al*, 2012; Wang *et al*, 2012). The prevalence rate is considered to be affected by the specific case selection, such as that collected by known gene alterations in the negative cohort. In the subgroup of consecutively resected ADC cases, the frequency of *RET* rearrangements was only 0.9%.

Patients with *RET* rearrangements displayed nearly equivalent gender distributions, were relatively younger in age, and had no history of smoking compared with patients without *RET* rearrangements. The young age of onset and non-smoking history in *RET*-rearranged NSCLCs is reminiscent of the patient characteristics of *ALK*-rearranged NSCLCs (Shaw *et al*, 2009). However, other investigators have observed no statistical differences in age, gender, smoking history, or tumour stage between *RET*-rearranged ADC and wild-type *RET* ADCs (Wang *et al*, 2012). As is well known in PTC, *RET* gene fusions are associated with radiation exposure (Nikiforov and Nikiforova, 2011). However, no exposure to radioactivity was detected in either the current or previously reported *RET*-rearranged NSCLCs (Suehara *et al*, 2012).

Consistent with previous studies, *RET* rearrangements were more common in ADCs. Wang *et al* (2012) also reported *RET* rearrangements in two cases of adenosquamous carcinomas. There have been no reported *RET* rearrangements in SQC, LCC, or SAC tumours. Based on IASLC/ATS/ERS classification, we and other investigators have reported on the association between papillary growth pattern and *RET* rearrangement (Suehara *et al*, 2012; Yokota *et al*, 2012). Recently, Wang *et al* (2012) reported that a solid pattern was most prevalent in *RET*-rearranged ADCs, with signet-ring cells also frequently observed (36.4%). In this study, although cytoplasmic mucin was present, at least focally, in the majority (59%) of cases, signet-ring cells were observed in only 27% of cases. Of note, the mucinous cribriform pattern – another characteristic morphology associated with *ALK*- and *ROS1*-rearranged lung cancers (Yoshida *et al*, 2011, 2013) – was also infrequently observed (13.6%) in the present cohort.

Determining the gold standard for FISH specificity is challenging, because there may be an unknown fusion partner in FISH-positive cases that could be detectable with RT–PCR or FISH for fusion probes. Therefore, in order to yield a more precise cutoff value for break-apart FISH probes, we used previously reported RNA sequence data as the gold standard. In the present study, FISH analysis with a break-apart probe of the *RET* gene is highly sensitive (100% sensitivity), but unlikely to be sufficient to define *RET*-positive cases because of the potential for false positivity



Variables	Total number	Event number	Censored number	%
RET-positive	22	5	17	68.8
RET-negative	1852	577	1275	77.3

Variables	Total number	Event number	Censored number	%
RET-positive	22	5	17	77.3
RET-negative	1598	463	1135	71.0

Variables	Total number	Event number	Censored number	%
RET-positive	7	5	2	28.6
RET-negative	823	314	509	61.8

Figure 4. Overall survival analysis in *RET*-rearranged lung carcinoma. (**A**) The overall survival curves for patients with *RET*-rearranged (green line) and *RET*-wild-type (blue line) non-small-cell lung carcinomas ($P=0.9613$). (**B**) The overall survival curves for patients with *RET*-rearranged (green line) and *RET*-wild-type (blue line) adenocarcinoma ($P=0.9665$). (**C**) The overall survival curves for patients with *RET*-rearranged (green line) and *RET*-wild-type (blue line) consecutively resected adenocarcinoma ($P=0.547$). A full colour version of this figure is available at the *British Journal of Cancer* journal online.

(58%) based on RT–PCR or break-apart FISH probes for known partners. Compared with the false-positive rate in our study, a slightly lower rate (41%) for *RET* break-apart FISH results has been reported with an alternate probe design (Takeuchi *et al*, 2012). The advantages of using a break-part FISH probe for gene translocation are that it can detect the translocations irrespective of fusion partners. Therefore, the current false-positive rate may be overestimated because it may include an unknown fusion partner for the *RET* gene. However, the possible effect of unknown fusion partners was not likely because the recently reported frequency of novel fusion partners of *RET*-rearranged lung carcinoma (i.e., *NCOA4* and *TRIM33*) was extremely low (Wang *et al*, 2012; Drilon *et al*, 2013). Similar to *EML4–ALK* translocation, there exists intrachromosomal proximity (10.6 Mbp) of *KIF5B* and *RET* genes that complicates the generation of a proper break-apart probe that is easily resolvable by contemporary *in situ* technology.

Although RET immunoreactivity was significantly associated with *RET* rearrangement, its test performance was poor with only 66.7% sensitivity and 86.1% specificity. Other investigators have also reported a slightly lower positivity rate (54%) of RET antibody for *RET*-rearranged NSCLCs (Wang *et al*, 2012). Therefore, we conclude that RET immunohistochemistry possesses limited value in detecting *RET*-rearranged NSCLCs, unlike, for example, immunohistochemistry for human epidermal growth factor 2 status for breast carcinoma (Jacobs *et al*, 1999). Interestingly, cytoplasmic staining was more specific to gene rearrangement than membranous staining, likely because the KIF5B–RET chimeric proteins (except fusion partner of K24;R8) lack a transmembrane domain.

The present survival analysis indicated that *RET* rearrangement was not associated with OS. Even when the analysis was limited to ADCs or cases of consecutively resected ADC, no association was observed, consistent with previous reports (Wang *et al*, 2012; Yokota *et al*, 2012). However, the number of cases that have been investigated has been too small (spanning stages I–III) to draw any definitive conclusions regarding survival of *RET*-rearranged NSCLCs.

In summary, *RET* rearrangements were observed in 1.2% of NSCLC cases. All *RET* rearrangements in NSCLC were observed in ADCs. The *RET* rearrangements were observed in younger patients, patients with no smoking history, and papillary-predominant tumours. Although, cytoplasmic mucin production was at least focally present in 54.5% of *RET*-rearranged ADCs, distinct histological features were not detected. Furthermore, immunohistochemistry for RET protein has limited value to detect *RET*-rearranged NSCLC. Finally, these fusion genes did not coexist with *EGFR* and *ALK* alterations.

ACKNOWLEDGEMENTS

We thank Yuko Adegawa, Shouichi Harada, and Susumu Wakai for their skillful technical assistance. The National Cancer Center Biobank is supported by the National Cancer Center Development Fund, Japan. This work was supported in part by the National Cancer Center Research and Development Fund (23-A-2), (23-A-11), (23-A-35), and (24-A-1), and Grant-in-Aid for Scientific Research (C) Grant Number 25460446.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Koji Tsuta and Takashi Kohno; financial support: Koji Tsuta and Takashi Kohno; administrative support: Koji Tsuta, Hisao Asamura, Takashi Kohno, and Ryoji Kushima; provision of study materials or patients: Koh Furuta and Hisao Asamura; data analysis and interpretation: Koji Tsuta, Yoko Shimada, Takashi Kohn, Koh Furuta, and Ryoji Kushima; manuscript writing: all authors; final approval of manuscript: all authors.

REFERENCES

Drilon A, Wang L, Hasanovic A, Suehara Y, Lipson D, Stephens PJ, Ross J, Miller VA, Ginsberg MS, Zakowski MF, Kris MG, Ladanyi M, Rizvi NA (2013) Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. *Cancer Discov* **3**: 630–635.

Fukui T, Ohe Y, Tsuta K, Furuta K, Sakamoto H, Takano T, Nokihara H, Yamamoto N, Sekine I, Kunitoh H, Asamura H, Tsuchida T, Kaneko M, Kusumoto M, Yamamoto S, Yoshida T, Tamura T (2008) Prospective study of the accuracy of EGFR mutational analysis by high-resolution melting analysis in small samples obtained from patients with non-small cell lung cancer. *Clin Cancer Res* **14**(15): 4751–4757.

Gautschi O, Zander T, Keller FA, Strobel K, Hirschmann A, Aebi S, Diebold J (2013) A patient with lung adenocarcinoma and RET fusion treated with vandetanib. *J Thorac Oncol* **8**(5): e43–e44.

Goldstraw P (2009) *International Association for the Study of Lung Cancer Staging Manual in Thoracic Oncology.* Editorial Rx Press: Florida.

Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della Porta G, Fusco A, Vecchio G (1990) PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. *Cell* **60**(4): 557–563.

Jacobs TW, Gown AM, Yaziji H, Barnes MJ, Schnitt SJ (1999) Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system. *J Clin Oncol* **17**(7): 1983–1987.

Ju YS, Lee WC, Shin JY, Lee S, Bleazard T, Won JK, Kim YT, Kim JI, Kang JH, Seo JS (2012) A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. *Genome Res* **22**(3): 436–445.

Knowles PP, Murray-Rust J, Kjaer S, Scott RP, Hanrahan S, Santoro M, Ibanez CF, McDonald NQ (2006) Structure and chemical inhibition of the RET tyrosine kinase domain. *J Biol Chem* **281**(44): 33577–33587.

Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, Sakamoto H, Tsuta K, Furuta K, Shimada Y, Iwakawa R, Ogiwara H, Oike T, Enari M, Schetter AJ, Okayama H, Haugen A, Skaug V, Chiku S, Yamanaka I, Arai Y, Watanabe S, Sekine I, Ogawa S, Harris CC, Tsuda H, Yoshida T, Yokota J, Shibata T (2012) KIF5B-RET fusions in lung adenocarcinoma. *Nat Med* **18**(3): 375–377.

Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, Choi HG, Kim J, Chiang D, Thomas R, Lee J, Richards WG, Sugarbaker DJ, Ducko C, Lindeman N, Marcoux JP, Engelman JA, Gray NS, Lee C, Meyerson M, Janne PA (2008) EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res* **14**(13): 4275–4283.

Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Janne PA, Costa DB, Varella-Garcia M, Kim WH, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW, Iafrate AJ (2010) Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* **363**(18): 1693–1703.

Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, Curran JA, Balasubramanian S, Bloom T, Brennan KW, Donahue A, Downing SR, Frampton GM, Garcia L, Juhn F, Mitchell KC, White E, White J, Zwirko Z, Peretz T, Nechushtan H, Soussan-Gutman L, Kim J, Sasaki H, Kim HR, Park SI, Ercan D, Sheehan CE, Ross JS, Cronin MT, Janne PA, Stephens PJ (2012) Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. *Nat Med* **18**(3): 382–384.

Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* **350**(21): 2129–2139.

Nikiforov YE, Nikiforova MN (2011) Molecular genetics and diagnosis of thyroid cancer. *Nat Rev Endocrinol* **7**(10): 569–580.

Pachnis V, Mankoo B, Costantini F (1993) Expression of the c-ret proto-oncogene during mouse embryogenesis. *Development* **119**(4): 1005–1017.

Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**(5676): 1497–1500.

Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kris M, Varmus H (2004) EGF 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 USA* **101**(36): 13306–13311.

Santoro M, Dathan NA, Berlingieri MT, Bongarzone I, Paulin C, Grieco M, Pierotti MA, Vecchio G, Fusco A (1994) Molecular characterization of RET/PTC3; a novel rearranged version of the RETproto-oncogene in a human thyroid papillary carcinoma. *Oncogene* **9**(2): 509–516.

Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, Solomon B, Stubbs H, Admane S, McDermott U, Settleman J, Kobayashi S, Mark EJ, Rodig SJ, Chirieac LR, Kwak EL, Lynch TJ, Iafrate AJ (2009) Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* **27**(26): 4247–4253.

Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, Bando M, Ohno S, Ishikawa Y, Aburatani H, Niki T, Sohara Y, Sugiyama Y, Mano H (2007) Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* **448**(7153): 561–566.

Suehara Y, Arcila M, Wang L, Hasanovic A, Ang D, Ito T, Kimura Y, Drilon A, Guha U, Rusch V, Kris MG, Zakowski MF, Rizvi N, Khanin R, Ladanyi M (2012) Identification of KIF5B-RET and GOPC-ROS1 fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions. *Clin Cancer Res* **18**: 6599–6608.

Takeuchi K, Soda M, Togashi Y, Suzuki R, Sakata S, Hatano S, Asaka R, Hamanaka W, Ninomiya H, Uehara H, Lim Choi Y, Satoh Y, Okumura S, Nakagawa K, Mano H, Ishikawa Y (2012) RET, ROS1 and ALK fusions in lung cancer. *Nat Med* **18**(3): 378–381.

Travis WD, Brambilla E, Müller-Hermelink HK, Harris CC (2004) Tumors of the lung. In: Kleihues P, Sobin LH (eds). *WHO Classification of Tumors. Pathology and Genetics of Tumors of the Lung, Pleura, Thymus and Heart.* IARC Press: Lyon, France, pp 9–124.

Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, Garg K, Austin JH, Asamura H, Rusch VW, Hirsch FR, Scagliotti G, Mitsudomi T, Huber RM, Ishikawa Y, Jett J, Sanchez-Cespedes M, Sculier JP, Takahashi T, Tsuboi M, Vansteenkiste J, Wistuba I, Yang PC, Aberle D, Brambilla C, Flieder D, Franklin W, Gazdar A, Gould M, Hasleton P, Henderson D, Johnson B, Johnson D, Kerr K, Kuriyama K, Lee JS, Miller VA, Petersen I, Roggli V, Rosell R, Saijo N, Thunnissen E, Tsao M, Yankelewitz D (2011) International association for the study of lung cancer/American Thoracic Society/European Respiratory Society International multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol* **6**(2): 244–285.