Disclosure of Potential Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

We thank Suenori Chiku and Hirohiko Totsuka for the analysis of sequencing data and Dai Suzuki, Kazuko Nagase, Sachiyo Mitani, Sumiko Ohnami, Yoko Odaka, and Misuzu Okuyama for technical assistance.

Grant Support

This work was supported in part by the Advanced Research for Medical Products Mining Program of the National Institute of Biomedical Innovation (NIBIO) and Grants-in-Aid from the Ministry of Health, Labor, and Welfare for the Third-term Comprehensive 10 year Strategy for Cancer Control.

Note: Supplementary information is available on the Clinical Cancer Research website.

References

1. Pao W, Hutchinson KE. Chipping away at the lung cancer genome. Nat Med. 2012;18:349-51.

2. Shaw AT, Engelman JA. ALK in lung cancer: past, present, and future. J Clin Oncol. 2013;31:1105-11.

3. Gautschi O, Zander T, Keller FA, Strobel K, Hirschmann A, Aebi S, et al. A patient with lung adenocarcinoma and RET fusion treated with vandetanib. J Thorac Oncol. 2013;8:e43-4.

4. Drilon A, Wang L, Hasanovic A, Suehara Y, Lipson D, Stephens P, et al. Response to Cabozantinib in Patients with RET Fusion-Positive Lung Adenocarcinomas. Cancer Discov. 2013;3:630-5.

5. Takeuchi K, Soda M, Togashi Y, Suzuki R, Sakata S, Hatano S, et al. RET, ROS1 and ALK fusions in lung cancer. Nat Med. 2012;18:378-81.

6. Mano H. ALKoma: a cancer subtype with a shared target. Cancer Discov. 2012;2:495-502.

7. Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med. 2012;18:382-4.

8. Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, et al. KIF5B-RET fusions in lung adenocarcinoma. Nat Med. 2012;18:375-7.

9. Ju YS, Lee WC, Shin JY, Lee S, Bleazard T, Won JK, 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.

10. Oxnard GR, Binder A, Janne PA. New targetable oncogenes in non-small-cell lung cancer. J Clin Oncol. 2013;31:1097-104.

11. Kohno T, Tsuta K, Tsuchihara K, Nakaoku T, Yoh K, Goto K. RET fusion gene: Translation to personalized lung cancer therapy. Cancer Sci. 2013.

12. Travis WD, Brambilla E, Riely GJ. New pathologic classification of lung cancer: relevance for clinical practice and clinical trials. J Clin Oncol. 2013;31:992-1001.

13. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol. 2011;6:244-85.

14. Tsuta K, Kawago M, Inoue E, Yoshida A, Takahashi F, Sakurai H, et al. The utility of the proposed IASLC/ATS/ERS lung adenocarcinoma subtypes for disease prognosis and correlation of driver gene alterations. Lung Cancer. 2013;81:371-6.

15. Warth A, Muley T, Meister M, Stenzinger A, Thomas M, Schirmacher P, et al. The novel histologic International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification system of lung adenocarcinoma is a stage-independent predictor of survival. J Clin Oncol. 2012;30:1438-46.

16. Yoshizawa A, Motoi N, Riely GJ, Sima CS, Gerald WL, Kris MG, et al. Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. Mod Pathol. 2011;24:653-64.

17. Travis WD, Brambilla, E., Muller-Hermelink, H.K. and Harris, C.C. , editor. *World Health Organization Classification of Tumors;* Pathology and Genetics, Tumours of Lung, Pleura, Thymus and Heart. Lyon: IARC Press; 2004.

18. Kim D, Salzberg SL. TopHat-Fusion: an algorithm for discovery of novel fusion transcripts. Genome Biol. 2011;12:R72.

19. Falls DL. Neuregulins: functions, forms, and signaling strategies. Exp Cell Res. 2003;284:14-30.

20. Fleck D, van Bebber F, Colombo A, Galante C, Schwenk BM, Rabe L, et al. Dual cleavage of neuregulin 1 type III by BACE1 and ADAM17 liberates its EGF-like domain and allows paracrine signaling. J Neurosci. 2013;33:7856-69.

21. Dislich B, Lichtenthaler SF. The Membrane-Bound Aspartyl Protease BACE1: Molecular and Functional Properties in Alzheimer's Disease and Beyond. Front Physiol. 2012;3:8.

22. Majem M, Pallares C. An update on molecularly targeted therapies in second- and third-line treatment in non-small cell lung cancer: focus on EGFR inhibitors and anti-angiogenic agents. Clin Transl Oncol. 2013;15:343-57.

23. Perez EA, Spano JP. Current and emerging targeted therapies for metastatic breast cancer. Cancer. 2012;118:3014-25.

24. Nelson V, Ziehr J, Agulnik M, Johnson M. Afatinib: emerging next-generation tyrosine kinase inhibitor for NSCLC. Onco Targets Ther. 2013;6:135-43.

25. Palanisamy N, Ateeq B, Kalyana-Sundaram S, Pflueger D, Ramnarayanan K, Shankar S, et al. Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. Nat Med. 2010;16:793-8.

26. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther. 2008;7:3129-40.

27. Kettle R, Simmons J, Schindler F, Jones P, Dicker T, Dubois G, et al. Regulation of neuregulin 1beta1-induced MUC5AC and MUC5B expression in human airway epithelium.

Am J Respir Cell Mol Biol. 2010;42:472-81.

Figure 1. Oncogenic fusions in invasive mucinous lung adenocarcinomas (IMAs). **A,** Schematic representations of the wild-type proteins (top rows of each section) followed by the fusion proteins identified in this study. The breakpoints for each variant are indicated by blue arrows. TM: transmembrane domain. Locations of putative cleavage sites in the NRG1 polypeptide are indicated by dashed green lines. **B,** Detection of gene-fusion transcripts by RT-PCR. RT-PCR products for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) are shown below. Six IMAs (T) positive for gene fusions are shown alongside their corresponding non-cancerous lung tissues (N); labels below the gel image indicate sample IDs (see **Table 1**). **C,** Pie chart showing the fraction of IMAs that harbor the indicated driver mutations.

Figure 2. Oncogenic properties of gene-fusion products. **A,** ERBB3 activation by *CD74-NRG1* fusion, demonstrated using the EFM-19 cell system. ERBB3, ERBB2, AKT, and ERK phosphorylation were examined in EFM-19 (reporter) cells treated for 30 min with conditioned media from H1299 cells exogenously expressing *CD74-NRG1* cDNA. Phosphorylation was suppressed by HER-TKIs. **B,** ERBB4 activation by *EZR-ERBB4* fusion. Stably transduced NIH3T3 cells were serum-starved for 24 h and treated for 2 h with DMSO (vehicle control) or TKIs. Phosphorylation of ERBB4 and ERK was suppressed by ERBB4-TKIs. EZR-ERBB4 protein was detected using an antibody recognizing ERBB4 polypeptides retained in the fusion protein. **C,** BRAF activation by *TRIM24-BRAF* fusion. Stably transduced NIH3T3 cells were serum-starved for 24 h and treated for 2 h with DMSO or kinase inhibitors. ERK phosphorylation

(activation) was suppressed by sorafenib, a kinase inhibitor targeting BRAF, as well as by U0126, a MEK inhibitor. TRIM24-BRAF protein was detected using an antibody recognizing BRAF polypeptides retained in the fusion protein. **D–F,** Anchorage-independent growth of NIH3T3 cells expressing *CD74-NRG1* (D), *EZR-ERBB4* (E), or *TRIM24-BRAF* (F) cDNA, and suppression of this growth by kinase inhibitors. Mock-, *CD74-NRG1*-, *EZR-ERBB4*-, and *TRIM24-BRAF*-transduced NIH3T3 cells were seeded in soft agar with DMSO alone or kinase inhibitors. Colonies > 100 μm in diameter were counted after 14 days. Column graphs show mean numbers of colonies ± S.E.M.

Figure 3. Tumorigenicity of NIH3T3 cells expressing *ERZ-ERBB4* or *TRIM24-BRAF* fusion cDNAs. **A,** Tumor growth in nude mice injected with NIH3T3 cells expressing empty vector, *EZR-ERBB4* fusion, or *TRIM24-BRAF* fusion. Cells were resuspended with 50% Matrigel and injected into the right flank of nude mice. Tumor size was measured twice weekly for 5 weeks. Data are shown as means ± S.E.M. **B,** Representative tumors were photographed on day 21. The numbers in parentheses indicate the ratio of the number of mice with tumors to the number of mice receiving cell injection.

Author Manuscript Published OnlineFirst on April 11, 2014; DOI: 10.1158/1078-0432.CCR-14-0107
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Table 1. Characteristics of invasive mucinous lung adenocarcinomas with novel gene fusions

No.	Sample	Sex	Age	Smoking (packs/year)	Gene fusion	Chromosome aberration	Oncogene mutation*	Pathological stage	TTF1	HNF4A
1	301T	M	55	Ever (47)	*CD74-NRG1*		None	1a	-	+
2	AD12-108T	F	68	Never	*CD74-NRG1*		None	2b	-	+
3	AD09-404T	F	78	Never	*CD74-NRG1*	t(5;8)(q32;p12)	None	1a	-	+
4	AD13-199T	F	47	Never	*CD74-NRG1*		None	1b	-	+
5	AD13-223T	F	53	Never	*CD74-NRG1*		None	1a	-	+
6	AD13-379T	F	66	Never	*SLC3A2-NRG1*	t(8;11)(p12;q13)	None	1b	Not tested	Not tested
7	436T	M	61	Ever (41)	*EZR-ERBB4*	t(2;6)(q25;q34)	None	1b	-	+
8	AD08_127T	F	66	Never	*TRIM24-BRAF*	inv7(q33;q34)	None	1a	+	+
9	AD12-119T	M	62	Current (63)	*KIAA1468-RET*	t(10;18)(q21;q11)	None	1a	+	-

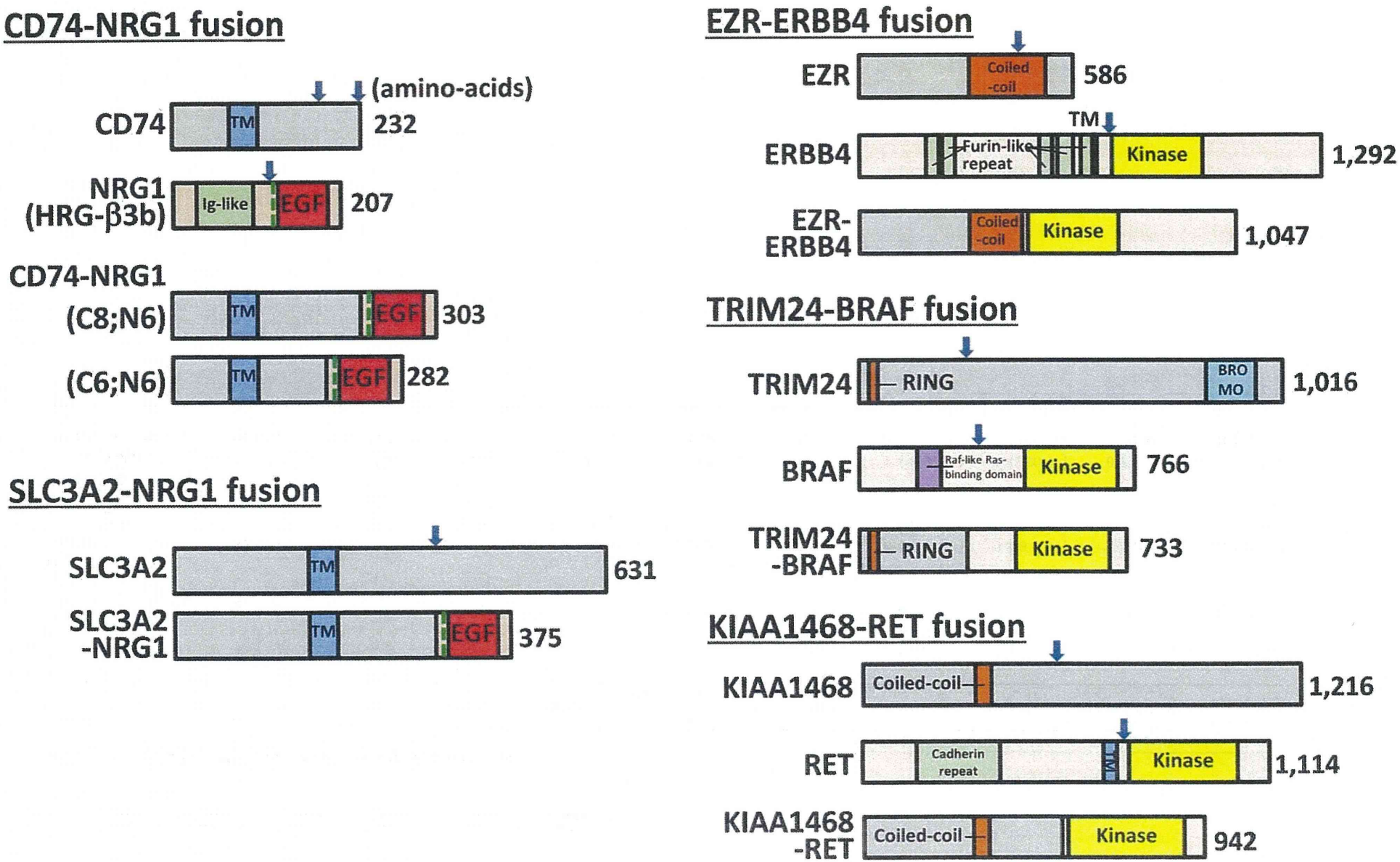
**EGFR*, *KRAS*, *BRAF*, and *HER2* mutations and *ALK*, *RET*, and *ROS1* fusions.

Author Manuscript Published OnlineFirst on April 11, 2014; DOI: 10.1158/1078-0432.CCR-14-0107
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Table 2. Characteristics of 90 invasive mucinous lung adenocarcinomas

Variable	All	Mutation			Fusion					None (%)
		KRAS	*BRAF*	*EGFR*	*CD74-NRG1 or SLC3A2-NRG1*	*EZR-ERBB4*	*TRIM24-BRAF*	*EML4-ALK*	*KIAA1468-RET*	
Total	90 (100)	56 (62.2)	2 (2.2)	1 (1.1)	6 (6.7)	1 (1.1)	1 (1.1)	1 (1.1)	1 (1.1)	21 (23.3)
Age (mean ± SD; years)	67.2 ± 9.7	68.1 ± 9.7	66.5 ± 3.5	50	61.2 ± 11.5	61	66	64	62	68.1 ± 9.6
Sex										
Male (%)	39 (43.3)	28 (50.0)	0 (0)	0 (0)	1 (16.7)	1 (100)	0 (0)	0 (0)	1 (100)	8 (38.1)
Female (%)	51 (56.7)	28 (50.0)	2 (100)	1 (100)	5 (83.3)	0 (0)	1 (100)	1 (100)	0 (0)	13 (61.9)
Smoking habit										
Never-smoker (%)	51 (56.7)	29 (51.8)	2 (100)	1 (100)	4 (66.7)	0 (0)	1 (100)	1 (100)	0 (0)	13 (61.9)
Ever-smoker (%)	39 (43.3)	27 (48.2)	0 (0)	0 (0)	2 (33.3)	1 (100)	0 (0)	0 (0)	1 (100)	8 (38.1)

A



B