Association of *BIM* Deletion Polymorphism and *BIM-γ* RNA Expression in NSCLC with *EGFR* Mutation

KAZUTOSHI ISOBE¹, ATSUSHI KAKIMOTO¹, TETSUO MIKAMI², KYOHEI KABURAKI¹,
HIROSHI KOBAYASHI¹, TAKAHIRO YOSHIZAWA¹, TAKASHI MAKINO³, HAJIME OTSUKA³,
GO SANO¹, KEISHI SUGINO¹, SUSUMU SAKAMOTO¹, YUJIRO TAKAI¹,
NAOBUMI TOCHIGI⁴, AKIRA IYODA² and SAKAE HOMMA¹

¹Division of Respiratory Medicine, Toho University School of Medicine, Tokyo, Japan;

²Division of Pathology, Toho University School of Medicine, Tokyo, Japan;

³Division of Chest Surgery, Toho University School of Medicine, Tokyo, Japan;

⁴Division of Surgical Pathology, Toho University School of Medicine, Tokyo, Japan

Abstract. Aim: This pilot study assessed the association of *BIM* deletion polymorphism and *BIM* RNA isoform in patients with *EGFR*-positive non-small cell lung cancer (NSCLC). Patients and Methods: The study included 33 patients with *EGFR*-positive NSCLC treated with gefitinib. *BIM* deletion polymorphism and *BIM* RNA isoform (EL/L/S/γ) were determined by polymerase chain reaction (PCR). Results: *BIM-γ* expression was significantly higher in patients with *BIM* deletion polymorphism inside tumors ($p=0.038$) and around tumors ($p=0.0024$). Relative *BIM-γ* expression was significantly higher in patients with *BIM* deletion polymorphism than among those without *BIM* deletion polymorphism ($p=0.0017$). Patients with *BIM-γ* had significantly shorter progression-free survival than those without *BIM-γ* (median: 304 vs. 732 days; $p=0.023$). Conclusion: Expression of *BIM-γ* mRNA and *BIM* deletion

polymorphism were strongly associated. *BIM-γ* overexpression may have a role in apoptosis related to *EGFR*-tyrosine kinase inhibitor.

Activating mutations in epidermal growth factor receptor (*EGFR*) are promising targets in the treatment of non-small cell lung cancer (NSCLC) (1, 2). The frequency of *EGFR* mutations varies by population. In North America and Western Europe, approximately 5-10% of patients with adenocarcinoma harbor mutations, whereas in East Asia approximately 60-70% of never-smokers have *EGFR* mutations (3, 4). *EGFR* tyrosine kinase inhibitors (*EGFR*-TKIs) induce marked radiographic and clinical improvement in patients with *EGFR* mutations. *EGFR*-TKIs such as gefitinib, erlotinib, and afatinib are recommended for treating *EGFR*-mutated NSCLC (5, 6). NSCLC patients with such mutations who were treated with an *EGFR*-TKI as first-line therapy had longer progression-free survival (PFS) than those who received platinum-based chemotherapy (7-11). Therefore, detection of *EGFR* mutations in patients with metastatic NSCLC is important for selecting individualized therapies.

Treatment resistance invariably develops within 10 to 16 months after initial *EGFR*-TKI treatment (12). Approximately 60% of patients with acquired resistance to *EGFR*-TKIs had an *EGFR* T790M mutation (13, 14). Other reported mechanisms underlying resistance are *MET* amplification, in 5-10% of cases (15, 16), and small-cell cancer transformation, in fewer than 5% of cases (17). However, approximately 30% of patients with *EGFR*-active mutations do not exhibit an objective response to *EGFR*-TKI, which is known as primary resistance (18-22). Although the mechanisms of primary resistance have been investigated in several preclinical and retrospective studies, the clinical and molecular characteristics of such resistance remain poorly understood.

Abbreviations: *BIM*, BCL2-like 11; BCL, B-cell CLL/lymphoma 2; BH3, BCL2 homology domain 3; NSCLC, non-small-cell lung cancer; *EGFR*, epidermal growth factor receptor; *EGFR*-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; FFPE, formalin-fixed paraffin-embedded; PFS, progression-free survival; OS, overall survival; PCR, polymerase chain reaction; RR, response rate; DCR, disease control rate; CTC, National Cancer Institute Common Terminology Criteria.

Correspondence to: Kazutoshi Isobe, Division of Respiratory Medicine, Toho University School of Medicine, 6-11-1 Omori-Nishi, Ota-ku, Tokyo 143-8541, Japan. Tel: +81 337624151 ext. 6555, Fax: +81 337663551, e-mail: kazutoshiisobe@med.toho-u.ac.jp

Key Words: *BIM*, non-small cell lung cancer, epidermal growth factor receptor tyrosine kinase inhibitor.

BCL2-like 11 (*BIM*) is a pro-apoptotic member of the B-cell CLL/lymphoma 2 (BCL2) family of proteins (23, 24) and is a key modulator of apoptosis triggered by EGFR-TKIs (25, 26). Faber *et al.* (27) used quantitative real-time polymerase chain reaction (PCR) and *BIM* immunohistochemistry to investigate *BIM* and β -actin RNA expression in pre-treatment tumors from 24 patients with *EGFR*-mutant lung cancer. The response rate to EGFR-TKIs was 44% in patients with low *BIM* expression and 77% in those with high *BIM* expression, although the difference was not significant. Recent data from the European Tarceva (EURTAC) trial showed that PFS and overall survival (OS) were shorter in patients with low/intermediate *BIM* mRNA levels in primary tumors than in those with high mRNA levels (PFS: 7.2 vs. 12.9 months, $p=0.0003$; OS: 22.1 vs. 28.6 months, $p=0.0364$) (28).

Ng *et al.* (29) reported a common intronic deletion polymorphism in the gene encoding *BIM*. This polymorphism switched *BIM* splicing from exon 4 to exon 3, which resulted in increased expression of *BIM* RNA isoforms lacking the proapoptotic BCL2-homology domain 3 (BH3), such as *BIM- γ* . The *BIM* isoforms with a BH3 domain were *BIM-EL*, *L*, and *S*. This *BIM* deletion polymorphism was absent in individuals from African and European populations but was present in 12% of an Asian population (29). After EGFR-TKI treatment, PFS was significantly shorter in patients with *BIM* deletion polymorphism than in those without this polymorphism, which suggests that reduced expression of *BIM* with a BH3 domain is associated with unfavorable response to EGFR-TKIs (29-33). However, few studies have examined the association between *BIM* polymorphism and expression of *BIM* RNA isoforms such as *BIM-EL*, *L*, *S*, and γ .

The present study investigated the association between *BIM* polymorphism and expression of the *BIM* RNA isoforms *BIM- γ* and *BIM-EL/L/S* in lung tissue from patients with *EGFR*-positive NSCLC.

Patients and Methods

Clinical samples. We studied 33 patients with *EGFR* mutation-positive NSCLC who were treated with EGFR-TKIs during the period from January 2008 to January 2016. *BIM* isoform and *BIM* deletion polymorphism were investigated by real-time PCR analysis of 33 formalin-fixed paraffin-embedded (FFPE) slides of surgical specimens of lung tissue.

Detection of *BIM* deletion polymorphism. To identify *BIM* deletion polymorphism, we performed 2 types of PCR analysis, using the method of Ng *et al.* (22). In brief, we used a single primer set that contained the deletion area in intron 2, as well as 2 separate primer sets designed for wild-type and deletion alleles. The DNA was subjected to PCR amplification using primers designed to detect the deletion site (2,903 bp) in intron 2 of the *BCL2L1* gene. The resulting PCR products from the deletion (1,285 bp) and wild-type (4,188 bp) alleles were analyzed on agarose gels. In addition, the

PCR products for the deletion (177 bp) and wild-type (174 bp) alleles were analyzed on agarose gels (30).

Detection of *BIM-EL/L/S* and *BIM- γ* . An miRNeasy FFPE Kit (Qiagen KK, Tokyo, Japan) was used to extract total RNA (including miRNA) from the FFPE sections of tumor tissue and non-tumor tissue. The extracted RNA was stored at -80°C until use. cDNA was synthesized using PrimeScriptRT MasterMix (PerfectRealTime, Takara Bio Inc., Otsu, Japan). Quantitative real-time PCR was performed in a Thermal Cycler Dice Real Time System TP800 (Takara Bio Inc.), using SYBR Premix Ex Taq II (Tli RNaseH Plus, Takara Bio Inc.).

Quantification of *BIM*, *BIM-EL/L/S*, and *BIM- γ* . The quantitative real-time PCR primers (forward and reverse) used Perfect Real Time Primer (Takara Bio Inc.). To correct for differences in quality and quantity between samples, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a reference gene. The targets were obtained from the same mRNA preparations. Relative expression of *BIM-EL/L/S* and *BIM- γ* in mRNA from tissue sections inside and around tumors, as normalized to the reference gene (*GAPDH* mRNA), was calculated by using the KCL22 cell line for calibration.

Clinical outcomes. We retrospectively analyzed the clinical characteristics, response rate (RR), disease control rate (DCR), and toxicity of gefitinib in patients with and without *BIM- γ* . We then estimated PFS and overall survival (OS) in the same groups. The PFS of patients treated with EGFR-TKI was assessed from the date gefitinib therapy started to the first sign of disease progression, as determined by computed tomographic or magnetic resonance imaging, according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. OS was defined as the interval from the date of diagnosis until death from any cause.

Statistical analysis. Statistical analyses were conducted using the SPSS software for Windows, version 12.0 (SPSS Inc., Tokyo, Japan). Differences in relative expressions of *BIM*, *BIM-EL/L/S*, and *BIM- γ* between patients with and without *BIM- γ* were compared with the Wilcoxon rank sum test. Differences in clinical characteristics, RR, and DCR, frequency of *BIM* deletion polymorphism, and *BIM- γ* expression between patients with and without *BIM- γ* were compared using the Fisher exact test. Survival curves were drawn by the Kaplan–Meier method, and statistical analysis was performed using the log-rank test. A p -value of less than 5% was considered statistically significant.

This single-center study was conducted at Toho University Omori Medical Center (Tokyo, Japan) and was approved by its Human Genome/Gene Analysis Research Ethical Committee (authorization number, 24-1).

Results

***BIM* deletion polymorphism in *EGFR*-positive NSCLC.** We analyzed *BIM* deletion polymorphism in 33 patients with *EGFR* mutation-positive NSCLC who were treated with gefitinib. *BIM* deletion polymorphism was present in 4 of the 33 patients (12.1%); heterozygous deletion was noted in all 4 patients (Table I).

Table I. Patient characteristics (N=33).

Age (years) range	25-82
Mean	64.7
Gender	
Male	26
Female	7
ECOG Performance status	
0	21
1	10
2	2
Histological pattern	
Ad	33
Clinical stage	
Rec	33
<i>EGFR</i> mutation at primary site	
19del	16
L858R	15
G719C	2
<i>BIM</i> deletion polymorphism	
Yes	4
No	29
Line of gefitinib therapy	
First	16
Second	16
Third	1

ECOG: Eastern Cooperative Oncology Group; Rec: recurrence after surgical resection; Ad: adenocarcinoma; *EGFR*: epidermal growth factor receptor; L858R: exon 21 L858R; 19del: exon 19 deletion; G719C: exon 18 G719C.

Clinical characteristics of patients with and without BIM deletion polymorphism. There was no significant difference in RR, DCR, or incidences of adverse events between patients with (n=8) and without (n=25) *BIM-γ* (Table II).

Association of BIM-EL/L/S and BIM-γ expression. Expression of *BIM-EL/L/S* mRNA was detected inside the tumor in 12 patients, around the tumor in 3 patients, and at both sites in 9 patients; 9 patients had no such expression. Expression of *BIM-γ* mRNA was detected inside the tumor in 5 patients and around the tumor in 3 patients; 25 patients had no such expression. There was no association between *BIM-EL/L/S* and *BIM-γ* expression (Table III). Relative expression was significantly higher for *BIM-γ* than for *BIM-EL/L/S* (276±163.6 vs. 12±15.1, $p=0.0018$) (Figure 1).

Association of BIM deletion polymorphism and BIM-EL/L/S expression. We compared *BIM-EL/L/S* expression in relation to the frequency of *BIM* polymorphism inside and/or around tumors. There was no significant difference in *BIM-EL/L/S* expression in any comparison (Table IV).

Association of BIM deletion polymorphism and BIM-γ expression. We compared the frequency of *BIM* deletion polymorphism and *BIM-γ* expression inside and/or around

Table II. Clinical response and adverse events after *EGFR*-TKI therapy (N=33).

	Patients with <i>BIM-γ</i> (N=8)	Patients without <i>BIM-γ</i> (N=25)	<i>p</i> -Value
Clinical response (%)			
RR	62.5	52	0.60
DCR	100	92	0.41
All adverse events (%)			
Rash	50.0	32.0	0.35
Diarrhea	37.5	24.0	0.45
AST/ALT	0	8.0	0.30
Appetite loss	37.5	16.0	0.20
Pneumonitis	0	12.0	0.30
CTC Grade 3-5 (%)			
Rash	12.5	8.0	0.69
Diarrhea	0	8.0	0.41
AST/ALT	0	4.0	0.56
Appetite loss	0	0	-
Pneumonitis	0	8.0	0.30

RR: Response rate, DCR: disease control rate, CTC: National Cancer Institute Common Terminology Criteria.

Table III. Association of *BIM-EL/L/S* and *BIM-γ* mRNA expression (N=33).

	<i>BIM-EL/L/S</i>			
	Inside tumor	Around tumor	Both sites	None
<i>BIM-γ</i>				
Inside tumor	3	0	2	0
Around tumor	1	0	1	1
Both sites	0	0	0	0
None	8	3	6	8

tumors. *BIM-γ* expression was significantly more frequent in patients with *BIM* deletion polymorphism than in those without *BIM* polymorphism inside tumors ($p=0.038$) and around tumors ($p=0.0024$). Absence of *BIM-γ* expression was significantly more frequent in patients without *BIM* polymorphism than in those with *BIM* polymorphism ($p=0.00016$) (Table V). Relative *BIM-γ* expression was significantly higher in patients with *BIM* deletion polymorphism than in those without *BIM* deletion polymorphism ($p=0.0017$; Figure 2).

Survival and indicators of shorter PFS. We estimated PFS and OS in patients with and without *BIM-γ*. Patients with *BIM-γ* had significantly shorter PFS than those without *BIM-γ* (median: 304 vs. 732 days; $p=0.023$; Figure 3). There was no significant difference in OS (median: 1,345 vs. 1,552 days, $p=0.24$; Figure 4).

Table IV. Association of *BIM* deletion polymorphism and *BIM-EL/L/S* expression (N=33).

	<i>BIM</i> polymorphism		<i>p</i> -Value
	Positive (N=4)	Negative (N=29)	
<i>BIM-EL/L/S</i>			
Inside tumor	2	10	0.55
Around tumor	1	2	0.23
Both sites	0	9	0.19
None	1	8	0.91

Table V. Association of *BIM* deletion polymorphism and *BIM-γ* expression (n=33).

	<i>BIM</i> polymorphism		<i>p</i> -Value
	Positive (n=4)	Negative (n=29)	
<i>BIM-γ</i>			
Inside tumor	2	3	0.038
Around tumor	2	1	0.0024
Both sites	0	0	-
None	0	25	0.00016

Figure 1. Relative expression was significantly higher for *BIM-γ* than for *BIM-EL/L/S* (276.3 ± 163.6 vs. 12.0 ± 15.1 , $p = 0.0018$).

Figure 2. Frequency of *BIM-γ* expression was significantly higher in patients with *BIM* polymorphism than in those without *BIM* polymorphism ($p = 0.0017$).

Discussion

The *BIM* deletion polymorphism is located in intron 2 of the *BIM* gene and results in expression of *BIM* isoforms lacking the BH3 domain, such as *BIM-γ*. However, we detected both

mRNA *BIM-γ* and *BIM-EL/L/S* expression in and around tumors in patients with and without *BIM* deletion polymorphism. We found no association between *BIM-EL/L/S* and *BIM-γ* expression, regardless of the status of *BIM* deletion polymorphism. Furthermore, relative expression was

Figure 3. Kaplan-Meier curves for progression-free survival. Patients with *BIM-γ* had significantly shorter progression-free survival than those without *BIM-γ* (median: 304 vs. 732 days; $p=0.023$).

Figure 4. Kaplan-Meier curves for overall survival. Overall survival did not significantly differ between patients with and without *BIM-γ* (median: 1,345 vs. 1,552 days; $p=0.24$).

significantly higher for *BIM-γ* than for *BIM-EL/L/S* (276 ± 163.6 vs. 12 ± 15.1 , $p=0.0018$). Faber *et al.* (24) reported that BIM levels were important in determining response to

targeted therapies in patients with solid tumors. This finding is consistent with research showing that cancer cells are sensitive to small changes in *BIM* protein concentration.

BIM-γ, a *BIM* isoform that lacks the BH3 domain, is up-regulated in most prostate cancer cell lines (34). *BIM-γ* inhibits clonal growth in prostate cancer and promotes apoptosis. Interestingly, *BIM-γ* was found in 13.7% (4 out of 29) of the present specimens without *BIM* deletion polymorphism. Relative *BIM-γ* expression in patients without *BIM* polymorphism was significantly lower than in those with *BIM* deletion polymorphism ($p=0.0017$). This suggests that, among the *BIM* isoforms, overexpression of *BIM-γ* suppresses TKI-related apoptosis. Further study of the mechanism of *BIM-γ* expression is warranted.

One hypothesis is that *BIM* deletion polymorphism itself results in relative resistance to EGFR-TKIs. Kuroda *et al.* (35) showed that cancer cells were sensitive to small changes in *BIM* protein concentrations and that *BIM* protein concentration had a dose-dependent effect on apoptosis and the degree of TKI resistance (35). We compared the frequency of *BIM* deletion polymorphism and *BIM-γ* inside and/or around tumors. Patients with *BIM* deletion polymorphism had significantly higher *BIM-γ* expression inside tumors ($p=0.038$) and around tumors ($p=0.0024$) than those without *BIM* deletion polymorphism. Absence of *BIM-γ* expression was significantly more frequent in patients without *BIM* polymorphism than among those with *BIM* polymorphism ($p=0.00016$). These findings suggest a strong association between an imbalance in *BIM* isoforms and *BIM* deletion polymorphism.

Clinical characteristics, response to EGFR-TKIs, and incidences of adverse events due to EGFR-TKI did not significantly differ among patients with and without *BIM-γ*. Thus, clinical characteristics are not sufficient to identify patients with and without *BIM-γ*. However, our analysis of PFS and OS in patients with and without *BIM-γ* showed that PFS was significantly shorter in patients with *BIM-γ* than in those without *BIM-γ* (median: 304 vs. 732 days; $p=0.023$). Future studies should attempt to clarify the association between *BIM-γ* and PFS in patients receiving gefitinib.

The major limitation of this study is that it was a retrospective single-center study with a small sample size. A large-scale multicenter study is thus needed in order to statistically confirm the validity of our results. Clinical application of our results would require a prospective study of patients receiving gefitinib for *EGFR* mutation-positive NSCLC with or without *BIM-γ* overexpression. Bean *et al.* (36) reported that *BIM* act as sentinels that interconnect kinase signaling networks and the mitochondria-dependent apoptotic program. Karachaliou *et al.* (37) reported that *BIM* and *mTOR* mRNA expression levels predict the outcome of erlotinib therapy in *EGFR*-mutant NSCLC. Future studies should examine the associations of *BIM-γ* with *PUMA*, *mTOR*, and other apoptosis markers.

In conclusion, the present study is the first to show that *BIM-γ* expression was strongly associated with *BIM* deletion polymorphism and that *BIM-γ* overexpression was associated

with TKI-related apoptosis. These findings may be useful in developing treatment strategies for patients receiving EGFR-TKIs for *EGFR* mutation-positive NSCLC.

Conflicts of Interest

The Authors declare no conflicts of interest.

Acknowledgements

The Authors thank Yoshihisa Otsuka of SRL Inc. (Tokyo, Japan) and Yusuke Hashizawa and Akimitsu Iwama of Astra Zeneca Inc. (Tokyo Japan). They are also grateful to David Kipler for his review of the language of this article. This study was supported by JSPS KAKENHI Grant Numbers JP15K09195, JP2642140, and a Research Promotion Grant from Toho University Graduate School of Medicine (No. 16-3, to K.I.).

References

- 1 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 and Haber DA: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 350: 2129-2139, 2004.
- 2 Paez JG, Jänne 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 and Meyerson M: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 304: 1497-1500, 2004.
- 3 Tan DS, Mok TS and Rebbeck TR: Cancer genomics: diversity and disparity across ethnicity and geography. *J Clin Oncol* 34: 91-101, 2016.
- 4 Tan DS, Yom SS, Tsao MS, Pass HI, Kelly K, Peled N, Yung RC, Wistuba II, Yatabe Y, Unger M, Mack PC, Wynes MW, Mitsudomi T, Weder W, Yankelevitz D, Herbst RS, Gandara DR, Carbone DP, Bunn PA Jr., Mok TS and Hirsch FR: The international association for the study of lung cancer consensus statement on optimizing management of EGFR mutation-positive non-small cell lung cancer: status in 2016. *J Thorac Oncol* 11: 946-963, 2016.
- 5 Masters GA, Temin S, Azzoli CG, Giaccone G, Baker S Jr, Brahmer JR, Ellis PM, Gajra A, Rackear N, Schiller JH, Smith TJ, Strawn JR, Trent D and Johnson DH: American society of clinical oncology clinical practice. systemic therapy for stage IV non-small-cell lung cancer: American society of clinical oncology clinical practice guideline update. *J Clin Oncol* 33: 3488-3515, 2015.
- 6 Kuan FC, Kuo LT, Chen MC, Yang CT, Shi CS, Teng D and Lee KD: Overall survival benefits of first-line EGFR tyrosine kinase inhibitors in EGFR-mutated non-small-cell lung cancers: a systematic review and meta-analysis. *Br J Cancer* 113: 1519-1528, 2015.
- 7 Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, and Fukuoka M: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 361: 947-957, 2009.

- 8 Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S and Nukiwa T: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 362: 2380-2388, 2010.
- 9 Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, Takada M, Yoshioka H, Shibata K, Kudoh S, Shimizu E, Saito H, Toyooka S, Nakagawa K and Fukuoka M: Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* 11: 121-128, 2010.
- 10 Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, Lu S, Zhang L, Hu C, Hu C, Luo Y, Chen L, Ye M, Huang J, Zhi X, Zhang Y, Xiu Q, Ma J, Zhang L and You C: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* 12: 735-742, 2011.
- 11 Yang JC, Wu YL, Schuler M, Sebastian M, Popat S, Yamamoto N, Zhou C, Hu CP, O'Byrne K, Feng J, Lu S, Huang Y, Geater SL, Lee KY, Tsai CM, Gorbunova V, Hirsh V, Bennouna J, Orlov S, Mok T, Boyer M, Su WC, Lee KH, Kato T, Massey D, Shahidi M, Zazulina V and Sequist LV: Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. *Lancet Oncol* 16: 141-151, 2015.
- 12 Ohashi K, Maruvka YE, Michor F and Pao W: Epidermal growth factor receptor tyrosine kinase inhibitor-resistant disease. *J Clin Oncol* 31: 1070-1080, 2013.
- 13 Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG and Halmos B: EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 352: 786-792, 2005.
- 14 Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG and Varmus H: Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2: e73, 2005.
- 15 Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC and Jänne PA: MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 316: 1039-1043, 2007.
- 16 Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, Chitale D, Motoi N, Szoke J, Broderick S, Balak M, Chang WC, Yu CJ, Gazdar A, Pass H, Rusch V, Gerald W, Huang SF, Yang PC, Miller V, Ladanyi M, Yang CH and Pao W: MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 104: 20932-20937, 2007.
- 17 Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, Bergethon K, Shaw AT, Gettinger S, Cospier AK, Akhavanfard S, Heist RS, Temel J, Christensen JG, Wain JC, Lynch TJ, Vernovsky K, Mark EJ, Lanuti M, Iafrate AJ, Mino-Kenudson M and Engelman JA: Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 3: 75ra26, 2011.
- 18 Wheeler DL, Dunn EF and Harari PM: Understanding resistance to EGFR inhibitors-impact on future treatment strategies. *Nat Rev Clin Oncol* 7: 493-507, 2010.
- 19 Takeda M, Okamoto I, Fujita Y, Arai T, Ito H, Fukuoka M, Nishio K and Nakagawa K: De novo resistance to epidermal growth factor receptor-tyrosine kinase inhibitors in EGFR mutation-positive patients with non-small cell lung cancer. *J Thorac Oncol* 5: 399-400, 2010.
- 20 Cappuzzo F, Jänne PA, Skokan M, Finocchiaro G, Rossi E, Ligorio C, Zucali PA, Terracciano L, Toschi L, Roncalli M, Destro A, Incarbone M, Alloisio M, Santoro A and Varela-Garcia M: MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. *Ann Oncol* 20: 298-304, 2009.
- 21 Tanaka A, Sueoka-Aragane N, Nakamura T, Takeda Y, Mitsuoaka M, Yamasaki F, Hayashi S, Sueoka E and Kimura S: Co-existence of positive MET FISH status with EGFR mutations signifies poor prognosis in lung adenocarcinoma patients. *Lung Cancer* 75: 89-94, 2012.
- 22 Sos ML, Koker M, Weir BA, Heynck S, Rabinovsky R, Zander T, Seeger JM, Weiss J, Fischer F, Frommolt P, Michel K, Peifer M, Mermel C, Girard L, Peyton M, Gazdar AF, Minna JD, Garraway LA, Kashkar H, Pao W, Meyerson M and Thomas RK: PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. *Cancer Res* 69: 3256-3261, 2009.
- 23 Youle RJ and Strasser A: The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 9: 47-59, 2008.
- 24 Faber AC, Ebi H, Costa C and Engelman JA: Apoptosis in targeted therapy responses: the role of BIM. *Adv Pharmacol* 65: 519-542, 2012.
- 25 Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, Tenen DG and Kobayashi S: BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med* 4: 1669-1779, 2007.
- 26 Gong Y, Somwar R, Politi K, Balak M, Chmielecki J, Jiang X and Pao W: Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. *PLoS Med* 4: e294, 2007.
- 27 Faber AC, Corcoran RB, Ebi H, Sequist LV, Waltman BA, Chung E, Incio J, Digumarthy SR, Pollack SF, Song Y, Muzikansky A, Lifshits E, Roberge S, Coffman EJ, Benes CH, Gómez HL, Baselga J, Arteaga CL, Rivera MN, Dias-Santagata D, Jain RK and Engelman JA: BIM expression in treatment-naïve cancers predicts responsiveness to kinase inhibitors. *Cancer Discov* 1: 352-365, 2011.
- 28 Costa C, Molina MA, Drozdowskyj A, Giménez-Capitán A, Bertran-Alamillo J, Karachaliou N, Gervais R, Massuti B, Wei J, Moran T, Majem M, Felip E, Carcereny E, Garcia-Campelo R, Viteri S, Taron M, Ono M, Giannikopoulos P, Bivona T and Rosell R: The impact of EGFR T790M mutations and BIM mRNA expression on outcome in patients with EGFR-mutant NSCLC treated with erlotinib or chemotherapy in the randomized phase III EORTC trial. *Clin Cancer Res* 20: 2001-2010, 2014.

- 29 Ng KP, Hillmer AM, Chuah CT, Juan WC, Ko TK, Teo AS, Ariyaratne PN, Takahashi N, Sawada K, Fei Y, Soh S, Lee WH, Huang JW, Allen JC Jr, Woo XY, Nagarajan N, Kumar V, Thalamuthu A, Poh WT, Ang AL, Mya HT, How GF, Yang LY, Koh LP, Chowbay B, Chang CT, Nadarajan VS, Chng WJ, Than H, Lim LC, Goh YT, Zhang S, Poh D, Tan P, Seet JE, Ang MK, Chau NM, Ng QS, Tan DS, Soda M, Isobe K, Nöthen MM, Wong TY, Shahab A, Ruan X, Cacheux-Rataboul V, Sung WK, Tan EH, Yatabe Y, Mano H, Soo RA, Chin TM, Lim WT, Ruan Y and Ong ST: A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. *Nat Med* 18: 521-528, 2012.
- 30 Isobe K, Hata Y, Tochigi N, Kaburaki K, Kobayashi H, Makino T, Otsuka H, Sato F, Ishida F, Kikuchi N, Hirota N, Sato K, Sano G, Sugino K, Sakamoto S, Takai Y, Shibuya K, Iyoda A and Homma S: Clinical significance of BIM deletion polymorphism in non-small-cell lung cancer with epidermal growth factor receptor mutation. *J Thorac Oncol* 9: 483-487, 2014.
- 31 Zhao M, Zhang Y, Cai W, Li J, Zhou F, Cheng N, Ren R, Zhao C, Li X, Ren S, Zhou C and Hirsch FR: The Bim deletion polymorphism clinical profile and its relation with tyrosine kinase inhibitor resistance in Chinese patients with non-small cell lung cancer. *Cancer* 120: 2299-2307, 2014.
- 32 Lee JH, Lin YL, Hsu WH, Chen HY, Chang YC, Yu CJ, Shih JY, Lin CC, Chen KY, Ho CC, Laio WY, Yang PC and Yang JC: Bcl-2-like protein 11 deletion polymorphism predicts survival in advanced non-small-cell lung cancer. *J Thorac Oncol* 9: 1385-1392, 2014.
- 33 Nie W, Tao X, Wei H, Chen WS and Li B: The BIM deletion polymorphism is a prognostic biomarker of EGFR-TKIs response in NSCLC: A systematic review and meta-analysis. *Oncotarget* 6: 25696-25700, 2015.
- 34 Liu JW, Chandra D, Tang SH, Chopra D and Tang DG: Identification and characterization of Bimgamma, a novel proapoptotic BH3-only splice variant of Bim. *Cancer Res* 62: 2976-2981, 2002.
- 35 Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC, Kimura S, Ottmann OG, Druker BJ, Villunger A, Roberts AW and Strasser A: Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. *Proc Natl Acad Sci USA* 103: 14907-14912, 2006.
- 36 Bean GR, Ganesan YT, Dong Y, Takeda S, Liu H, Chan PM, Huang Y, Chodosh LA, Zambetti GP, Hsieh JJ and Cheng EH: PUMA and BIM are required for oncogene inactivation-induced apoptosis. *Sci Signal* 6: ra20, 2013.
- 37 Karachaliou N, Codony-Servat J, Teixidó C, Pilotto S, Drozdowskyj A, Codony-Servat C, Giménez-Capitán A, Molina-Vila MA, Bertrán-Alamillo J, Gervais R, Massuti B, Morán T, Majem M, Felip E, Carcereny E, García-Campelo R, Viteri S, González-Cao M, Morales-Espinosa D, Verlicchi A, Crisetti E, Chaib I, Santarpia M, Luis Ramírez J, Bosch-Barrera J, Felipe Cardona A, de Marinis F, López-Vivanco G, Miguel Sánchez J, Vergnenegre A, Sánchez Hernández JJ, Sperduti I, Bria E and Rosell R: BIM and mTOR expression levels predict outcome to erlotinib in EGFR-mutant non-small-cell lung cancer. *Sci Rep* 5: 17499, 2015.

Received August 15, 2016

Revised September 10, 2016

Accepted September 21, 2016