Epidermal Growth Factor Receptor Gene Amplification in Patients with Advanced-stage NSCLC

ONDREJ FIALA[1,2], MILOS PESEK[3], JINDRICH FINEK[1], MAREK MINARIK[4,5], LUCIE BENESOVA[4], ONDREJ SOREJS[1], MARTIN SVATON[3], ZBYNEK BORTLICEK[6], RADEK KUCERA[7] and ONDREJ TOPOLCAN[7]

Departments of [1]Oncology and Radiotherapy, [3]Pneumology and [7]Nuclear Medicine, Medical School and Teaching Hospital Pilsen, Charles University in Prague, Prague, Czech Republic; [2]Biomedical Center, Faculty of Medicine in Pilsen, and [5]Department of Analytical Chemistry, Faculty of Sciences, Charles University in Prague, Prague, Czech Republic; [4]Center for Applied Genomics of Solid Tumours, Genomac Research Institute, Prague, Czech Republic; [6]Institute of Biostatistics and Analysis, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Abstract. *Background: Tyrosine kinase inhibitors (TKIs) targeting epidermal growth factor receptor (EGFR) represent novel, effective tools in the management of advanced-stage non-small cell lung cancer (NSCLC). We aimed to evaluate the incidence and predictive role of EGFR gene amplification in patients with advanced-stage NSCLC treated with EGFR-TKIs. Patients and Methods: The study included 290 patients with advanced-stage (IIIB or IV) NSCLC. Multiplex ligation-dependent probe amplification (MLPA) and polymerase chain reaction (PCR) were used for detection of EGFR gene amplification and EGFR mutations, respectively. Results: EGFR amplification was detected in 26 (9.0%) patients. EGFR amplification was found more frequently in patients harboring the EGFR mutation (p<0.001). No significant corelation between EGFR gene amplification and survival was observed. Conclusion: EGFR gene amplification is associated with EGFR gene mutation. EGFR gene amplification is not a feasible predictive biomarker for treatment with EGFR-TKIs in patients with advanced-stage NSCLC.*

Lung cancer is the most common cause of cancer-related death worldwide, while non-small cell lung cancer (NSCLC) is the most frequent histological type (1, 2). Treatment with low-molecular weight tyrosine kinase inhibitor (TKI) targeting epidermal growth factor receptor (EGFR) signaling pathway represents a novel, effective tool in the management of locally advanced or metastatic NSCLC. Amplification of the human epidermal growth factor receptor 2 *(HER2)/NEU* gene has since long been used as a predictive biomarker for targeted therapy of breast and gastric cancer by effective blockage of HER2-signaling by anti-HER2 antibody (3, 4). Considering HER2/NEU and EGFR are members of the same human epidermal growth factor receptor (ERBB) superfamily, it is reasonable to expect that *EGFR* gene amplification indicates a better response to anti-EGFR therapy in NSCLC. The aim of the present study was, therefore, to evaluate the predictive role of *EGFR* gene amplification in patients with advanced-stage NSCLC treated with EGFR-TKIs.

Correspondence to: Ondrej Fiala, MD, Ph.D., Department of Oncology and Radiotherapy, Medical School and Teaching Hospital Pilsen, Charles University Prague, alej Svobody 80, CZ-304 60 Pilsen, Czech Republic. Tel: +42 0728655488, e-mail: fiala.o@centrum.cz

Key Words: Non-small cell lung cancer, epidermal growth factor gene, gene amplification, tyrosine kinase inhibitors.

Patients and Methods

Patients and treatment. The study included 290 patients with cytologically or histologically confirmed locally advanced (stage IIIB) or metastatic (stage IV) NSCLC tested for *EGFR* gene amplification and activating *EGFR* gene mutations (exon 19 deletion or exon 21 L858R mutation). A sub-group including 177 patients was treated with EGFR-TKIs (erlotinib or gefitinib). All patients were diagnosed and treated between 2004 and 2012. The baseline patient characteristics are summarized in Table I.

Both erlotinib and gefitinib were administered orally at the standard approved doses of 150 mg and 250 mg daily, respectively. The treatment was continued until disease progression or development of intolerable adverse effects. Dose interruption or reduction was permitted in the event of treatment-related toxicity.

Clinical monitoring and statistics. The treatment was prospectively monitored and the clinical course of patients was continuously assessed at specific time points. Clinical follow-up including physical examination, plain chest X-ray and routine laboratory tests was performed every 3-4 weeks; computed tomography (CT) or positron-emission tomography-CT was performed after two or three months of the treatment. Progression-free survival (PFS) was

Table I. *Baseline patient characteristics*.

	Overall (n=290)	Patients treated with EGFR-TKIs (n=177)
Gender		
Female	109 (37.6%)	75 (42.4%)
Male	181 (62.4%)	102 (57.6%)
Median age (min-max), years	63 (28-88)	63 (28-83)
Smoking status		
Never-smoker	64 (22.1%)	42 (23.7%)
Current/former smoker	215 (74.1%)	133 (75.1%)
Unknown	11 (3.8%)	2 (1.2%)
Histological type		
Adenocarcinoma	170 (58.6%)	106 (59.9%)
Squamous-cell carcinoma	143 (31.92%)	59 (33.3%)
NOS	27 (16.07%)	33 (6.8%)
EGFR-TKI treatment		
Erlotinib	-	169 (95.5%)
Gefitinib		8 (4.5%)
EGFR gene mutation status		
Mutation	17 (5.9%)	14 (7.9%)
Wild-type	273 (94.1%)	163 (92.1%)
EGFR gene amplification status		
Amplification	26 (9.0%)	22 (12.4%)
Without amplification	264 (91.0%)	155 (87.6%)

NOS: Not otherwise specified; EGFR-TKI: epidermal growth factor receptor tyrosine kinase inhibitor.

determined from the date of erlotinib or gefitinib initiation until the date of first documented progression or death. Overall survival (OS) was determined from the date of erlotinib or gefitinib initiation until the date of death.

Data source. The clinical registry TULUNG (http:// tulung.registry.cz/), in which Faculty Hospital Pilsen has participated since its creation, is a non-interventional post-registration database of epidemiological and clinical data of patients with advanced-stage NSCLC treated with targeted therapies in the Czech Republic. The registry contains anonymized individual patient data including demographic parameters, initial staging and disease characteristics, baseline patient information at the start of targeted therapy, as well as data on survival and adverse events which is updated at least twice a year. The protocol was approved by the independent Ethics Committee (130515_ TULUNG_IBA_ FNBRNO) and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws.

Statistical analysis. Standard summary statistics were used to describe the sample data set. The significance of differences in the incidence of *EGFR* gene amplification according to patient clinical characteristics was assessed using Fisher's exact test. PFS and OS were estimated using Kaplan–Meier method and all point estimates were accompanied by 95% confidence intervals. Statistical significance of the differences in Kaplan–Meier estimates was assessed using the log-rank test. As a level of statistical significance α=0.05 was used.

EGFR mutation and amplification analysis. The tumor specimens acquired during initial bronchoscopy were evaluated by a senior cytologist using standard giemsa staining. In a few cases, a tumor biopsy was processed into formalin-fixed paraffin-embedded (FFPE) histological sections. The cytology slides or, eventually, the FFPE sections, were submitted for molecular genetic testing, which included detection of somatic mutations in *EGFR* exons 19 and 21 and detection of *EGFR* gene amplification. If necessary, tumor cells were carefully selected and removed from the samples by laser microdissection using a P.A.L.M. microlaser instrument (Carl Zeiss MicroImaging GmbH, Jena, Germany). The microdissected cells were collected directly into the polymerase chain reaction buffer and processed without a special DNA extraction step. In all other cases, the DNA was extracted from tissue cells by a standard spin-column procedure using JetQuick Tissue DNA Issolation Kit (Genomed GmbH, Loehne, Germany). Mutations in exons 19 and 21 of the *EGFR* gene were tested by Genoscan mutation detection kits (Genomac International, Prague, Czech Republic) utilizing a denaturing capillary electrophoresis technique on an ABI PRISM 3100 16-capillary genetic analyzer (Applied Biosystems, Foster City, CA, USA). Detected mutations were confirmed by Sanger DNA sequencing using a BigDye v 3.0 chemistry (Applied Biosystems). In rare cases, where the overall fraction of mutated DNA was below the 20% threshold for DNA sequencing, mutation was identified indirectly after forming only a homoduplex fragment with a given known mutation reference standard. *EGFR* amplification was evaluated by Multiplex ligation-dependent probe amplification (MLPA) technique using SALSA MLPA KIT P315-A1 (MRC Holland, Amsterdam, the Netherlands), which contains 28 probes for each of the *EGFR* exons (chromosome 7) in addition to nine reference probes on other chromosomes. A standard manufacturer protocol was followed to generate MLPA products, which were then again analyzed on the ABI PRISM 3100.

Results

Incidence of EGFR gene amplification. *EGFR* gene amplification was detected in 26 (9.0%) patients and *EGFR* gene mutation was detected in 17 (5.86%) patients out of all 290 tested. *EGFR* mutations included 10 (58.8%) cases of exon 19 deletion and 7 (41.2%) cases of exon 21 L858R substitution. *EGFR* gene amplification was found more frequently in patients harboring *EGFR* mutation compared to those harboring wild-type *EGFR* gene (41.2 *vs*. 7.0%); the difference was statistically significant (p<0.001) (Figure 1). We did not observe any significant correlation between the incidence of *EGFR* gene amplification and clinical characteristics including: sex (7.3% in women *vs*. 9.9% in men; p=0.529), smoking status (8.4% in current or former smokers *vs*. 12.5% in non-smokers; p=0.331) and histological type of NSCLC (11.2% in adenocarcinomas *vs*. 7.5% in squamous-cell carcinomas; p=0.135).

Relation between EGFR gene amplification and survival. The median PFS and OS for patients with *EGFR* gene amplification

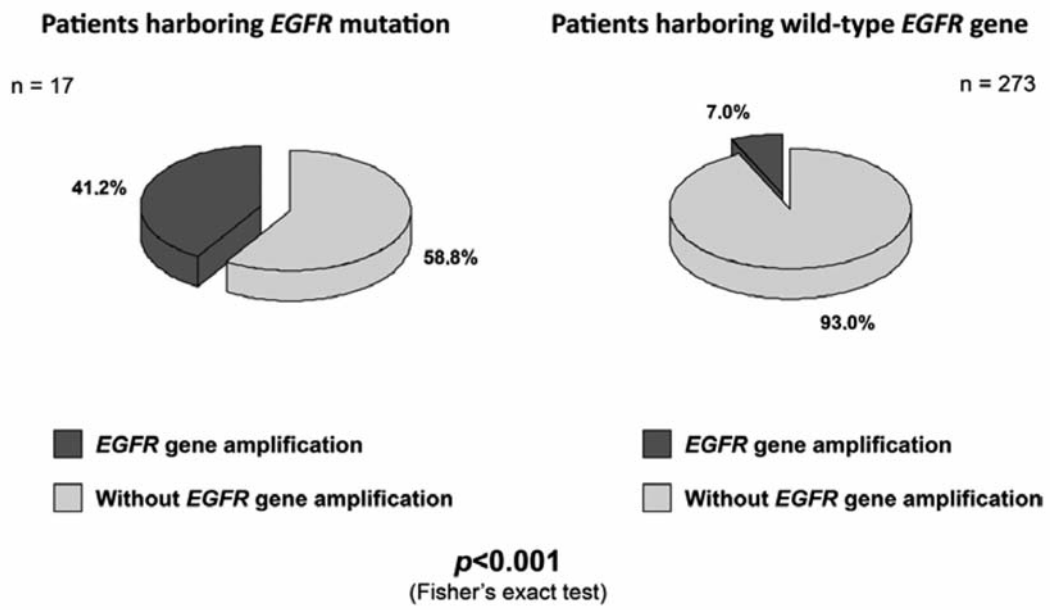

Figure 1. *Comparison of incidence of epidermal growth factor receptor (EGFR) gene amplification according to EGFR mutation.*

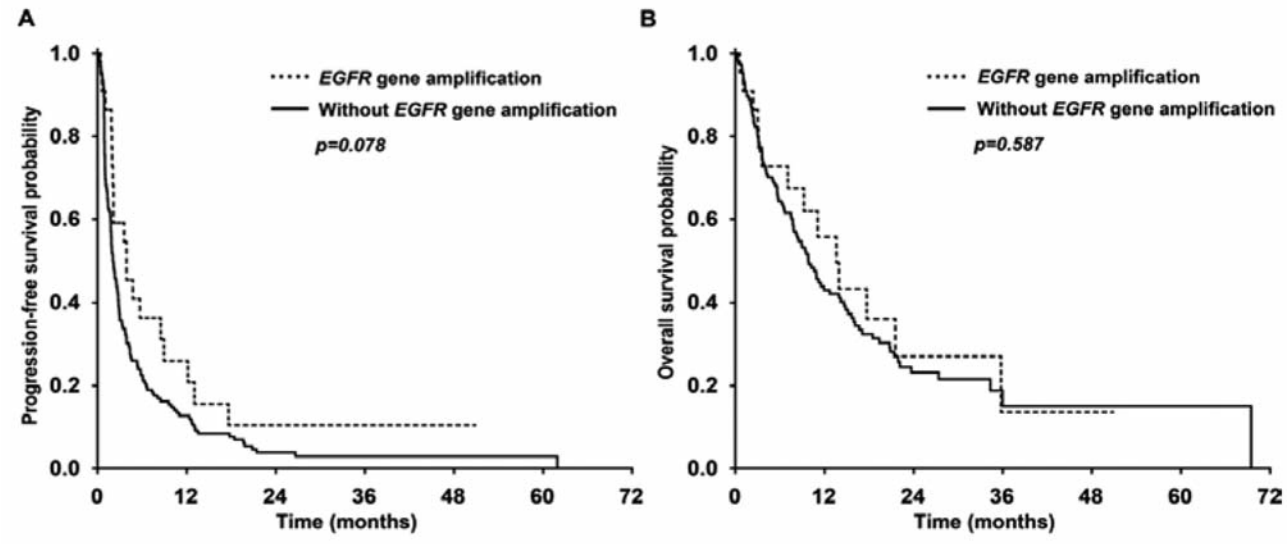

Figure 2. *Kaplan–Meier plots showing progression-free (PFS) (A) and overall (OS) (B) survival according to epidermal growth factor receptor (EGFR) gene amplification.*

(n=22) was 3.9 and 13.6 compared to 2.1 and 9.8 months (p=0.078 and p=0.587) for those without *EGFR* gene amplification (n=155) (Figure 2). Among patients harboring wild-type *EGFR* gene, the median PFS and OS for patients with *EGFR* gene amplification (n=18) was 2.1 and 13.6 compared to 1.9 and 9.8 months (p=0.172 and p=0.737) for those without *EGFR* gene amplification (n=145) (Figure 3).

Discussion

The efficacy and safety of EGFR-TKIs in the treatment of patients with advanced-stage NSCLC have been demonstrated in various phase III clinical trials (5-9). Several molecular biomarkers predicting the treatment efficacy of EGFR-TKIs have been established in recent years. *EGFR*

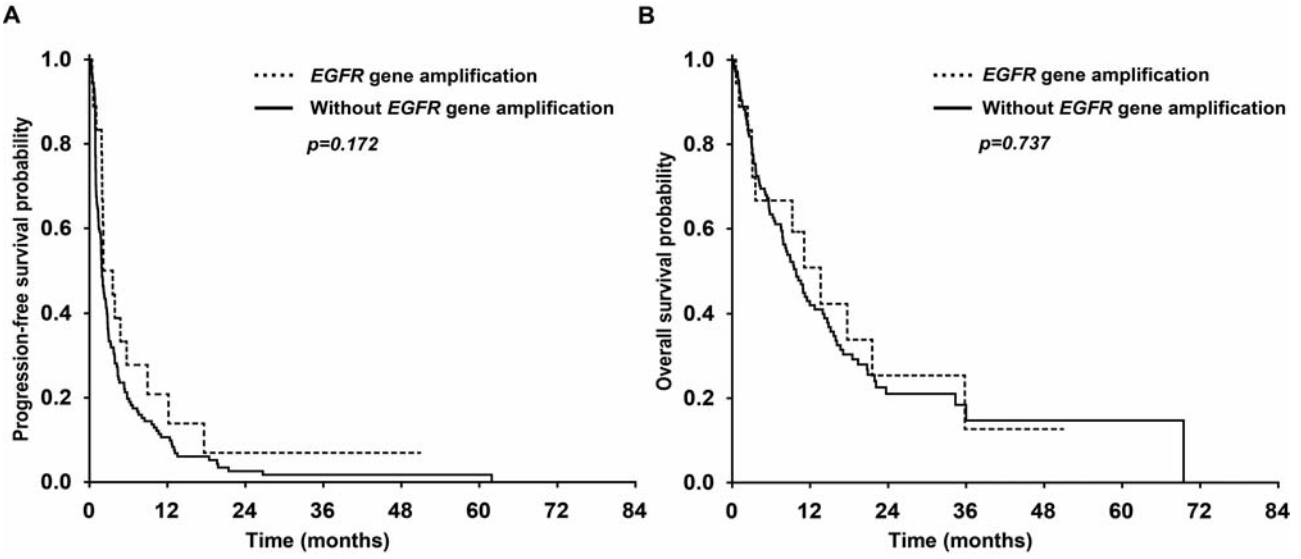

Figure 3. *Kaplan–Meier plots showing progression-free (PFS) (A) and overall (OS) (B) survival according to epidermal growth factor receptor (EGFR) gene amplification in patients harboring wild-type EGFR gene.*

gene mutations, occurring approximately in 5-20% of patients (predominantly exon 19 deletions or a point-mutation in exon 21 termed *L858R*), represent the strongest predictor of a good treatment response to EGFR-TKIs (10-13). Based on that finding, patients with advanced-stage NSCLC are selected for first-line treatment according to the presence of activating *EGFR* gene mutation. Because the response to other targeted agents, such as trastuzumab and tamoxifen, depends mainly on the level of expression of the target, several studies focused on the predictive role of EGFR protein expression and *EGFR* gene copy number have recently been conducted, although the results are conflicting.

In the present study, we aimed to evaluate the incidence and predictive role of *EGFR* gene amplification in patients with advanced-stage NSCLC treated with EGFR-TKIs. We were able to detect *EGFR* gene amplification in 9% of patients, that is similar to the findings by Hirsch *et al*. (14) and Dacic *et al*. (15), who detected *EGFR* gene amplification in 9% and 10% of patients, respectively.

Fluorescence *in situ* hybridization (FISH) has since long been the golden standard for detection of gene amplification in various tumor types, including breast, stomach and brain tissues (16). More recently multiplex ligation-dependent probe amplification (MLPA) has been demonstrated as an alternative semi-automatic approach with low demand on sample consumption (17). MLPA analysis can also be seemingly integrated with other molecular-genetic testing, most importantly the predictive testing of activating *EGFR* mutations (18). Using MLPA in the present study, we obtained results equivalent to FISH in terms of the frequency of *EGFR* amplifications reported in the literature (14-21). We observed higher frequency of *EGFR* gene amplifications in patients harboring *EGFR* gene mutation compared to those harboring wild-type *EGFR* gene (41.2 *vs*. 7.0%, $p<0.001$), which is in agreement with the findings previously reported by Hirsch *et al*. (22). Such a finding is important for evaluation of the predictive role of *EGFR* gene amplification. We did not observe any significant correlation between the incidence of *EGFR* gene amplification and basic clinical characteristics such as sex ($p=0.529$), smoking status ($p=0.331$) and histological type of NSCLC ($p=0.135$). Our results did not confirm higher frequency of *EGFR* gene amplification in patients with squamous cell carcinoma, which has been reported by others (14, 18, 21).

We observed a trend for longer PFS and OS for patients with *EGFR* amplification (3.9 and 13.6 months) compared to those without *EGFR* amplification (2.1 and 9.8 months), although the differences did not reach statistical significance ($p=0.078$ and $p=0.587$). Given the previously mentioned finding, we suggest that the trend for longer survival may be caused by the frequent coincidence of *EGFR* gene amplification and *EGFR* gene mutation. The basis for such a hypothesis becomes more apparent when evaluating survival data for the sub-group of patients harboring wild-type *EGFR* gene, where we recorded almost equivalent PFS (2.1 *vs*. 1.9 months, $p=0.172$). Thus, we did not confirm the predictive role of *EGFR* gene amplification reported in several previous studies (19, 22, 23). Our results are similar to those reported by Fukuoka *et al*., who analyzed biomarkers in phase III randomized study comparing gefitinib with chemotherapy in

patients with advanced NSCLC (IPASS), the study results suggested that the predictive value of *EGFR* gene copy number was driven by coexisting *EGFR* gene mutation (24). The principal limitations of our study are its retrospective design and limited number of patients included.

In conclusion, using MLPA, we confirmed the incidence of *EGFR* gene amplification reported in previous studies using FISH. We recorded frequent coincidence of *EGFR* gene amplification and *EGFR* gene mutation. The results of this study suggest that *EGFR* gene amplification is not a feasible biomarker for prediction of treatment efficacy EGFR-TKIs in patients with advanced-stage NSCLC.

Conflicts of Interest

JF has received honoraria from Astra Zeneca, Roche and Novartis for consultations and lectures unrelated to this project. OF, MP, MM, LB, OS, MS, ZB, RK and OT declare that they have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence this work.

Acknowledgements

The Authors would like to thank all patients voluntarily taking part in this study. This study was supported by the National Sustainability Program I (NPU I) No. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic.

References

1 Parkin DM: Global cancer statistics in the year 2000. Lancet Oncol *2*: 533-543, 2001.

2 Brambilla E, Travis WD, Colby TV, Corrin B and Shimosato Y: The new World Health Organization classification of lung tumours. Eur Respir J *18*: 1059-1068, 2001.

3 Krishnamurti U and Silverman JF: HER2 in breast cancer: a review and update. Adv Anat Pathol *21*: 100-107, 2014.

4 de Mello RA, Marques AM and Araújo A: HER2 therapies and gastric cancer: a step forward. World J Gastroenterol *19*: 6165-6169, 2013.

5 Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V and Carroll K: Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet *366*: 1527-1537, 2005.

6 Kim ES, Hirsh V, Mok T, Socinski MA, Gervais R, Wu YL, Li LY, Watkins CL, Sellers MV, Lowe ES, Sun Y, Liao ML, Osterlind K, Reck M, Armour AA, Shepherd FA, Lippman SM and Douillard JY: Gefitinib *versus* docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet *372*: 1809-1818, 2008.

7 Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabárbara P, Seymour L and National Cancer Institute of Canada Clinical Trials Group: Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med *353*: 123-132, 2005.

8 Ciuleanu T, Stelmakh L, Cicenas S, Miliauskas S, Grigorescu AC, Hillenbach C, Johannsdottir HK, Klughammer B and Gonzalez EE: Efficacy and safety of erlotinib *versus* chemotherapy in second-line treatment of patients with advanced, non-small-cell lung cancer with poor prognosis (TITAN): a randomised multicentre, open-label, phase 3 study. Lancet Oncol *13*: 300-308, 2012.

9 Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki 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.

10 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.

11 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.

12 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 III study. Lancet Oncol *12*: 735-742, 2011.

13 Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, Porta R, Cobo M, Garrido P, Longo F, Moran T, Insa A, De Marinis F, Corre R, Bover I, Illiano A, Dansin E, de Castro J, Milella M, Reguart N, Altavilla G, Jimenez U, Provencio M, Moreno MA, Terrasa J, Muñoz-Langa J, Valdivia J, Isla D, Domine M, Molinier O, Mazieres J, Baize N, Garcia-Campelo R, Robinet G, Rodriguez-Abreu D, Lopez-Vivanco G, Gebbia V, Ferrera-Delgado L, Bombaron P, Bernabe R, Bearz A, Artal A, Cortesi E, Rolfo C, Sanchez-Ronco M, Drozdowskyj A, Queralt C, de Aguirre I, Ramirez JL, Sanchez JJ, Molina MA, Taron M, Paz-Ares L; Spanish Lung Cancer Group in collaboration with Groupe Français de Pneumo-Cancérologie and Associazione Italiana Oncologia Toracica: Erlotinib *versus* standard chemotherapy as first-line treatment for European patients with advanced *EGFR* mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase III trial. Lancet Oncol *13*: 239-246, 2012.

14 Hirsch FR, Varella-Garcia M, Bunn PA Jr, Di Maria MV, Veve R, Bremmes RM, Barón AE, Zeng C and Franklin WA: Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol *21*: 3798-3807, 2003.

15 Dacic S, Flanagan M, Cieply K, Ramalingam S, Luketich J, Belani C and Yousem SA: Significance of EGFR protein expression and gene amplification in non-small cell lung carcinoma. Am J Clin Pathol *125*: 860-365, 2006.

16 Tanas MR, Goldblum JR: Fluorescence *in situ* hybridization in the diagnosis of soft tissue neoplasms: a review. Adv Anat Pathol *16*: 383-391, 2009.

17 Farshid G, Cheetham G, Davies R, Moore S and Rudzki Z: Validation of the multiplex ligation-dependent probe amplification (MLPA) technique for the determination of *HER2* gene amplification in breast cancer. Diagn Mol Pathol *20*: 11-17, 2011.

18 Minarik M, Gassman M, Belsanova B, Pesek M, Schouten J, Chudoba R, Gas B and Benesova L: A novel high-resolution chipCE assay for rapid detection of *EGFR* gene mutations and amplifications in lung cancer therapy by a combination of fragment analysis, denaturing CE and MLPA. Electrophoresis *31*: 3518-3524, 2010.

19 Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, Haney J, Witta S, Danenberg K, Domenichini I, Ludovini V, Magrini E, Gregorc V, Doglioni C, Sidoni A, Tonato M, Franklin WA, Crino L, Bunn PA Jr. and Varella-Garcia M: Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst *97*: 643-655, 2005.

20 Jeon YK, Sung SW, Chung JH, Park WS, Seo JW, Kim CW and Chung DH: Clinicopathologic features and prognostic implications of epidermal growth factor receptor (*EGFR*) gene copynumber and protein expression in non-small cell lung cancer. Lung Cancer *54*: 387-398, 2006.

21 Lee HJ, Xu X, Choe G, Chung DH, Seo JW, Lee JH, Lee CT, Jheon S, Sung SW and Chung JH: Protein overexpression and gene amplification of epidermal growth factor receptor in nonsmall cell lung carcinomas: Comparison of four commercially available antibodies by immunohistochemistry and fluorescence *in situ* hybridization study. Lung Cancer *68*: 375-382, 2010.

22 Hirsch FR, Varella-Garcia M, Bunn PA Jr., Franklin WA, Dziadziuszko R, Thatcher N, Chang A, Parikh P, Pereira JR., Ciuleanu T, von Pawel J, Watkins C, Flannery A, Ellison G, Donald E, Knight L, Parums D, Botwood N, Holloway B: Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. J Clin Oncol *24*: 5034-5042, 2006.

23 Zhu CQ, da Cunha Santos G, Ding K, Sakurada A, Cutz JC, Liu N, Zhang T, Marrano P, Whitehead M, Squire JA, Kamel-Reid S, Seymour L, Shepherd FA, Tsao MS; National Cancer Institute of Canada Clinical Trials Group Study BR.21: Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR. 21. J Clin Oncol *26*: 4268-4275, 2008.

24 Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, Chao TY, Nakagawa K, Chu DT, Saijo N, Duffield EL, Rukazenkov Y, Speake G, Jiang H, Armour AA, To KF, Yang JC and Mok TS: Biomarker analyses and final overall survival results from phase III, randomized, open-label, first-line study of gefitinib *versus* carboplatin/paclitaxel selected patients with advanced non-small-cell lung cancer inAsia (IPASS). J Clin Oncol *29*: 2866-2874, 2011.

Received October 27, 2015
Revised December 1, 2015
Accepted December 4, 2015