The Role of Neuron-specific Enolase (NSE) and Thimidine Kinase (TK) Levels in Prediction of Efficacy of EGFR-TKIs in Patients with Advanced-stage NSCLC

ONDREJ FIALA[1], MILOS PESEK[2], JINDRICH FINEK[1], LUCIE BENESOVA[3], MAREK MINARIK[3], ZBYNEK BORTLICEK[4] and ONDREJ TOPOLCAN[5]

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

Abstract. *Background/Aim: Tumor biomarkers are used for diagnostics and follow-up monitoring of patients with non-small cell lung cancer (NSCLC). We focused on the predictive role of neuron-specific enolase (NSE) and thimidine kinase (TK) in patients with advanced-stage NSCLC treated with epidermal growth factor tyrosine kinase inhibitors (EGFR-TKIs). Patients and Methods: In a total of 163 patients with advanced-stage (IIIB or IV) NSCLC treated with EGFR-TKIs (erlotinib or gefitinib), pre-treatment levels of NSE and TK were measured. Results: We observed significantly shorter progression-free (PFS) and overall survival (OS) in patients with high NSE levels (p=0.002; p=0.003) and also in those with high TK levels (p=0.026; p=0.020). The multivariate Cox proportional hazards model confirmed that high NSE is a strong independent predictive factor for short PFS (hazard ratio; HR=2.36; p=0.003). Conclusion: High pre-treatment serum levels of NSE is an independent biomarker predicting poor outcome of patients with NSCLC treated with EGFR-TKIs.*

Lung cancer is the most common cause of cancer-related deaths worldwide (1). Non-small cell lung cancer (NSCLC) is the most frequent histological type of lung cancer, representing approximately 85% of cases (2). Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) are novel effective agents used for the treatment of locally-advanced or metastatic-stage NSCLC. Erlotinib and gefitinib are orally-administered low-molecular weight EGFR-TKIs. Randomised phase III clinical trials demonstrated efficacy and safety of erlotinib and gefitinib in the treatment of patients with advanced-stage NSCLC (3-7). The aim of our study was to evaluate the predictive role of pretreatment serum levels of neuron specific enolase (NSE) and thimidine kinase (TK) in patients with advanced-stage NSCLC treated with EGFR-TKIs.

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

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Patients and Methods

Patients and treatment. We analysed clinical data of 163 patients with cytologically or histologically confirmed locally-advanced (IIIB) or metastatic stage (IV) NSCLC treated with erlotinib or gefitinib. Patients were treated between 2003 and 2013. 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 toxic effects. Dose interruption or reduction was permitted in the event of treatment-related toxicity.

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

Tumor marker measurement. Serum samples for measurement of tumor markers were collected within one month before EGFR-TKI treatment. Serum levels of NSE were measured using a immunoradiometric titration method (IRMA) (Beckman-Immunotech, city, county, USA). Serum levels of TK were measured using radioenzymatic assay (REA) (Beckman-Immunotech). The measurement was performed in the Central Immunoanalytic Laboratory at the Department of Nuclear Medicine,

Table I. *Baseline patient characteristics*.

Overall survival (OS)	n	Median OS (95% CI)	6-months survival	1-year survival	Log-rank
Neuron-specific enolase (NSE)					
<12.5 μg/l	132 (88.0)	11.6 months (7.4; 15.9)	71.1 (63.1; 79.1)	49.5 (40.1; 58.9)	0.003
≥12.5 μg/l	18 (12.0)	3.7 months (3.2; 4.2)	25.3 (1.7; 48.8)	25.3 (1.7; 48.8)	
Thimidine kinase (TK)					
<8 U/l	65 (39.9)	17.4 months (5.1; 29.7)	77.8 (67.5; 88.1)	60.9 (50.7; 71.2)	0.02
≥8 U/l	98 (60.1)	8.5 months (4.1; 12.9)	58.0 (45.3; 70.7)	43.2 (32.0; 54.4)	
Progression-free survival (PFS)	**n**	**Median PFS (95% CI)**	**3-months survival (%; 95% CI)**	**6-months survival (%; 95% CI)**	**Log-rank *p*-Value**
NSE					
<12.5 μg/l	132 (88.0)	2.6 months (1.8; 3.4)	45.4 (36.8; 54.0)	23.2 (15.8; 30.7)	0.002
≥12.5 μg/l	18 (12.0)	1.1 months (0.8; 1.3)	11.1 (0.1; 25.6)	5.6 (0.1; 16.1)	
TK					
<8 U/l	65 (39.9)	2.9 months (0.9; 5.0)	48.9 (36.7; 61.2)	30.0 (18.8; 41.2)	0.026
≥8 U/l	98 (60.1)	2.1 months (1.7; 2.6)	35.5 (25.8; 45.1)	16.3 (8.7; 24.0)	

using the following cut-off values: NSE: 12.5 μg/l and TK: 8 IU/l Statistical analysis. Standard summary statistics were used to describe the sample data set. PFS and OS were calculated using the Kaplan Meier method and all point estimates were accompanied by 95% confidence intervals (CI). Statistical significance of the differences in Kaplan-Meier estimates was assessed using the log-rank test. Multivariate Cox proportional hazards model (hazard ratio; HR) was used to evaluate influence of all potential predictive and prognostic factors on the survival measures $p=0.05$ was used as a level of statistical significance.

EGFR mutation analysis. The tumor specimens acquired during initial bronschoscopy 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* genes. 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 (PCR) 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 the JetQuick Tissue DNA Issolation Kit (Genomed GmbH, Loehne, Germany). Mutations in exons 19 and 21 of the *EGFR* gene were tested by the Genoscan mutation detection kits (Genomac International, Prague, Czech Republic) utilizing a denaturing capillary electrophoresis (DCE) 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.

Results

Patients' characteristics. The study included 163 patients. The median age was 64 years (range 28-88 years). Ninety-nine (60.7%) patients were male, 66 (40.5%) had a positive smoking history, 85 (52.1%) had adenocarcinoma, 138 (84.7%) had stage IV disease at EGFR-TKI treatment initiation, 90 (55.2%) had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1 and 118 (72.4%) patients had received at least one previous chemotherapy regimen. One hundred and forty-seven (90.2%) patients were treated with erlotinib and 16 (9.8%) patients were treated with gefitinib. Ninety-three patients were tested for activating *EGFR* mutation, 77 (82.8%) of them were wild-type *EGFR* and 16 (17.2%) were *EGFR* mutation-positive. The baseline patients' characteristics are summarized in Table I.

Pre-treatment levels of NSE and TK. Before the beginning of EGFR-TKI treatment, a high serum level of NSE (≥12.5 μg/l) was measured in 18 (12.0%) patients and a low serum level of NSE (<12.5 μg/l) was measured in 132 (88.0%) patients; a high serum level of TK (≥8 U/I) was measured in 98 (60.1%) patients and a low serum level of TK (<8 U/I) was measured in 65 (39.9%) patients.

Relation between NSE and TK levels and survival. The median PFS and OS for patients with high NSE was 1.1 and

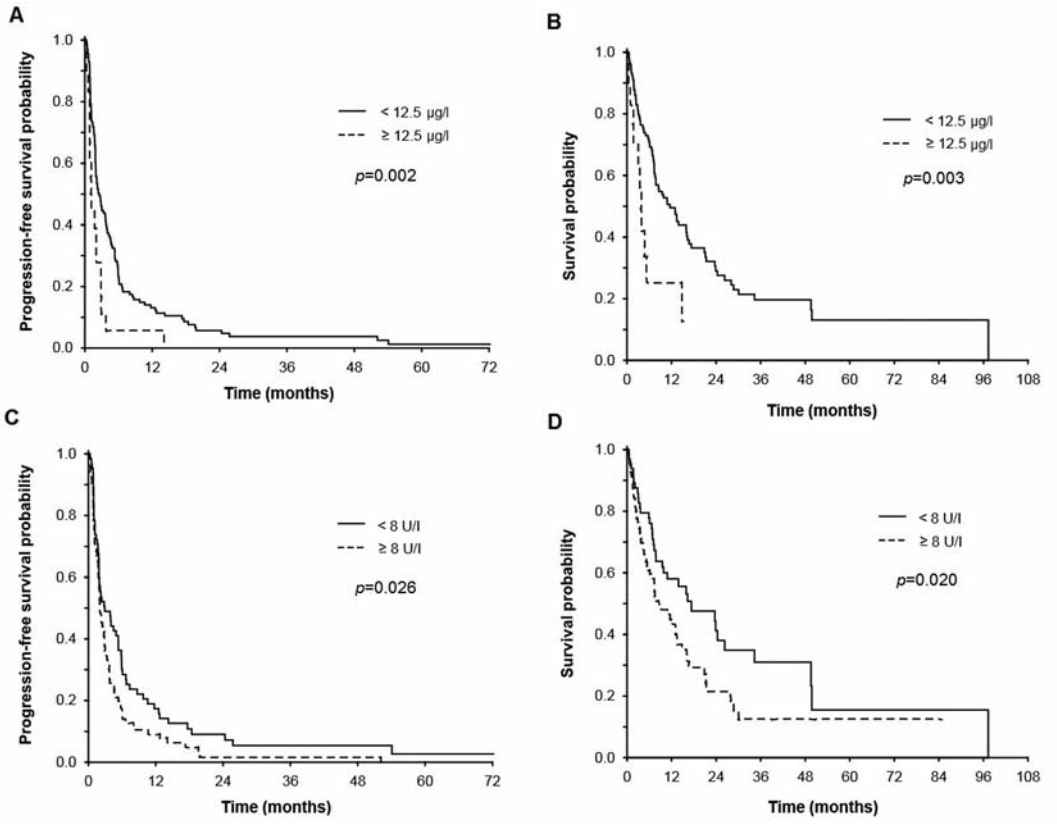

Figure 1. *Kaplan-Meier plots showing progression-free (PFS) and overall (OS) survival according to pretreatment levels of NSE (A, B) and TK (C, D).*

3.7 compared to 2.6 and 11.6 months for patients with low NSE (p=0.002 and p=0.003) (Figure 1A, B). The median PFS and OS for patients with high TK was 2.1 and 8.5 compared to 2.9 and 17.4 months for patients with low TK (p=0.026 and p=0.020) (Figure 1C, D). The PFS and OS data are summarized in Table II. The multivariate Cox proportional hazards model revealed that the *EGFR* mutation status (HR=0.31; CI: 0.16-0.61; p=0.001) and pre-treatment levels of NSE (HR=2.36; CI: 1.34-4.17; p=0.003) were significant independent predictive factors for PFS, whereas *EGFR* mutation status (HR=0.40; 0.18-0.90; p=0.028) and PS (HR=1.89; 1.20-2.97; p=0.006) were significant independent predictive factors for OS (Table III).

Discussion

Considerable progress in the field of molecular biology led to identification of several biomarkers predicting for treatment efficacy of EGFR-TKIs. The presence of activating *EGFR* mutations (predominantly exon 19 deletions or a point-mutation in exon 21 termed L858R) is currently the strongest predictor of a good treatment response (8-12) to EGFR-TKIs and patients are selected for first-line treatment according to the presence of activating *EGFR* mutation. On the other hand the majority of NSCLC patients harbor wild-type *EGFR* gene and moreover there is still a large proportion of patients in whom it is not feasible to acquire an adequate tissue for *EGFR* mutation analysis. Therefore, new predictive tools are required. Serum tumor markers NSE and TK have been shown to be promising candidates for the improvement of diagnosis, histological differentiation and staging of lung cancer.

NSE is a glycolytic enzyme largely expressed in neuroendocrine tumors, particularly in small-cell lung cancer (SCLC) (13). Neuroendocrine markers are not commonly expressed in NSCLC, except for a subset of 10-20% of cases with neuroendocrine differentiation (14). In our study, we observed high NSE serum levels in 12% of patients which is consistent with the commonly reported rate of neuroendocrine differentiation in NSCLC (14). Pujol *et al*. have reported that high NSE levels is a negative prognostic factor in a large study including 621 patients (all stages) and similar results were reported by others (15-17). In our study, we observed significantly shorter PFS (1.1 *vs*. 2.6 months; p=0.002) and

Table II. *Progression-free (PFS) and overall (OS) survival data according to pretreatment levels of NSE and TK.*

Parameter	Category	n	Overall survival		Progression-free survival	
			Hazard Ratio (95% CI)	p-Value	Hazard Ratio (95% CI)	p-Value
Gender	Males	94	1.45 (0.88; 2.38)	0.141	1.11 (0.73; 1.68)	0.622
	Females	56				
Age	≥65 years	74	0.84 (0.53; 1.32)	0.446	0.80 (0.55; 1.15)	0.228
	<65 years	76				
Smoking	Current or former smoker	114	0.79 (0.44; 1.44)	0.446	0.77 (0.46; 1.29)	0.318
	Never smoker	36				
Histology	Adenocarcinoma	79	1.42 (0.90; 2.25)	0.135	1.01 (0.69; 1.50)	0.947
	Other	71				
Stage	IV	129	1.92 (0.87; 4.21)	0.105	0.92 (0.55; 1.53)	0.746
	IIIB	21				
Performance status	PS 2 or PS 3	69	1.89 (1.20; 2.97)	0.006	1.16 (0.80; 1.67)	0.435
	PS 0 or PS 1	81				
Line	3rd or higher	36	1.02 (0.61; 1.72)	0.927	0.98 (0.64; 1.50)	0.924
	1st or 2nd	114				
Neuron-specific enolase (NSE)	≥12.5 ng/ml	18	1.90 (0.95; 3.80)	0.071	2.36 (1.34; 4.17)	0.003
	<12.5 ng/ml	132				
Thimidine kinase (TK)	≥8 U/I	90	1.24 (0.78; 1.98)	0.356	1.07 (0.73; 1.56)	0.738
	<8 U/I	60				
Epidermal growth factor receptor (EGFR) mutation	Mutated	16	0.40 (0.18; 0.90)	0.028	0.31 (0.16; 0.61)	0.001
	Wild-type or unknown	134				

also significantly shorter OS (3.7 *vs*. 11.6 months; p=0.003) for patients with high NSE levels compared to those with low NSE levels. The multivariate Cox proportional hazards model confirmed that high NSE is a strong independent predictive factor for short PFS (HR=2.36; CI: 1.34-4.17; p=0.003), but not for OS (HR=1.90; CI: 0.95-3.80; p=0.071). It has been suggested that the expression of neuroendocrine markers could predict for better response to chemotherapy in NSCLC patients (18-20), although that question still seems to be controversial. On the other hand, very little is known about the relation between neuroendocrine markers, particularly NSE, and response to EGFR-TKIs. Wang *et al*. have recently reported that NSE mRNA expression was inversely correlated with sensitivity to gefitinib in NSCLC patients (21). However, in our study we used routine laboratory assessment of *NSE* serum levels and our results are in agreement with those reported by Wang *et al*. According to the results of our study, we suggest that high pretreatment NSE levels predicts *de novo* resistance to EGFR-TKIs in patients with advanced-stage NSCLC.

TK is an enzyme present in most cells, indicating their proliferative characteristics. It has two isoforms, TK I and TK II, different in chemical structure and biological function. TK I is the one most important, commonly used for detection and estimation of prognosis in cancer. TK I appears during cell dividion in the G_1 and S phase while it is absent in resting cells (22). High TK levels have been previously reported as a negative prognostic factor for both SCLC and NSCLC by several authors (23, 24). The relation between TK levels and response to EGFR-TKIs is still unclear. However we observed significantly shorter PFS (2.1 *vs*. 2.9 months; p=0.026) and also significantly shorter OS (8.5 *vs*. 17.4 months; p=0.020) for patients with high TK levels compared to those with low TK levels. The multivariate Cox proportional hazards model did not confirm that high TK is an independent predictive factor for short PFS (HR=1.07; CI: 0.73-1.56; p=0.738) nor for OS (HR=1.24, CI: 0.78-1.98; p=0.356). The results of our study indicate no relation between pretreatment TK levels and response to the treatment with EGFR-TKIs. A high TK level is a negative prognostic factor, even if not independent. The principal limitations of our study are its retrospective design and relatively small number of patients included.

In conclusion, the results of the present study clearly showed that a high pre-treatment level of NSE, as a marker of neuroendocrine differentiation, predicts for poor outcome of patients with advanced-stage NSCLC treated with EGFR-TKIs. Thus, we suggest that the pre-treatment NSE level is a cheap and easily measurable independent predictive biomarker, feasible for the use in the routine clinical practice. This is the first study to show a negative predictive role of high pre-treatment serum NSE levels in NSCLC patients treated with EGFR-TKIs. Further studies should be

Table III. *Multivariate Cox proportional hazards model.*

	Total (n=163)
Gender, n (%)	
Males	99 (60.7)
Females	64 (39.3)
Age (years)	
Median, (min-max)	64 (28-88)
Smoking status, n (%)	
Smoker	66 (40.5)
Former smoker	57 (35.0)
Non smoker	40 (24.5)
Histology, n (%)	
Adenocarcinoma	85 (52.1)
Squamous-cell carcinoma	67 (41.1)
Other	11 (6.7)
Epidermal growth factor receptor (*EGFR*) mutation	
Wild-type	77 (47.2)
Mutated	16 (9.8)
Unknown	70 (42.9)
Treatment	
Erlotinib	147 (90.2)
Gefitinib	16 (9.8)
Stage at treatment initiation, n (%)	
IIIB	25 (15.3)
IV	138 (84.7)
Performance status (PS) at treatment initiation, n (%)	
PS 0	2 (1.2)
PS 1	88 (54.0)
PS 2	67 (41.1)
PS 3	6 (3.7)
Line of treatment	
1st	45 (27.6)
2nd	77 (47.2)
3rd	36 (22.1)
Higher	5 (3.1)

performed to confirm these results. We hope that our findings could have a valuable impact on the treatment of patients with advanced-stage NSCLC in the future.

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 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.

4 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.

5 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.

6 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.

7 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.

8 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.

9 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 3 trial. Lancet Oncol *13*: 239-246, 2012.

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 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 and 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.

13 Cooper EH, Splinter TA, Brown DA, Muers MF, Peake MD and Pearson SL: Evaluation of a radioimmunoassay for neuron specific enolase in small cell lung cancer. Br J Cancer *52*: 333-338, 1985.

14 Travis W: The concept of pulmonary neuroendocrine tumours. In: Pathology and genetics of tumours of the lung, pleura, thymus and heart (Travis W, Brambilla E, Müller-Hermelink H and Harris CC (eds.). Lyon, WHO Press, pp. 19-20, 2004.

15 Pujol JL, Boher JM, Grenier J and Quantin X: Cyfra 21–1, neuron specific enolase and prognosis of non-small cell lung cancer: Prospective study in 621 patients. Lung Cancer *31*: 221-231, 2001.

16 Barlési F, Gimenez C, Torre JP, Doddoli C, Mancini J, Greillier L, Roux F and Kleisbauer JP: Prognostic value of combination of Cyfra 21–1, CEA and NSE in patients with advanced non-small cell lung cancer. Respir Med *98*: 357-362, 2004.

17 Nisman B, Heching N, Biran H, Barak V and Peretz T: The prognostic significance of circulating neuroendocrine markers chromogranin a, pro-gastrin-releasing peptide and neuron-specific enolase in patients with advanced non-small-cell lung cancer. Tumour Biol *27*: 8-16, 2006.

18 van Zandwijk N, Jassem E, Bonfrer JM, Mooi WJ and van Tinteren H: Serum neuron specific enolase and lactate dehydrogenase as predictors of response to chemotherapy and survival in non-small cell lung cancer. Semin Oncol *19*: 37-43, 1992.

19 Zych J, Szturmowicz M, Sakowicz A, Słodkowska J, Załeska M, Radzikowska E, Załeska J, Jodkiewicz Z and Rowińska-Zakrzewska E: Neuron-specific enolase (NSE) serum level as a prognostic factor in non-small cell lung cancer. Pneumonol Alergol Pol *70*: 278-283, 2002.

20 Viñolas N, Molina R, Galán MC, Casas F, Callejas MA, Filella X, Grau JJ, Ballesta AM and Estape J: Tumor markers in response monitoring and prognosis of non-small cell lung cancer: preliminary report. Anticancer Res *18*: 631-634, 1998.

21 Wang Y, Tang D, Sui A, Jiao W, Luo Y, Wang M, Yang R, Wang Z and Shen Y: Prognostic significance of NSE mRNA in advanced NSCLC treated with gefitinib. Clin Transl Oncol *15*: 384-390, 2013.

22 Zhou J, He E and Skog S: The proliferation marker thymidine kinase 1 in clinical use. Mol Clin Oncol *1*: 18-28, 2013.

23 Holdenrieder S, Von Pawel J, Duell T, Feldmann K, Raith H, Schollen A, Nagel D and Stieber P: Clinical relevance of thymidine kinase for the diagnosis, therapy monitoring and prognosis of non-operable lung cancer. Anticancer Res *30*: 1855-1862, 2010.

24 Li HX, Lei DS, Wang XQ, Skog S and He Q: Serum thymidine kinase 1 is a prognostic and monitoring factor in patients with non-small cell lung cancer. Oncol Rep *13*: 145-149, 2005.

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