Predictive Role of CEA and CYFRA 21-1 in Patients with Advanced-stage NSCLC Treated with Erlotinib

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Abstract. *Background: Tumor biomarkers are used for predicting therapy effect and prognosis of patients with non-small cell lung cancer (NSCLC). We focused on their potential role in prediction of the efficacy of erlotinib. Patients and Methods: In a total of 144 patients with advanced-stage (IIIB or IV) NSCLC treated with erlotinib, pre-treatment levels of soluble carcinoembryonic antigen (CEA) and cytokeratin markers in serum were measured. Results: The median progression-free and overall survival for patients with a high level of carcinoembryonic antigen (CEA) was 1.9 and 8.6 vs. 2.9 and 16.1 months for patients with low CEA (p=0.046 and p=0.116). The respective medians for patients with a high level of cytokeratin-19 fragment were 1.9 and 6.1 vs. 3.4 and 23.8 months for patients with the low cytokeratin-19 fragment (p<0.001 and p<0.001). Conclusion: High pre-treatment serum levels of one or both biomarkers are associated with poor outcome of patients with NSCLC treated with erlotinib.*

Non-small cell lung cancer (NSCLC) is the most frequent histological type of lung cancer (1), which is one of the most common human malignant diseases and the leading cause of cancer-related deaths worldwide (2). Targeted-treatment based on tyrozine kinase inhibitors (TKI) directed at epidermal growth factor receptor (EGFR) represents a novel effective tool in management of advanced-stage NSCLC. The aim of our study was to evaluate the predictive role of pre-treatment serum levels of carcinoembryonic antigen (CEA) and cytokeratin-19 fragments (CYFRA 21-1) in patients with advanced-stage NSCLC treated with erlotinib.

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Patients and Methods

Patients' characteristics. The study included 144 patients. The median age was 64 years (range 28-84 years). 85 (59.0%) patients were male, 109 (75.7%) patients had a history of smoking , 73 (50.7%) patients had adenocarcinoma, 121 (84.0%) patients had stage IV disease, 77 (53.5%) patients had ECOG PS 0 or 1 and 113 (78.5%) patients had received at least one previous chemotherapy regimen. A total of 78 patients were tested for activating *EGFR* mutation, 70 of them were wild-type *EGFR* and eight were *EGFR* mutation-positive. The baseline patient characteristics are summarized in Table I.

Study design and treatment. We retrospectively analyzed clinical and laboratory data of patients with cytologically- or histologically-confirmed advanced-stage (stage IIIB or IV) NSCLC treated with erlotinib between 2006 and 2013 at the Department of Pneumology in Pilsen. Erlotinib was administered orally at the standard approved dose of 150 mg daily; dose interruption or reduction was permitted in the event of treatment-related toxicity. The treatment was continued until disease progression or development of intolerable toxic effects.

Clinical monitoring. The treatment was prospectively monitored and the clinical course of patients was continuously assessed at specific time points. Clinical follow-up examinations including physical examination, plain chest X-ray and routine laboratory tests were performed every 3-4 weeks; computed tomography (CT) or positron-emission tomography (PET)-CT was performed after two or three months of treatment with erlotinib. The objective tumor response was assessed by investigators in terms of complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) using Response Evaluation Criteria in Solid Tumours (RECIST) (3). The disease control rate (DCR) was defined as the sum of CR, PR and SD. Progression-free survival (PFS) was determined from the date of erlotinib initiation until the date of first documented progression or death. Overall survival (OS) was determined from the date of erlotinib initiation until the date of death.

Tumor marker assessment. Serum samples for measurement of tumor markers were collected within one month before erlotinib treatment. Serum levels of CEA were measured using chemiluminiscent method on an DXI 800i analyzer (Beckman, USA). Serum levels of CYFRA 21-1 were measured using immunoradiometric titration method (IRMA) (Beckman-Immunotech, USA). The measurement was performed in Central Immunoanalytic Laboratory at Department of Nuclear Medicine, using following cut-off values: CEA: 3 μg/l and CYFRA 21-1: 2.5 μg/l.

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, 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 (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.

Statistics. Standard frequency tables and descriptive statistics were used to characterize sample data set. The significance of differences between baseline characteristics, as well as treatment response, and level of tumor markers, was determined using the Fisher's exact test. PFS and OS were calculated 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. Univariate and multivariate Cox proportional hazards model was used to evaluate influence of all potential predictive and prognostic factors on the survival measures. Based on univariate results, variables with the greatest effect on survival measures (EGFR mutation status, stage and PS) were involved in multivariate Cox model to adjust results in terms of tumor markers. As a level of statistical significance, $p=0.05$ was used.

Table I. *Baseline patients' characteristics*.

Patient characteristics	Total (n=144)
Gender, n (%)	
Male	85 (59.0)
Female	59 (41.0)
Age (years)	
Median (5-95%)	64 (28-84)
Smoking history, n (%)	
Current smoker	60 (41.7)
Former smoker	49 (34.0)
Never smoker	35 (24.3)
Histology, n (%)	
Adenocarcinoma	73 (50.7)
Squamous-cell carcinoma	61 (42.4)
Other	10 (6.9)
EGFR mutation status, n (%)	
Wild-type	70 (48.6)
Activating mutation	8 (5.6)
Unknown	66 (45.8)
Stage, n (%)	
IIIB	23 (16.0)
IV	121 (84.0)
Performance status, n (%)	
PS 0	2 (1.4)
PS 1	75 (52.1)
PS 2	61 (42.4)
PS 3	6 (4.2)
Prior chemotherapy regimens, n (%)	
None	31 (21.5)
One	72 (50.0)
Two	35 (24.3)
More	6 (4.2)

Results

CEA and CYFRA 21-1 levels before the treatment. Before the begining of erlotinib treatment, high CEA (≥3 μg/l) was measured in 99 (68.8%) patients, high CYFRA 21-1 (≥2.5 μg/l) was measured in 83 (57.6%) patients and high CEA and CYFRA 21-1 were measured in 59 (41%) patients. Adenocarcinoma histology was significantly associated with high CEA levels ($p=0.019$); PS 2 or 3 was significantly associated with high CYFRA 21-1 levels ($p=0.002$) and and stage IV was significantly associated with both high CEA ($p=0.026$) and high CYFRA 21-1 ($p=0.021$) (data not shown).

Relation between CEA and CYFRA 21-1 levels and treatment efficacy. For patients with high CEA the DCR was 51.5% compared to 75.6% for patients with low CEA ($p=0.01$). For patients with high CYFRA 21-1 the DCR was 45.8% compared to 77.0% for patients with low CYFRA 21-1 ($p<0.001$). For patients with high CEA the median PFS and OS was 1.9 and 8.6 compared to 2.9 and 16.1 months for patiens with low CEA ($p=0.046$ and $p=0.116$) (Figure 1A, B). For patients with high CYFRA 21-1 the median PFS and OS was 1.9 and 6.1 compared to 3.4 and 23.8 months for patiens with low CYFRA 21-1 ($p<0.001$ and $p<0.001$) (Figure 1C, D). The univariate Cox proportional hazards model revealed that *EGFR* mutation status (HR=0.20, $p=0.001$), CEA (HR=1.44, $p=0.049$) and CYFRA 21-1

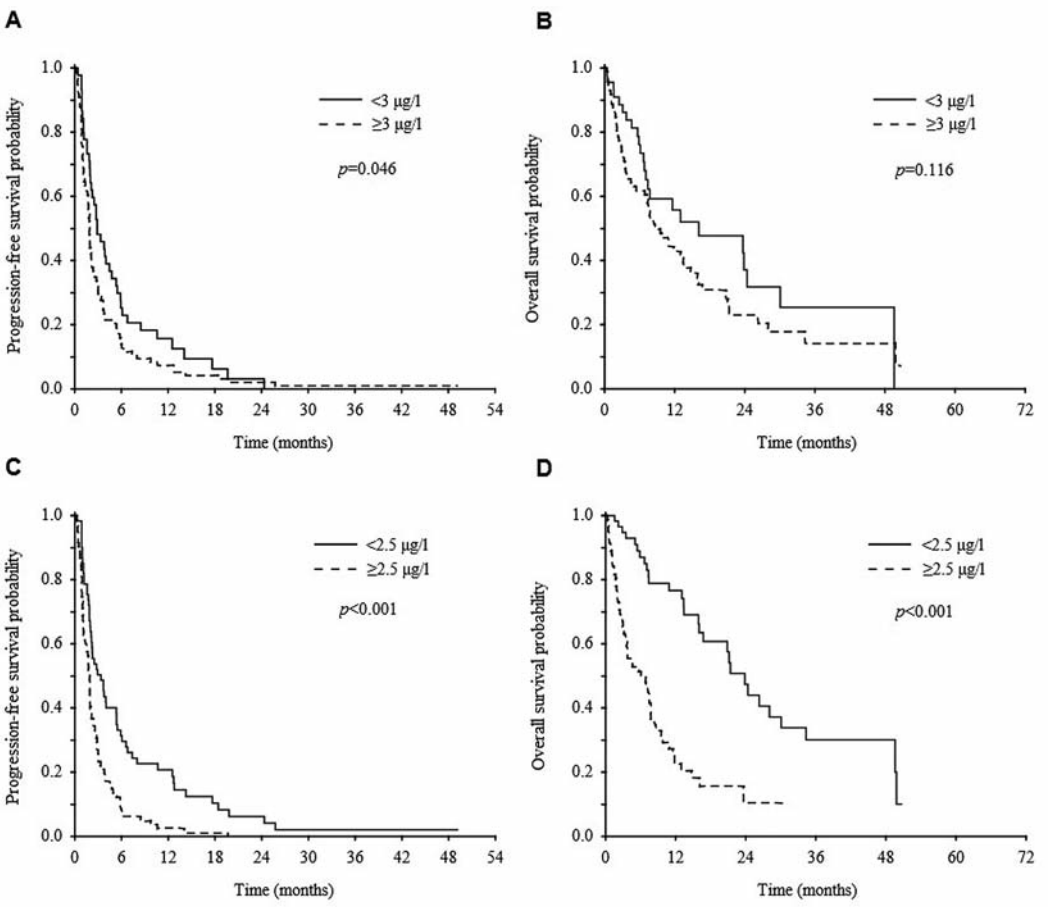

Figure 1. *Kaplan Meier plots showing the comparison of progression-free survival (PFS) and overall survival (OS) according to CEA (A, B) and CYFRA 21-1 (C, D) levels.*

(HR=2.06, $p<0.001$) were significant factors for PFS, whereas *EGFR* mutation status (HR=0.25, $p=0.019$), stage (HR=3.36, $p=0.004$,); PS (HR=2.36, $p<0.001$) and CYFRA 21-1 (HR=3.73, $p<0.001$) were significant factors for OS (Table II). Finally the multivariate Cox proportional hazards model revealed that *EGFR* mutation status (HR=0.21, $p=0.001$), CEA (HR=1.72, $p=0.007$) and CYFRA 21-1 (HR=2.17, $p<0.001$) were independent predictive factors for PFS, whereas *EGFR* mutation status (HR=0.30, $p=0.044$), and CYFRA 21-1 (HR=2.74, $p<0.001$) were independent predictive factors for OS (Table III).

Discussion

Erlotinib is a low-molecular EGFR-TKI approved for the treatment of patients with locally-advanced or metastatic NSCLC. It has been proven that EGFR-TKIs are highly efficient particularly in patients harboring activating *EGFR* mutations, occuring approximately in 5-20% of patients (4-7). Activating *EGFR* mutations are the strongest predictor of efficacy of EGFR-TKIs, however the majority of NSCLC patiens harbor a wild-type *EGFR* gene. Moreover there is still a large proportion of patients in whom it is not feasible to acquire an adequate tissue for *EGFR* mutation analysis. Hence looking for other predictive biomarkers is required. Both CEA and CYFRA 21-1 are serum tumor markers commonly used for diagnostics and follow-up monitoring of patients with NSCLC. In the present study we focused on their potential role in prediction of efficacy of erlotinib in patients with advanced-stage NSCLC.

CEA is thought to play a role in cell-to-cell adhesion and has a dominant effect in blocking cell differentiation (8) and it cooperates with Myc and Bcl-2 in cellular tranformation (9). Additionally, it can also inhibit cell death induced by a loss of anchorage to the extracellular matrix (anoikis) (10). High CEA levels have been described as a significant marker of poor prognosis in patients with NSCLC regardless of treatment (11-13). In our study, we observed significantly lower DCR (51.5%

Table II. *Univariate Cox proportional hazards model.*

Parameter	Category	n	OS		PFS	
			HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Gender	Male	85	1.40 (0.91; 2.15)	0.131	1.37 (0.97-1.92)	0.075
	Female	59				
Age	≥65 years	68	0.94 (0.61-1.43)	0.759	0.73 (0.52-1.03)	0.074
	<65 years	76				
Smoking history	Current/former	109	1.10 (0.68-1.79)	0.696	1.36 (0.9-2.06)	0.147
	Never	35				
Histology	Adenocarcinoma	73	1.26 (0.82-1.93)	0.292	0.91 (0.65-1.29)	0.607
	Other	71				
Stage	IV	121	3.36 (1.46-7.71)	0.004	1.26 (0.79-2.02)	0.323
	IIIB	23				
PS	PS 2/3	67	2.36 (1.53-3.65)	<0.001	1.18 (0.85-1.66)	0.326
	PS 0/1	77				
Previous chemotherapy	Three/more	41	1.03 (0.65-1.63)	0.902	1.20 (0.83-1.74)	0.341
	One/two	103				
CEA	≥3 μg/l	99	1.46 (0.91-2.33)	0.119	1.44 (1.00-2.08)	0.049
	<3 μg/l	45				
CYFRA	≥2.5 μg/l	83	3.73 (2.30-6.07)	<0.001	2.06 (1.45-2.95)	<0.001
	<2.5 μg/l	61				
EGFR mutation status	Mutation	8	0.25 (0.08-0.80)	0.019	0.20 (0.08-0.50)	0.001
	Wild-type/unknown	136				

Table III. *Multivariate Cox proportional hazards model.*

Parameter	Category	OS		PFS	
		HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
CEA	≥3 μg/l	1.38 (0.85-2.24)	0.200	1.72 (1.16-2.56)	0.007
	<3 μg/l				
CYFRA	≥2.5 μg/l	2.74 (1.63-4.61)	<0.001	2.17 (1.48-3.19)	<0.001
	<2.5 μg/l				
EGFR mutation status	Mutant	0.30 (0.09-0.97)	0.044	0.21 (0.08-0.53)	0.001
	Wild-type/unknown				

Only statistically significant ($p \leq 0.05$) values are shown.

vs. 75.6%; $p=0.010$) and shorter PFS (1.9 *vs*. 2.9 months; $p=0.046$) for patients with high CEA levels compared to those with low CEA levels. The multivariate Cox proportional hazards model confirmed that high CEA is an independent predictive factor for short PFS (HR=1.72, $p=0.007$). On the contrary, Okamoto *et al*. and Jung *et al*. have recently reported that high CEA levels predict a good outcome of advanced-stage NSCLC patients treated with EGFR-TKIs (14, 15). Okamoto *et al*. hypothesized that up-regulated expression of the anti-apoptotic protein CEA could be caused by the aberrant activation of mutated EGFR *via* activation of downstream molecules such as Akt and STAT3/5 which play crucial role in the anti-apoptotic patways (14). The hypothesis is in accordance with findings reported by Shoji *et al*. who has shown an association between high CEA and presence of activating *EGFR* mutations (16). In our study, no association with the presence of activating *EGFR* mutations was observed. The reason why the results of our study did not confirm the previously-published notion could be addressed to the different study populations, while both studies by Okamoto *et al*. and Jung *et al*. included Asians compared to our study including Caucasians. There is a notable difference in the presence of various driving mutations between Asians and Caucasians. Activating *EGFR* mutations predicting high efficacy of EGFR-TKIs (4-7) are frequently found in Asians whereas rarely in Caucasians (17). On the other hand, *KRAS* and *BRAF* mutations conferring resistance to EGFR-TKIs (18-20) are more

frequently found in Caucasians whereas rarely in Asians (17, 21, 22). In accordance with the hypothesis by Okamoto mentioned above, we hypothesize that up-regulated expression of CEA also could be caused by the aberrant activation of several molecules downstream *EGFR*, such as *KRAS* and *BRAF* mutations, respectively. Thus high CEA in Caucasians could be associated predominantly with *KRAS* and *BRAF* mutations resulting in a poor outcome of patients treated with erlotinib.

CYFRA 21-1, a fragment of cytokeratin subunit 19 has been previously extensively evaluated in the setting of NSCLC. Various studies has shown an unfavourable prognosis of patients with high CYFRA 21-1 levels regardless of treatment (23-25). In our study, we observed lower DCR (45.8% *vs*. 77.0%, $p<0.001$), shorter PFS (1.9 *vs*. 3.4 months, $p<0.001$) and also shorter OS (6.1 *vs*. 23.8 months, $p<0.001$) for patients with high CYFRA 21-1 levels compared to those with low CYFRA 21-1 levels. The results were confirmed by multivariate Cox proportional hazards model (PFS: HR=2.17, $p<0.001$; OS: HR=2.74, $p<0.001$). Our findings clearly confirmed results of several previously published studies showing negative predictive role of high CYFRA 21-1 levels in NSCLC patients treated with EGFR-TKIs (15, 26, 27).

In conclusion, this is the first study focusing on the predictive role of pre-treatment levels of CEA and CYFRA 21-1 in the Caucasian population. CEA and CYFRA 21-1 are commonly used serum tumor markers which are simple and easy to detect. We observed that high pre-treatment serum levels of CEA and/or CYFRA 21-1 are associated with poor outcome of NSCLC patients treated with erlotinib. Based on the present study results, one can say that pre-treatment serum levels of CEA and CYFRA 21-1 are feasible diagnostic and also predictive tools in patients with advanced-stage NSCLC. Futher research is required to elucidate the different predictive role of CEA between Asian and Caucasian populations.

Conflicts of Interest

All Authors 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.

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References

1 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.

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

3 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumours. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst *3*: 205-216, 2000.

4 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.

5 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.

6 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 and 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.

7 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.

8 Benchimol S, Fuks A, Jothy S, Beauchemin N, Shirota K and Stanners CP: Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell *57*: 327-334, 1989.

9 Screaton RA, Penn LZ, Stanners CP. Carcinoembryonic antigen, a human tumor marker, cooperates with Myc and Bcl-2 in cellular transformation. J Cell Biol *137*: 939-952, 1997.

10 Ordoñez C, Screaton RA, Ilantzis C and Stanners CP: Human carcinoembryonic antigen functions as a general inhibitor of anoikis. Cancer Res *60*: 3419-3424, 2000.

11 Okada M, Nishio W, Sakamoto T, Uchino K, Yuki T, Nakagawa A and Tsubota N: Prognostic significance of perioperative serum carcinoembryonic antigen in non-small cell lung cancer: analysis of 1,000 consecutive resections for clinical stage I disease. Ann Thorac Surg *78*: 216-221, 2004.

12 Matsuoka K, Sumitomo S, Nakashima N, Nakajima D and Misaki N: Prognostic value of carcinoembryonic antigen and CYFRA21-1 in patients with pathological stage I non-small cell lung cancer. Eur J Cardiothorac Surg *32*: 435-439, 2007.

13 Concannon JP, Dalbow MH, Davis W, Hodgson SE, Mitchell J and Markopoulos E: Immunoprofile studies for patients with bronchogenic carcinoma--III. Multivariate analysis of immune tests in correlation with survival. Int J Radiat Oncol Biol Phys *4*: 255-261, 1978.

14 Okamoto T, Nakamura T, Ikeda J, Maruyama R, Shoji F, Miyake T, Wataya H and Ichinose Y: Serum carcinoembryonic antigen as a predictive marker for sensitivity to gefitinib in advanced non-small cell lung cancer. Eur J Cancer *41*: 1286-1290, 2005.

15 Jung M, Kim SH, Lee YJ, Hong S, Kang YA, Kim SK, Chang J, Rha SY, Kim JH, Kim DJ and Cho BC: Prognostic and predictive value of CEA and CYFRA 21-1 levels in advanced non-small cell lung cancer patients treated with gefitinib or erlotinib. Exp Ther Med *2*: 685-693, 2011.

16 Shoji F, Yoshino I, Yano T, Kometani T, Ohba T, Kouso H, Takenaka T, Miura N, Okazaki H and Maehara Y: Serum carcinoembryonic antigen level is associated with epidermal growth factor receptor mutations in recurrent lung adenocarcinomas. Cancer *110*: 2793-2798, 2008.

17 Dearden S, Stevens J, Wu YL and Blowers D: Mutation incidence and coincidence in non small-cell lung cancer: meta-analyses by ethnicity and histology (mutMap). Ann Oncol *24*: 2371-2376, 2013.

18 Massarelli E, Varella-Garcia M, Tang X, Xavier AC, Ozburn NC, Liu DD, Bekele BN, Herbst RS and Wistuba II: KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. Clin Cancer Res *13*: 2890-2896, 2005.

19 Baselga J and Rosen N: Determinants of RASistance to anti-epidermal growth factor receptor agents. J Clin Oncol *26*: 1582-1584, 2008.

20 Pratilas CA, Hanrahan AJ, Halilovic E, Persaud Y, Soh J, Chitale D, Shigematsu H, Yamamoto H, Sawai A, Janakiraman M, Taylor BS, Pao W, Toyooka S, Ladanyi M, Gazdar A, Rosen N and Solit DB: Genetic predictors of MEK dependence in non-small cell lung cancer. Cancer Res *68*: 9375-9383, 2008.

21 Paik PK, Arcila ME, Fara M, Sima CS, Miller VA, Kris MG, Ladanyi M and Riely GJ: Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. J Clin Oncol *29*: 2046-2051, 2011.

22 Sasaki H, Shitara M, Yokota K, Okuda K, Hikosaka Y, Moriyama S, Yano M and Fujii Y: Braf and erbB2 mutations correlate with smoking status in lung cancer patients. Exp Ther Med *3*: 771-775, 2012.

23 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.

24 Pujol JL, Molinier O, Ebert W, Daurès JP, Barlesi F, Buccheri G, Paesmans M, Quoix E, Moro-Sibilot D, Szturmowicz M, Bréchot JM, Muley T and Grenier J: CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: results of a meta-analysis in 2063 patients. Br J Cancer *90*: 2097-2105, 2004.

25 Edelman MJ, Hodgson L, Rosenblatt PY, Christenson RH, Vokes EE, Wang X, Kratzke R: CYFRA 21-1 as a prognostic and predictive marker in advanced non-small-cell lung cancer in a prospective trial: CALGB 150304. J Thorac Oncol *7*: 649-654, 2012..

26 Barlési F, Tchouhadjian C, Doddoli C, Torre JP, Astoul P and Kleisbauer JP: CYFRA 21-1 level predicts survival in non-small-cell lung cancer patients receiving gefitinib as third-line therapy. Br J Cancer *92*: 13-14, 2005.

27 Chen F, Luo X, Zhang J, Lu Y and Luo R: Elevated serum levels of TPS and CYFRA 21-1 predict poor prognosis in advanced non-small-cell lung cancer patients treated with gefitinib. Med Oncol *27*: 950-957, 2010.

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