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Carcinoembryonic antigen-related cell adhesion molecules as surrogate markers for EGFR inhibitor sensitivity in human lung adenocarcinoma

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BACKGROUND: Lung adenocarcinoma (LADCA) patients with epidermal growth factor receptor (EGFR) mutations are in general associated with relatively high clinical response rate to EGFR-tyrosine kinase inhibitors (TKIs) but not all responded to TKI. It has therefore become important to identify the additional surrogate markers regarding EGFR-TKI sensitivity.

METHODS: We first examined the effects of EGFR-TKIs, gefitinib and erlotinib, upon cell proliferation of lung adenocarcinoma cell lines. We then evaluated the gene profiles related to EGFR-TKI sensitivity using a microarray analysis. Results of microarray analysis led us to focus on carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family, CEACAM 3, 5, 6, 7, and 19, as potential further surrogate markers of EGFR-TKI sensitivity. We then examined the correlation between the status of CEACAM 3, 5, 6, 7, and 19 immunoreactivity in LADCA and clinicopathological parameters of individual cases.

RESULTS: In the cases with EGFR mutations, the status of all CEACAMs examined was significantly higher than that in EGFR wild-type patients, but there were no significant differences in the status of CEACAMs between TKI responder and nonresponder among 22 patients who received gefitinib therapy. However, among 115 EGFR mutation-negative LADCA patients, both CEACAM6 and CEACAM3 were significantly associated with adverse clinical outcome (CEACAM6) and better clinical outcome (CEACAM3).

CONCLUSION: CEACAMs examined in this study could be related to the presence of EGFR mutation in adenocarcinoma cells but not represent the effective surrogate marker of EGFR-TKI in LADCA patients. However, immunohistochemical evaluation of CEACAM3/6 in LADCA patients could provide important information on their clinical outcome.

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Epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) has been reported to provide therapeutic benefits to NSCLC patients associated with EGFR gene mutations (Lynch *et al*, 2004; Paez *et al*, 2004) and also to female and nonsmoker patients (Thatcher *et al*, 2005). The response rate to EGFR-TKI among EGFR gene mutation-positive NSCLC patients has been reported as >70% and progression-free survival (PFS) as 9 to 10 months (Asahina *et al*, 2006; Inoue *et al*, 2006). Gefitinib did demonstrate a therapeutic effectiveness at least equivalent to docetaxel as the second-line chemotherapy in these patients with EGFR gene mutations (Niho *et al*, 2007). In addition, EGFR-TKI as the first-line therapy was reported to have extended the PFS of the EGFR mutation-positive lung cancer cases more significantly than the conventional chemotherapy (Mok *et al*, 2009; Maemondo *et al*, 2010). Erlotinib has also been reported to demonstrate a potential therapeutic benefit to the gefitinib-resistant EGFR mutation-positive lung cancer patients (Cho *et al*, 2007).

It has then become important to evaluate the potential surrogate markers of these EGFR-TKI agents in addition to the presence or absence of EGFR mutation(s) in order to increase the response rate to these agents. The first potential surrogate marker for primary resistance to EGFR-TKI reported in the literature was KRAS mutations in the EGFR mutations-negative cases (Shigematsu *et al*, 2005). Acquired clinical resistance to EGFR-TKI was also documented in lung cancer patients, who had an EGFR mutation in exon 20 (T790M) (Bell *et al*, 2005). In addition, the resistance to gefitinib and erlotinib in NSCLC cell lines was reported to be associated with epithelial-to-mesenchymal transition (EMT) of these cell lines examined (Thomson *et al*, 2005; Yauch *et al*, 2005; Witta *et al*, 2006). Therefore, in this study, we first examined the

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effects of gefitinib or erlotinib on cell proliferation of the cell lines including those originated from lung adenocarcinoma (LADCA). We then evaluated gene profiles of EGFR-TKI-sensitive cells using a microarray analysis in order to further characterise the possible differential mRNA expression patterns among EGFR-TKI-sensitive cells. These results of microarray analysis led us to focus on carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family including carcinoembryonic antigen (CEA) as a potential surrogate marker of EGFR-TKI sensitivity. However, it is also true that the biological or clinical significance of CEACAM family expression including CEA in NSCLC has not necessarily been well characterised. Therefore, we also examined the relationship between the expression of CEACAM family and clinicopathological factors including patient outcome, EGFR mutation, and EGFR-TKI response in human LADCA cases in our present study.

MATERIALS AND METHODS

Cell lines

In this study, we used the following cell lines: A549, LCSC#1, RERF-LC-OK, LK87, and LCAM1. The original tissues, sources, and medium employed in these cell lines above are summarised in Supplementary Table S1. EGFR mutations in exons 18, 19, 20, and 21, which confer sensitivity to EGFR-TKI, were identified by the PCR-Invader assay (BML, Inc., Tokyo, Japan). Cells were maintained in each medium supplemented with 10% fetal bovine serum (Nichirei Co. Ltd, Tokyo, Japan). All the cells were maintained in culture at 37 °C, 95% relative humidity, and 5% CO_2 at room air.

EGFR-TKI sensitivity test

Gefitinib was commercially obtained from Biaffin GmbH (Kassel, Germany). Erlotinib was kindly provided by Roche Diagnostics GmbH (Mannheim, Germany). Each cell lines above were cultured in a 96-well culture plate. At 72 h after gefitinib or erlotinib treatment, the cell number was evaluated using a Cell Counting Kit (DOJINDO LABORATORIES, Kumamoto, Japan) (Isobe *et al*, 1999). Then, 10 μl of 5 mM WST-8 was added to these cells, which were then incubated for 2 h at 37 °C. Optical densities (OD, 450 nm) were obtained with microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The status of cell proliferation (%) was calculated according to the following equation: (cell OD value after test materials treated/vehicle control cell OD value) × 100.

Microarray analysis

Cell lysates were prepared using RLT buffer (QIAGEN GmbH, Hilden, Germany). Total RNA was extracted using RNeasy Mini Kit (QIAGEN). First-strand cDNA was synthesised by incubating 5 μg of total RNA with 200 U SuperScript II reverse transcriptase (Invitrogen Corporation, Carlsbad, CA, USA) and 100 pmol T7-(dT)24 primer (Invitrogen). Ten units of T4 DNA polymerase (Invitrogen) were then added, and the dsDNA was mixed with T7 RNA polymerase (Invitrogen). The purified cRNA was fragmented at 300–500 bp as target solution. Both test and reference samples were labelled with cyanine-5 (Cy5)-labelled CTP (PerkinElmer Inc., Waltham, MA, USA). The Cy5-labelled cRNA probes were subsequently hybridised on the Human 1A version 2.0 (Agilent Technologies, Inc., Santa Clara, CA, USA) including 22 000 genes. The reacted arrays were then scanned as digital image files with GenePix 4000A (Axon Instruments, Foster City, CA, USA). Results were extracted using Agilent Feature Extraction software version 9.5.3.1 (Agilent Technologies) and analysed using Gene Spring GX 7.3.1 software (Agilent Technologies) in order to obtain gene expression ratios. Raw microarray data were normalised and analysed using the Gene Spring GX 7.3.1 software (Agilent Technologies). Expression data were median centred.

Patients and tissue specimens

A total of 165 specimens of LADCA were obtained from the patients who underwent surgical resection from 2000 to 2006 in the Department of Surgery, Tohoku University Hospital and Miyagi Cancer Center. Clinicopathological features of the cases examined in this study are summarised in Supplementary Tables S2 and S3. A total of 115 cases were EGFR mutation-negative cases and had not received chemotherapy at all. Of the 165 LADCA patients, 50 were known to have EGFR mutations (exon 19 deletion, $n=28$; exon 21 point mutation, $n=22$; Supplementary Table S3). Among 50 LADCA cases, the response of gefitinib treatment was evaluated in 22 cases (responder (PR), $n=15$; nonresponder (SD), $n=7$; Supplementary Table S3). Time to progression was available in 17 out of these 22 cases who received gefitinib treatment, and hence 5 cases whom we lost afterward were treated as censored cases. Other EGFR mutation-positive 28 cases did not receive gefitinib treatment or no recurrence in their clinical course. All the specimens studied had been fixed in 10% formalin and embedded in paraffin wax. Research protocols for this study were approved by the Ethics Committee at Tohoku University School of Medicine (2009-380) and Miyagi Cancer Center (No. 34), respectively.

Immunohistochemistry

Primary antibodies used in this study were as follows: CEA/CEACAM5 (monoclonal CEM010; 1:1500 dilution; Mochida Pharmaceutical Co., Ltd, Tokyo, Japan), CEACAM6 (polyclonal, 1:200 dilution; Aviva Systems Biology, Corp., San Diego, CA, USA), CEACAM3 (polyclonal, 1:200 dilution; Sigma-Aldrich Corporation, St Louis, MO, USA), CEACAM7 (monoclonal BAC2, 1:200 dilution; Abcam plc, Cambridge, UK), and CEACAM19 (monoclonal HY-8H10, 1:300 dilution; Abcam). Streptavidin-biotin amplification method was employed for immunostaining using a Histofine Kit (Nichirei). The antigen–antibody complex was subsequently visualised with 3,3'-diaminobenzidine solution and counterstained with haematoxylin.

Evaluation of CEACAM immunohistochemistry was performed based on the staining proportion scoring systems used for CEACAM1 immunohistochemistry (Sienel *et al*, 2003; Dango *et al*, 2008) with some modifications. Immunoreactivity was examined independently by two of the authors (MK and YM) who were unaware of the clinical data. CEACAM immunoreactivity of tumour cells was compared with that in normal lung epithelial cells that were negative for immunoreactivity. CEACAM-positive rate was categorised according to the percentage of positive tumour cells into 'negative' (<40% positive carcinoma cells) and 'positive' (≥40% positive carcinoma cells) (Sienel *et al*, 2003; Dango *et al*, 2008). Specificity of immunohistochemistry was assessed by evaluating the negative controls. For monoclonal antibodies, the primary antibodies had been replaced with normal rabbit nonimmune IgG. For polyclonal antibodies, immunoabsorption test using the corresponding antigens was conducted as a negative control.

Statistical analysis

The duration of disease-free survival (DFS) or PFS was calculated from the date of diagnosis to that of relapse or death, whichever first occurred, or to the last follow-up information for living patients (censored case). The duration of PFS was calculated from the date of start medication to that of progression, or to the last follow-up information for living patients (censored case). DFS and PFS data were graphically presented using the Kaplan–Meier method and were also compared with immunoreactivity of each CEACAM (positive *vs* negative) using the log-rank test. The 5-year DFS and PFS values were obtained from the Kaplan–Meier curves. The differences of positive rates of CEACAMs by each variant were

assessed by Mann–Whitney *U*-test. The influence of each variable on the positive rate of each CEACAM was assessed by multinomial logistic regression model, and the survival of the patients was assessed by the Cox proportional hazards model. All statistical analyses were performed using Statview for windows (version 5.0; SAS Institute Inc., Cary, NC, USA). The accepted level of significance was $P<0.05$.

RESULTS

EGFR-TKI sensitivity test

Results of the cell proliferation assays are summarised in Figure 1A. There was a significant decrease in the cell number after 48 h in RERF-LC-OK, A549, LCSC#1, and LK87 cells treated with 1 μM (LCSC#1) or 10 μM of erlotinib. There was a significant decrease in the cell number after 72 h in LCAM1, RERF-LC-OK, A549, LCSC#1, and LK87 cells treated with 1 μM (LCSC#1 and LK87) or 10 μM of gefitinib. There were no EGFR mutations in all these cell lines examined.

The order of sensitivity to EGFR-TKI in the cells examined was as follows: LCSC#1, LK87, A549, RERF-LC-OK, and LCAM1.

Analysis of EGFR-TKI sensitivity-related genes using cDNA microarray

Each cell line was arranged according to the sensitivity of EGFR-TKI evaluated by EGFR-TKI sensitivity assay described above. We therefore searched gene expression similar to EGFR-TKI sensitivity patterns in five adenocarcinoma cell lines above (Figure 1B). In our present study, we focussed on four genes (*CGM1* (CEACAM3), *CD66c* (CEACAM6), *CGM2* (CEACAM7), and *CEACAM19*) of the CEACAM family.

CEACAMs in LADCA cases

We further examined the clinical significance of CEACAMs, especially that of CEA/CEACAM5, which has been known to be related to EGFR mutation (Okamoto *et al*, 2005; Shoji *et al*, 2007) in 115 EGFR mutation-negative LADCA cases using immunohistochemistry.

All CEACAMs examined were detected in cytoplasm and/or cell membrane of carcinoma cells (Figure 2). The positive cases of each CEACAM in 115 LADCA patients were summarised as follows: 36 cases (CEACAM5), 53 cases (CEACAM6), 57 cases (CEACAM3), 16 cases (CEACAM7), and 13 cases (CEACAM19). Results of an association between the status of CEACAM immunoreactivity and clinicopathological parameters in 115 LADCA patients are also summarised in Table 1. CEACAM3 status was significantly associated with gender or lymph node metastasis in 115 LADCA patients. There were no statistically significant association between other CEACAMs and clinicopathological parameters of individual patients.

The association between CEACAM expression and the 5-year DFS of the patients was evaluated using Kaplan–Meier survival curves and log-rank test. The statistical analysis demonstrated that the status of CEACAM3 in 115 LADCA patients examined in this study was significantly associated with better DFS, whereas that of CEACAM6 with poor DFS (Figure 3). The 5-year DFS rate of CEACAM6-negative patients was 74.2%, whereas that of CEACAM6-positive patients was 49.1%. Also, the 5-year DFS rate of CEACAM3-negative patients was 46.6%, whereas that of CEACAM3-positive patients was 78.9%. Using the Cox proportional hazards model, we performed multivariate analysis to assess the independent predictive value of CEACAM status for the DFS of patients with LADCA. The following prognostic variables were also included in this study: age, sex, tumour size, and lymph node metastasis. Results of this multivariate analysis demonstrated that CEACAM6 positive ($P=0.0003$), CEACAM3 positive ($P=0.0008$),

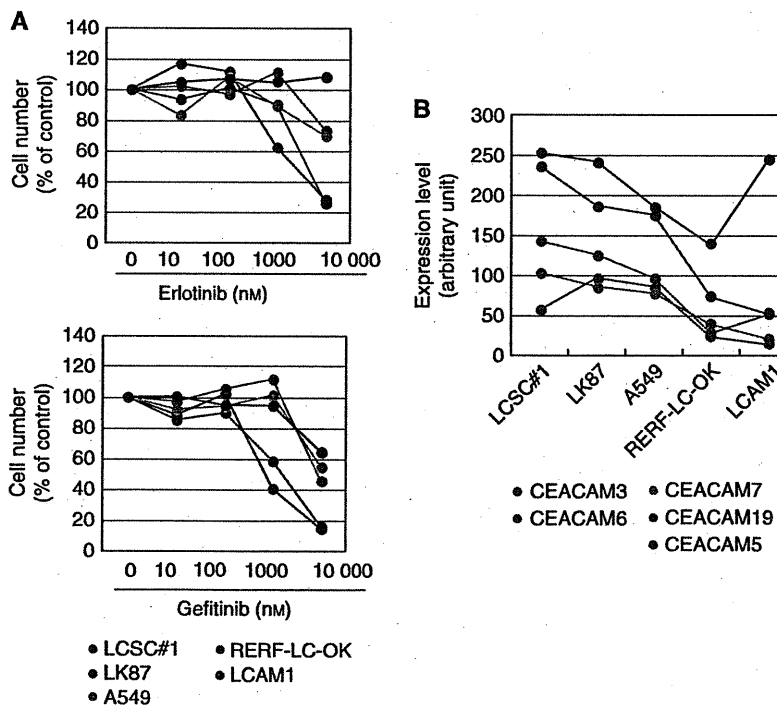

Figure 1 (**A**) Effects of EGFR-TKI on cell proliferation of the lung adenocarcinoma cell lines. Data are expressed as mean ($n=3$). (**B**) Each cell line was arranged according to the sensitivity of EGFR-TKI evaluated by EGFR-TKI sensitivity assay. We therefore searched gene expression similar to EGFR-TKI sensitivity patterns in five adenocarcinoma cell lines. In our present study, we focussed on four genes (*CGM1* (CEACAM3), *CD66c* (CEACAM6), *CGM2* (CEACAM7), and *CEACAM19*) of the CEACAM family.

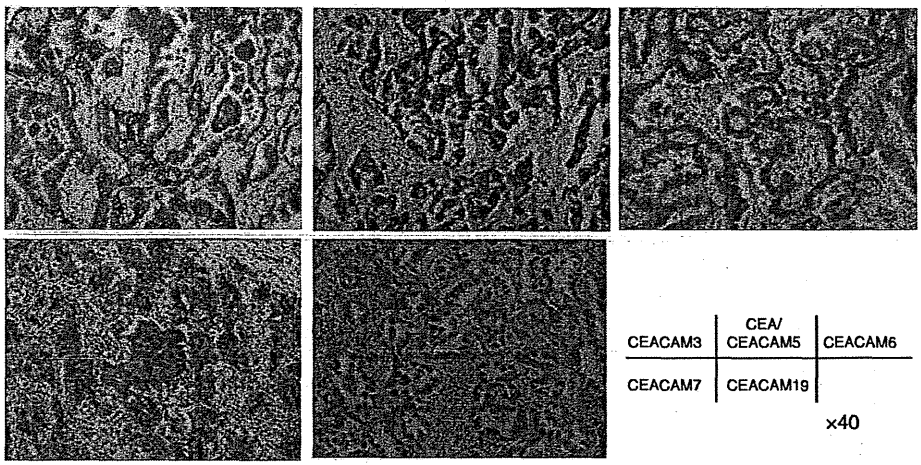

Figure 2 Representative illustrations of CEACAM 3, 5, 6, 7, and 19 immunohistochemistry in LADCA cases. Each CEACAM immunoreactivity was detected in cytoplasm and/or cell membrane of carcinoma cells.

Table 1 Multivariate analysis of characteristic factors influencing positive rate of each CEACAM

	CEACAM5		CEACAM3		CEACAM6		CEACAM7		CEACAM19	
Factors	**OR**	***P***	**OR**	***P***	**OR**	***P***	**OR**	***P***	**OR**	***P***
Age (⩽75 *vs* >75 years)	1.03	0.12	1.01	0.14	1.01	0.30	0.99	0.62	0.85	0.16
Sex (male *vs* female)	1.01	0.09	0.97	*0.0013*	1.00	0.95	1.00	0.96	1.00	0.87
Tumour size (<30 *vs* ⩾30)	1.02	0.20	1.01	0.67	0.99	0.44	0.96	0.86	1.01	0.12
LN (positive *vs* negative)	0.95	0.43	0.95	*0.045*	1.01	0.57	0.99	0.91	1.04	0.32
Stage (I *vs* II or IIIA)	0.98	0.16	0.97	0.82	0.96	0.15	1.82	0.99	0.96	0.53

Abbreviations: CEACAM = carcinoembryonic antigen-related cell adhesion molecule; OR = odds ratio; LN = lymph node metastasis. Multinomial logistic regression model. Italic entries indicate $P<0.05$.

age ($P=0.032$), tumour size ($P=0.015$), and lymph node metastasis ($P=0.0001$) were all turned out independent prognostic factors, respectively (Table 2).

Association between CEACAM status and EGFR mutation in LADCA cases

All CEACAM (CEA, CEACAM6, CEACAM7, CEACAM7, CEACAM19) immunoreactivity in EGFR mutation-positive cases was significantly higher than that in EGFR mutation-negative cases (Figure 4). However, there were no statistically significant differences in the status of CEACAMs between responder and nonresponder patients, and also in EGFR mutations between exon 19 and exon 21 among 22 EGFR mutation-positive LADCA patients (Figures 5 and 6). The association between CEACAM status and the PFS of the patients was evaluated using Kaplan–Meier survival curves and log-rank test. Results of univariate analysis demonstrated that the positive CEACAM6 status was associated with an increased PFS in EGFR mutation-positive LADCA patients with EGFR-TKI treatment (Figure 7). We also performed multivariate analysis, including age and gender, to assess the independent predictive value of CEACAM6 expression for PFS of the patients receiving EGFR-TKI treatment using Cox proportional hazards model but no significant correlations were detected (Supplementary Table S4).

DISCUSSION

CEA is one of the most extensively studied tumour markers and belongs to the CEACAM family members. These groups of protein are typically cell membrane-associated glycoproteins, and are part of the immunoglobulin superfamily (Gold and Freedman, 1965). Among these CEACAM family, CEACAM5, also well known as CEA, was reported to be overexpressed in a majority of carcinomas including those of the gastrointestinal tract, the respiratory systems, and the breast (Hansen *et al*, 1974; Kuroki *et al*, 1992, Lamerz, 1999). CEACAM6 (CD66c, NCA-90) is a nonspecific crossreacting glycoprotein antigen that shares some antigenic determinants with CEACAM5 (Kuespert *et al*, 2006). CEACAM6 is also reported to be expressed in granulocytes and epithelia from various organs (Kuespert *et al*, 2006). Overexpression of CEACAM6 has been demonstrated to result in cell proliferation and invasion of breast and pancreatic cancer (Kuespert *et al*, 2006; Lewis-Wambi *et al*, 2008; Maraqa *et al* 2008). CEACAM3 is also present in neutrophils and considered to play an important role in the process of phagocytosis (Chen and Gotschlich, 1996). CEACAM7 expression was also very recently reported to be significantly low in rectal adenocarcinoma compared with that in normal mucosa (Messick *et al*, 2010). CEACAM19 has functional immunoreceptor tyrosine-based activation motifs in cytoplasmic domain (Kuespert *et al*, 2006), but it is also true that the physiological or pathological functions of CEACAM19 have remained entirely unknown at this juncture. CEACAMs have been also recently demonstrated to play important roles in several types of human malignancies, but the roles of CEACAMs have remained largely unknown in lung cancer.

In this study, we first demonstrated that the expression of CEACAM family (CEACAM 3, 6, 7, and 19) was associated with EGFR-TKI sensitivity in microarray analysis *in vitro*. The status of these CEACAMs was also significantly higher in EGFR mutation-positive cases than in negative LADCA cases. Shoji *et al* (2007) reported that serum CEA/CEACAM5 level was significantly higher in EGFR mutation-positive lung cancer cases than in wild-type

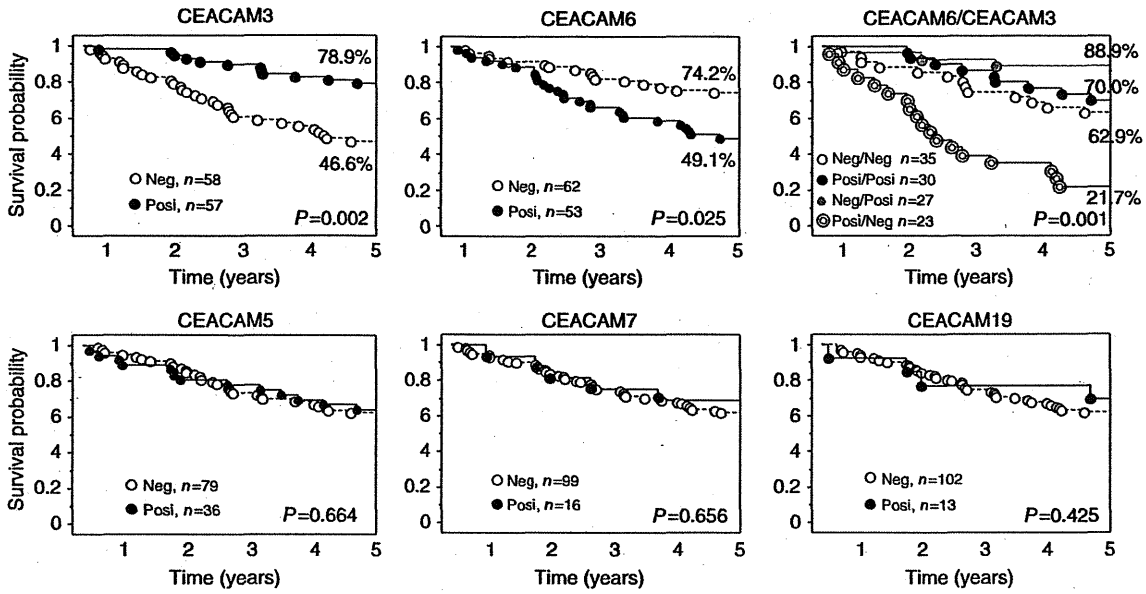

Figure 3 Kaplan–Meier survival curves of 115 LADCA patients according to the status of each CEACAM. The *P*-value is from the log-rank test. In CEACAM6/CEACAM3, 'neg' represented negative cases, and 'posi' positive cases of CEACAM6 or CEACAM3.

Table 2 Multivariate analysis of prognostic factors influencing survival of EGFR mutation-negative 115 patientsCox proportional hazards model.

Factors	s.e.	Hazard ratio	95% CI	*P*-value
CEACAM5[a]	0.35	0.75	0.38–1.48	0.40
CEACAM3[a]	0.38	3.81	1.83–7.96	*0.0004*
CEACAM6[a]	0.32	0.36	0.17–0.61	*0.0005*
CEACAM7[a]	0.53	0.86	0.30–2.47	0.77
CEACAM19[a]	0.68	2.01	0.54–7.87	0.29
Age (⩽75 *vs* >75 years)	0.38	0.42	0.20–0.88	*0.020*
Sex (male *vs* female)	0.35	0.79	0.40–1.58	0.51
Tumour size (<30 *vs* ⩾30)	0.30	0.37	0.20–0.67	*0.0011*
Lymph node metastasis[a]	0.64	0.09	0.03–0.34	*0.0003*
Stage (I *vs* II or IIIA)	0.64	3.47	0.98–11.9	0.054

Abbreviations: CI = confidence interval; CEACAM = carcinoembryonic antigen-related cell adhesion molecule; EGFR = epidermal growth factor receptor. [a]Positive *vs* negative. Italic entries indicate $P<0.05$.

cases. In addition, Okamoto *et al* (2005) demonstrated that in LADCA patients, serum CEA/CEACAM5 concentration of $\geqslant 5\,ng\,ml^{-1}$ turned out to be more sensitive to gefitinib treatment than those of $\leqslant 5\,ng\,ml^{-1}$. It is true that CEA/CEACAM5 was not included in EGFR-TKI sensitivity molecules examined by microarray analysis in our present study but CEA/CEACAM5 expression was significantly higher in EGFR mutation cases as well as other CEACAMs examined in our study compared with EGFR wild-type cases. There were, however, no significant statistical associations between the status of CEACAMs examined in primary tumour of the patients and clinical response of gefitinib treatment in 22 LADCA patients. Therefore, it awaits further investigations including the validation in a larger number of the cases in different institutions to clarify whether the status of these CEACAMs in adenocarcinoma cases actually results in EGFR TKI-sensitivity in LADCA patients or not.

In this study, we also examined the clinicopathological significance of CEACAMs in LADCA patients. Among 5 CEACAMs above, both CEACAM3 and CEACAM6 demonstrated the most significant clinical significance in terms of clinical outcome of the patients. Results of our present study clearly demonstrated that the positive rate of CEACAM3 was significantly higher in female or lymph node metastasis-negative LADCA patients. In addition, CEACAM3 and CEACAM6 positivity in carcinoma cells turned out to be independent prognostic factors in LADCA patients examined in this study, that is, CEACAM3 positivity was associated with significantly better prognosis and CEACAM6 positivity with significantly worse prognosis. CEACAM3 is well known to be present as transmembrane protein, whereas CEACAM6 is linked to membrane via glycosyl-phosphatidylinositol anchor in neutrophils (Kuespert *et al*, 2006). CEACAM6 also acts as an inducer of cell proliferation in A549 cells (Singer *et al*, 2010). A549 cells expressed significant amounts of nonmembrane-anchored variants of CEACAM6 as well as CEA/CEACAM5, representing a putative source for the increased CEACAM5/6 serum levels frequently detected in lung cancer patients (Singer *et al*, 2010). In our present study, CEACAM6-positive/CEACAM3-negative cases were significantly associated with poor clinical outcome compared with CEACAM6-negative/CEACAM3-positive, double-positive, and double-negative cases. CEACAM3, which is anchored in cell membrane, was also reported to form heterodimer with other CEACAM family including CEACAM6 (Skubitz and Skubitz, 2008). These findings, including results of our present study, all indicated that CEACAM3 may inhibit the dissociation of CEACAM6 from the cell membrane and the stimulatory effects upon cell proliferation of CEACAM6 in LADCA patients. In addition, CACAM3 status of the primary tumour turned out to be an independent factor of good prognosis in LADCA cases examined in this study. CEACAM3 may inhibit cell proliferation/invasion of LADCA cells as a binding protein, but further investigations are required for clarification. Results of the univariate analysis in our present study did demonstrate that CEACAM6 was associated with an increased PFS for EGFR mutation-positive adenocarcinoma patients undergoing EGFR-TKI treatment. EGFR gene mutation-positive lung cancer was also reported to demonstrate better treatment responses than EGFR mutation-negative lung cancers following EGFR-TKI treatment (Takano *et al*, 2008). These findings all suggest that CEACAM3 overexpression was associated with better prognosis or clinical outcome, and CEACAM6 overexpression could account for protecting EGFR-TKI resistance (Lo and Hung, 2006) in the EGFR mutation-positive LADCA patients. This is

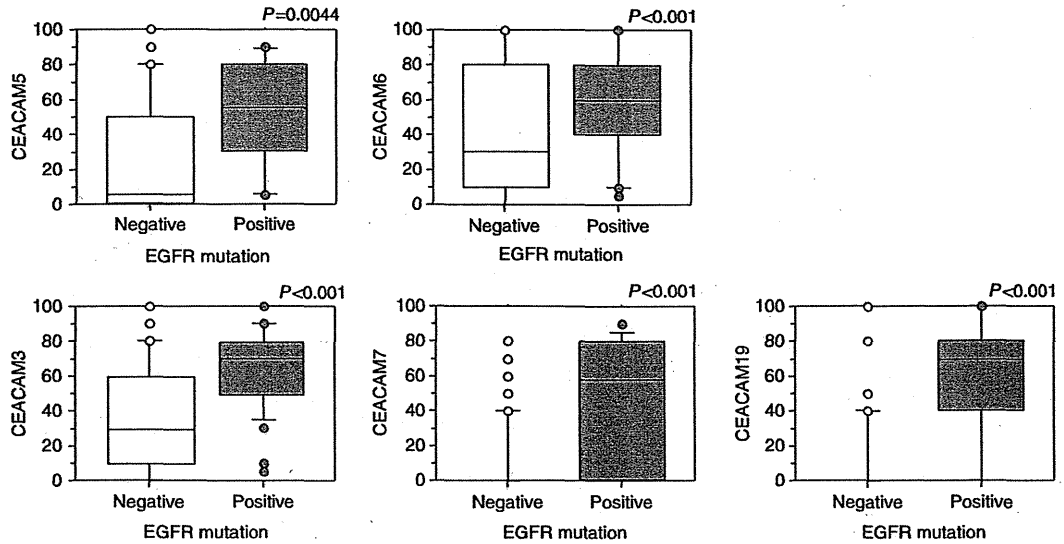

Figure 4 Box-plot of positive rate of each CEACAM according to the status of EGFR mutation (upper and bottom left and middle). The *P*-value is from the Mann–Whitney *U*-test.

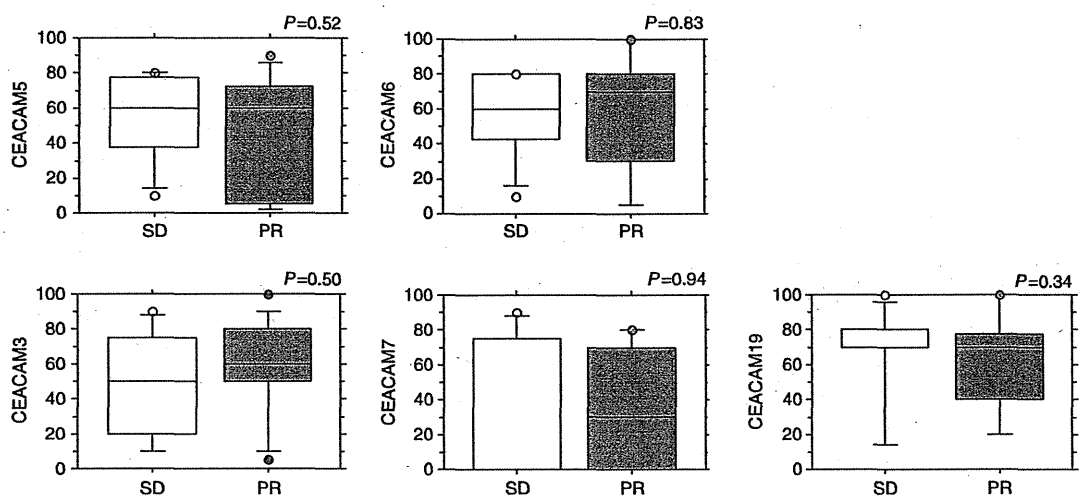

Figure 5 Box-plot of positive rate of each CEACAM according to the response to gefitinib in EGFR mutation-positive lung adenocarcinoma. The *P*-value is from the Mann–Whitney *U*-test.

because CEACAM3- and CEACAM6-positive rates in EGFR mutation-positive cases were significantly higher than that in EGFR mutation-negative cases, and CEACAM6-positive cases receiving gefitinib therapy were associated with a relatively long PFS in their clinical course. In the present study, we did not get significant collection between CEACAM6 and the response to EGFR-TKI, but we found relatively high CEACAM6 expression in PR cases. Because we only dealt with SD or PR cases, or we did not have enough cases to assess, we thought of the possibility that there was no significant difference. We think it is necessary to assess the CEACAM6 expression of PD cases, T790M positive cases, and an independent larger set to confirm the assumption.

Abdel-Aziz *et al* (2009) reported that a double-positive status of CEA/CEACAM5 and EGFR expression was detected in the majority of patients (81%) with colorectal cancers. Abou-Rjaily *et al* (2004) also reported that CEACAM1 was closely associated with EGFR actions and may reduce the EGFR-mediated cell proliferation following EGF binding, and that the CEACAM1 effects upon EGF-dependent hepatocyte proliferation are mediated by its ability to bind to and sequester Shc, thus uncoupling EGFR signalling from the Ras/Raf/MAP kinase pathway (Abou-Rjaily *et al*, 2004). Therefore, the CEACAMs examined in our present study are reasonably postulated to be associated directly with EGFR and to modify the anti-tumour effects of EGFR-TKI in LADCA patients. Choi *et al* (2007) recently reported that CEACAM6 was decreased by gefitinib treatment and abundantly expressed in EGFR-mutant lung cancer cell lines. They also suggested that CEACAM6 could serve as a potentially important EGFR transcriptional target in these cell lines (Choi *et al*, 2007). Results of several previous studies also demonstrated the translocation of EGFR in the nucleus as full-length receptors (Marti *et al*, 1991; Lin *et al*, 2001; Li *et al*, 2009). Li *et al* (2009) demonstrated that expression of a nuclear localisation sequence-tagged EGFR in cetuximab-sensitive cells increased resistance to cetuximab, both *in vitro* and in mouse xenografts. These results as well as results of our own study all indicated that CEACAMs could interact with EGFR and subsequently stabilise EGFR on the cell membrane, and maintain the sensitivity of EGFR-TKI. Further investigations are, however,

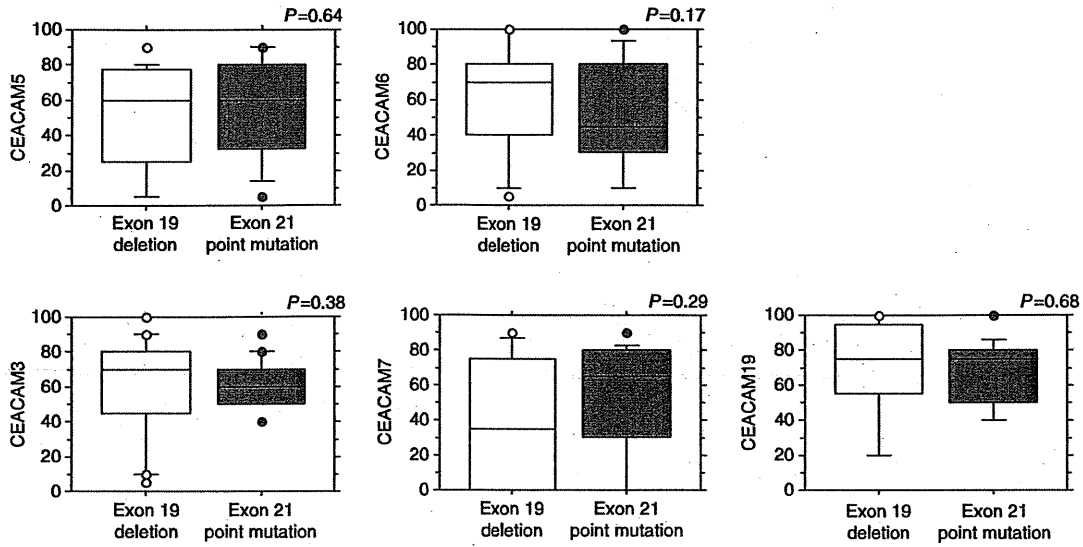

Figure 6 Box-plot of positive rate of each CEACAM according to the response to mutation site in EGFR mutation-positive lung adenocarcinoma. The *P*-value is from the Mann–Whitney *U*-test.

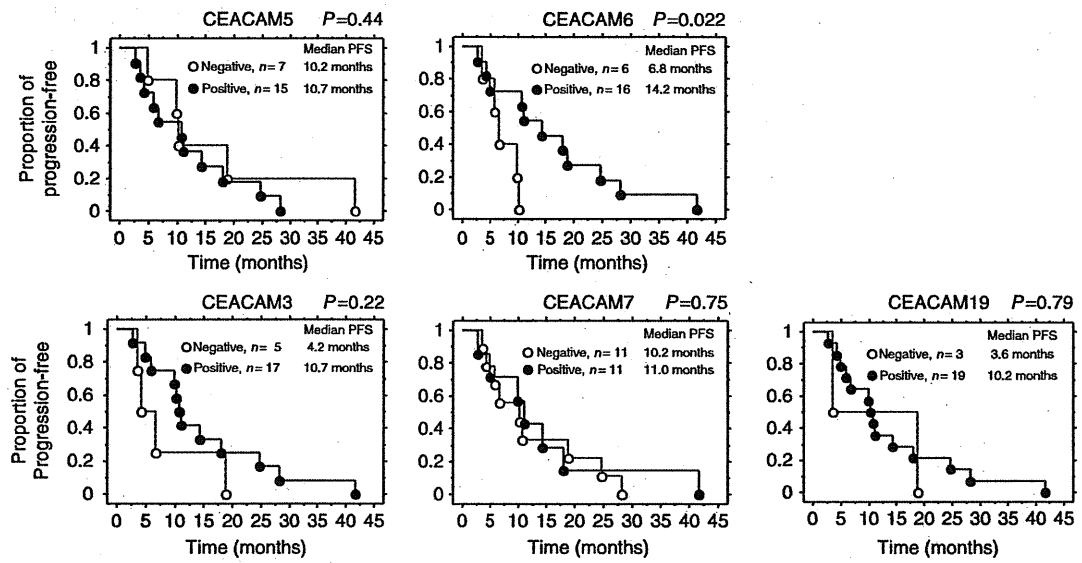

Figure 7 The PFS of 22 EGFR mutation-positive lung adenocarcinoma patients who had gefitinib therapy according to the status of each CEACAM. The *P*-value is from the log-rank test.

required to clarify further details of the biological correlations between CEACAMs and EGFR toward the development of much more effective EGFR-TKI therapy of NSCLC patients.

STATEMENT OF TRANSLATIONAL RELEVANCE

The CEACAMs (CEACAM 5, 3,6, 7, and 19) examined in this study could be effective surrogate markers for prediction of EGFR gene mutation. Among these five CEACAMs above, immunohistochemical evaluation of CEACAM3/6 in LADCA patients could contribute to predicting their clinical outcome.

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Conflict of interest

The authors (KM and HY-O) have ownership interest in CHUGAI Pharmaceutical Company, Shizuoka, Japan.

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REFERENCES

Abdel-Aziz MM, Lotfy M, El-Kady IM, Abozaid M (2009) Mutant p53 protein in the serum of patients with colorectal cancer: correlation with the level of carcinoembryonic antigen and serum epidermal growth factor receptor. *Cancer Detect Prev* **32**: 329–335

Abou-Rjaily GA, Lee SJ, May D, Al-Share QY, Deangelis AM, Ruch RJ, Neumaier M, Kalthoff H, Lin SH, Najjar SM (2004) CEACAM1 modulates epidermal growth factor receptor–mediated cell proliferation. *J Clin Invest* **114**: 944–952

Asahina H, Yamazaki K, Kinoshita I, Sukoh N, Harada M, Yokouchi H, Ishida T, Ogura S, Kojima T, Okamoto Y, Fujita Y, Dosaka-Akita H, Isobe H, Nishimura M (2006) A phase II trial of gefitinib as firstline therapy for advanced non-small cell lung cancer with epidermal growth factor receptor mutations. *Br J Cancer* **95**: 998–1004

Bell DW, Gore I, Okimoto RA, Godin-Heymann N, Sordella R, Mulloy R, Sharma SV, Brannigan BW, Mohapatra G, Settleman J, Haber DA (2005) Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. *Nat Genet* **37**: 1315–1316

Chen T, Gotschlich EC (1996) CGM1a antigen of neutrophils, a receptor of gonococcal opacity proteins. *Proc Natl Acad Sci USA*. **93**: 14851–14856

Cho BC, Im CK, Park MS, Kim SK, Chang J, Park JP, Choi HJ, Kim YJ, Shin SJ, Sohn JH, Kim H, Kim JH (2007) Phase II study of erlotinib in advanced non-small-cell lung cancer after failure of gefitinib. *J Clin Oncol* **25**: 2528–2533

Choi K, Creighton CJ, Stivers D, Fujimoto N, Kurie JM (2007) Transcriptional profiling of non-small cell lung cancer cells with activating EGFR somatic mutations. *PLoS One* **2**: e1226

Dango S, Sienel W, Schreiber M, Stremmel C, Kirschbaum A, Pantel K, Passlick B (2008) Elevated expression of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1) is associated with increased angiogenic potential in non-small-cell lung cancer. *Lung Cancer* **60**: 426–433

Gold P, Freedman SO (1965) Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. *J Exp Med* **121**: 439–462

Hansen HJ, Snyder JJ, Miller E, Vandevoorde JP, Miller ON, Hines LR, Burns JJ (1974) Carcinoembryonic antigen (CEA) assay. A laboratory adjunct in the diagnosis and management of cancer. *Hum Pathol* **5**: 139–147

Inoue A, Suzuki T, Fukuhara T, Maemondo M, Kimura Y, Morikawa N, Watanabe H, Saijo Y, Nukiwa T (2006) Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. *J Clin Oncol* **24**: 3340–3346

Isobe I, Michikawa M, Yanagisawa K (1999) Enhancement of MTT, a tetrazolium salt, exocytosis by amyroid β-protein and chloroquine in cultured rat astrocytes. *Neurosci Lett* **266**: 129–132

Kuespert K, Pils S, Hauck CR (2006) CEACAMs: their role in physiology and pathophysiology. *Curr Opin Cell Biol* **18**: 565–571

Kuroki M, Matsuo Y, Kinugasa T, Matsuoka Y (1992) Three different NCA species, CGM6/CD67, NCA-95, and NCA-90, are comprised in the major 90 to 100-kDa band of granulocyte NCA detectable upon SDS-polyacrylamide gel electrophoresis. *Biochem Biophys Res Commun* **182**: 501–506

Lamerz R (1999) Role of tumour markers, cytogenetics. *Ann Oncol* **10**: 145–149

Lewis-Wambi JS, Cunliffe HE, Kim HR, Willis AL, Jordan VC (2008) Overexpression of CEACAM6 promotes migration and invasion of oestrogen-deprived breast cancer cells. *Eur J Cancer* **44**: 1770–1779

Li C, Iida M, Dunn EF, Ghia AJ, Wheeler DL (2009) Nuclear EGFR contributes to acquired resistance to cetuximab. *Oncogene* **28**: 3801–3813

Lin SY, Makino K, Xia W, Matin A, Wen Y, Kwong KY, Bourguignon L, Hung MC (2001) Nuclear localization of EGF receptor and its potential new role as a transcription factor. *Nat Cell Biol* **3**: 802–808

Lo HW, Hung MC (2006) Nuclear EGFR signalling network in cancers: linking EGFR pathway to cell cycle progression, nitric oxide pathway and patient survival. *Br J Cancer* **94**: 184–188

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, Haber DA (2004) 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

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, Nukiwa T, North-East Japan Study Group (2010) Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* **362**: 2380–2388

Maraqa L, Cummings M, Peter MB, Shaaban AM, Horgan K, Hanby AM, Speirs V (2008) Carcinoembryonic antigen cell adhesion molecule 6 predicts breast cancer recurrence following adjuvant tamoxifen. *Clin Cancer Res* **14**: 405–411

Marti U, Burwen SJ, Wells A, Barker ME, Huling S, Feren AM, Jones AL (1991) Localization of epidermal growth factor receptor in hepatocyte nuclei. *Hepatology* **13**: 15–20

Messick CA, Sanchez J, Dejulius KL, Hammel J, Ishwaran H, Kalady MF (2010) CEACAM-7: a predictive marker for rectal cancer recurrence. *Surgery* **147**: 713–719

Niho S, Ichinose Y, Tamura T, Yamamoto N, Tsuboi M, Nakagawa K, Shinkai T, Jiang H, Nishiwaki Y, Fukuoka M (2007) Results of a randomized phase III study to compare the overall survival of gefitinib versus docetaxel in Japanese patients with non-small cell lung cancer who failed one or two chemotherapy regimens. *J Clin Oncol* **25**: LBA7509 (2007 ASCO Annual Meeting Proceedings)

Okamoto T, Nakamura T, Ikeda J, Maruyama R, Shoji F, Miyake T, Wataya H, Ichinose Y (2005) The serum CEA level can be a predictive factor for the efficacy of gefitinib in NSCLC patients. *Eur J Cancer* **41**: 1286–1290

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, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**: 1497–1500

Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD, Gazdar AF (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* **97**: 326–328

Shoji F, Yoshino I, Yano T, Kometani T, Ohba T, Kouso H, Takenaka T, Miura N, Okazaki H, Maehara Y (2007) Serum carcinoembryonic antigen level is associated with epidermal growth factor receptor mutations in recurrent lung adenocarcinomas. *Cancer* **110**: 2793–2798

Sienel W, Dango S, Woelfle U, Morresi-Hauf A, Wagener C, Brümmer J, Mutschler W, Passlick B, Pantel K (2003) Elevated expression of carcinoembryonic antigen-related cell adhesion molecule 1 promotes progression of non-small cell lung cancer. *Clin Cancer Res* **9**: 2260–2266

Singer BB, Scheffrahn I, Kammerer R, Suttorp N, Ergun S, Slevogt H (2010) Deregulation of the CEACAM expression pattern causes undifferentiated cell growth in human lung adenocarcinoma cells. *PLoS One* **5**: e8747

Skubitz KM, Skubitz AP (2008) Interdependency of CEACAM-1, -3, -6, and -8 induced human neutrophil adhesion to endothelial cells. *J Transl Med* **6**: 78

Takano T, Fukui T, Ohe Y, Tsuta K, Yamamoto S, Nokihara H, Yamamoto N, Sekine I, Kunitoh H, Furuta K, Tamura T (2008) EGFR mutations predict survival benefit from gefitinib in patients with advanced lung adenocarcinoma: a historical comparison of patients treated before and after gefitinib approval in Japan. *J Clin Oncol* **26**: 5589–5595

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

Thomson S, Buck E, Petti F, Griffin G, Brown E, Ramnarine N, Iwata KK, Gibson N, Haley JD (2005) Epithelial to mesenchymal transition is a determinant of sensitivity of non-small-cell lung carcinoma cell lines and xenografts to epidermal growth factor receptor inhibition. *Cancer Res* **65**: 9455–9462

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, Fukuoka M (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* **361**: 947–957

Witta SE, Gemmill RM, Hirsch FR, Coldren CD, Hedman K, Ravdel L, Helfrich B, Dziadziuszko R, Chan DC, Sugita M, Chan Z, Baron A, Franklin W, Drabkin HA, Girard L, Gazdar AF, Minna JD, Bunn Jr PA (2006) Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. *Cancer Res* **66**: 944–950

Yauch RL, Januario T, Eberhard DA, Cavet G, Zhu W, Fu L, Pham TQ, Soriano R, Stinson J, Seshagiri S, Modrusan Z, Lin CY, O'Neill V, Amler LC (2005) Epithelial versus mesenchymal phenotype determines in vitro sensitivity and predicts clinical activity of erlotinib in lung cancer patients. *Clin Cancer Res* **11**: 8686–8698

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Lung Cancer

Quality of Life with Gefitinib in Patients with *EGFR*-Mutated Non-Small Cell Lung Cancer: Quality of Life Analysis of North East Japan Study Group 002 Trial

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Key Words. Lung carcinoma • Epidermal growth factor receptor • EGFR • Tyrosine kinase inhibitor • TKI • Gefitinib • Quality of life • QoL

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ABSTRACT

Background. **For non-small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (*EGFR*) mutations, first-line gefitinib produced a longer progression-free survival interval than first-line carboplatin plus paclitaxel but did not show any survival advantage in the North East Japan 002 study. This report describes the quality of life (QoL) analysis of that study.**

Methods. **Chemotherapy-naïve patients with sensitive *EGFR*-mutated, advanced NSCLC were randomized to receive gefitinib or chemotherapy (carboplatin and paclitaxel). Patient QoL was assessed weekly using the Care Notebook, and the primary endpoint of the QoL analysis was time to deterioration from baseline on each of the physical, mental, and life well-being QoL scales. Kaplan–Meier probability curves and log-rank tests were employed to clarify differences.**

Results. **QoL data from 148 patients (72 in the gefitinib arm and 76 in the carboplatin plus paclitaxel arm) were analyzed. Time to defined deterioration in physical and life well-being significantly favored gefitinib over chemotherapy (hazard ratio [HR] of time to deterioration, 0.34; 95% confidence interval [CI], 0.23–0.50; $p < .0001$ and HR, 0.43; 95% CI, 0.28–0.65; $p < .0001$, respectively).**

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Conclusion. **QoL was maintained much longer in patients treated with gefitinib than in patients treated with standard chemotherapy, indicating that gefitinib should be considered as the standard first-line therapy for advanced *EGFR*-mutated NSCLC in spite of no survival advantage.**
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INTRODUCTION

Dysregulation of protein kinases is frequently observed in cancer cells. Therefore, protein kinases are attractive targets in the development of anticancer drugs such as small molecule inhibitors that block binding of ATP to the catalytic domain of the tyrosine kinase. In 2004, three groups of researchers reported that activating mutations of the epidermal growth factor receptor gene (*EGFR*) were present in a subset of non-small cell lung cancer (NSCLC) tumors, and that tumors with *EGFR* mutations were highly sensitive to EGFR tyrosine kinase inhibitors (TKIs) [1–3]. Since then, our multiple phase II studies confirmed a striking response to EGFR TKIs in this population [4–8].

In phase III NSCLC trials, EGFR TKIs such as gefitinib or erlotinib were compared with conventional chemotherapies initially in unselected patients [9–11], next on the basis of clinical characteristics [12], and subsequently using molecular selection [13–16]. Among them, the pivotal phase III study North East Japan (NEJ) 002 compared gefitinib with chemotherapy in first-line therapy for patients with NSCLC with mutated *EGFR* and confirmed, as the primary endpoint, that the progression-free survival (PFS) interval in the gefitinib group was significantly longer than that in the carboplatin plus paclitaxel group (10.8 months versus 5.4 months, hazard ratio [HR], 0.30; $p < .001$) [13]. A subgroup analysis of the Iressa® Pan-Asia Study (IPASS) [12] and similar phase III studies—the West Japan Thoracic Oncology Group 3405 trial [14], the OPTIMAL trial [15], and European Randomised Trial of Tarceva versus Chemotherapy [16]—also demonstrated a superior PFS outcome in patients treated with EGFR TKIs than in those treated with standard chemotherapies. However, the IPASS and NEJ 002 trials showed identical overall survival (OS) outcomes using gefitinib and chemotherapy in the first-line treatment of NSCLC patients harboring sensitive *EGFR* mutations [17, 18].

When the OS time is identical in the two arms, improvements in quality of life (QoL) and disease-related symptoms are among the key goals of treatment for NSCLC. However, there has been no prospective report describing QoL in NSCLC patients with sensitive *EGFR* mutations who were treated using an EGFR TKI. This QoL analysis was prospectively conducted as a secondary endpoint in the NEJ 002 study.

METHODS

This study was performed in accordance with the Helsinki Declaration (1964, amended in 2000) of the World Medical Association. The participating institutions received approval from their institutional ethics review boards. The details regarding patient eligibility and treatment were described previously [13]. Briefly, eligibility stipulated the presence of advanced NSCLC harboring a sensitive *EGFR* mutation, the absence of the resistant *EGFR* mutation T790M, no history of chemotherapy, and age ≤75 years. *EGFR* mutation status was examined using the peptide nucleic acid-locked nucleic acid polymerase chain reaction (PNA-LNA PCR) clamp method [19]. Eligible patients were randomly assigned to receive either gefitinib (at a dose of 250 mg/day orally) or standard chemotherapy. Standard chemotherapy consisted of paclitaxel (at a dose of 200 mg/m^2 i.v.) and carboplatin (area under the concentration–time curve of 6), both administered on the first day of every 3-week cycle. Randomization was balanced by institution, sex, and stage. The primary endpoint was the PFS interval; secondary endpoints included the OS time, response rate, toxic effects, and QoL.

QoL Assessment

The Care Notebook (supplemental online Fig. 1) [20], which has been previously validated and reported [21, 22], was used to assess QoL. The Care Notebook is a self-administered, cancer-specific questionnaire that asks about cancer patients' conditions during 1 week regarding 24 items that are structured in multidimensional scales. The questionnaire consists of three major scales: physical well-being, mental well-being, and life well-being. These major scales are divided into several subscales. Physical well-being has three multi-item subscales, which are appetite loss (items P3, P4, P7), constipation (P6, P8), and fatigue (P9, P10), and three single-item measures, which are pain (item P1), shortness of breath (item P2), and sleeping trouble (P5). Mental well-being has three multi-item subscales, which are anxiety (M1, M2), irritation (M3, M5), and depression (M4, M6). Life well-being has three multi-item subscales, which are daily functioning (L1, L2), social functioning (L3, L4), and subjective QoL (L5–L8), which consists of peace of mind (L5), feeling of happiness (L6), QoL functioning (L7), and satisfaction with daily life (L8). Each item is asked using one word or a short phrase and employs an 11-point linear analog scale (0–10). A score of 10 in physical well-being and mental well-being indicates the heaviest burden. A score of 10 in life well-being indicates the best possible function or QoL; thus, the polarity of the data for life well-being was reversed before the analysis so that a greater score indicated a poorer QoL in all items of the questionnaire.

Seventy sheets of the Care Notebook were bundled as a booklet. Patients started answering the questionnaire before starting therapy and answered it once a week during first-line treatment. After completion of the questionnaire, the booklets were collected by the patients' doctors and sent to the QoL data center (Saitama Medical University).

Statistical Analyses

The primary endpoint in the QoL analysis, which was prospectively defined in the protocol of the clinical trial, was the time from random assignment of treatment to deterioration in the

following, which are clinically relevant and are frequently observed in patients with advanced NSCLC: (a) pain and shortness of breath (P1 and P2), (b) anxiety (M1 and M2), and (c) daily functioning (L1 and L2). From previous studies [23, 24], deterioration was recognized when the score changed from baseline by one of 11 points (9.1%) in a direction indicating a worse QoL at any timepoint. This primary analysis was performed for 20 weeks after the initiation of first-line therapy. All patients who had a baseline plus at least one follow-up QoL assessment were included in the time-to-deterioration analysis. Patients who had not deteriorated were censored at the time of the last QoL questionnaire completion. Kaplan–Meier curves and the log-rank test were used to compare the time to deterioration in each subscale between the two treatment arms. Also, more severe deterioration was defined as a score change of three of 11 points (27.3%) [23, 24].

In addition, we performed a secondary analysis using QoL data according to the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) standard method [25]. During the initial 20 weeks from the start of treatment, we first checked whether or not the scores showed an improvement at any time in a subscale by ≥9.1% (one point or more) from baseline. In such cases, the response was judged to be "improved" even if the scores were initially or subsequently below the lower boundary, that is, −9.1%. If the response was not classified as improved, we next checked whether or not the scores showed a worsening in a subscale by ≥−9.1% from baseline, resulting in the response being classified as "worsened." In cases that were classified as neither improved nor worsened, the response was classified as "stable." A χ^2 test was used for comparisons between the two arms.

Results

Summary of Clinical Outcomes

In the NEJ 002 study [13], 230 patients who had sensitive *EGFR* mutations were enrolled and were randomly assigned to either gefitinib (n = 115) or carboplatin plus paclitaxel (n = 115), and 114 and 110 patients, respectively, were included in the PFS analysis (Fig. 1). Patients in the gefitinib arm had a significantly longer PFS time (median PFS time, 10.8 months versus 5.4 months; HR, 0.30; 95% CI, 0.22–0.41; $p < .001$) and a higher response rate (73.7% versus 30.7%; $p < .001$) than patients in the chemotherapy arm. Second-line gefitinib was administered to 98.2% of patients in the carboplatin plus paclitaxel arm after disease progression. As a result, the median OS time was 27.7 months in the gefitinib arm and 26.6 months in the chemotherapy arm, with the difference in survival time not statistically significant (p = .48) [18]. The most common adverse events of any grade were rash (71.1%) and aspartate aminotransferase or alkaline phosphatase elevation (55.3%) in the gefitinib arm and neutropenia (77.0%), anemia (64.6%), appetite loss (56.6%), and sensory neuropathy (54.9%) in the chemotherapy arm [13].

Baseline QoL

Of the 224 patients, the QoL booklets of 163 patients (73%) were collected by their doctors and sent to the QoL data center.

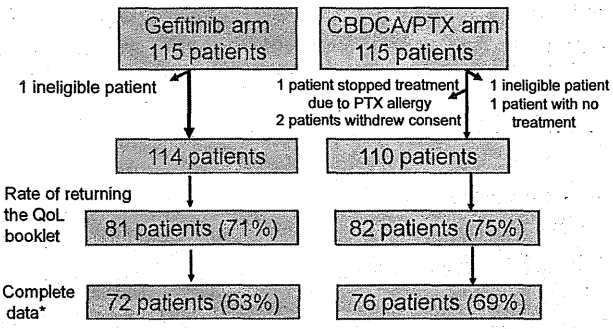

Figure 1. Patient disposition.

*The complete dataset was defined as having both a pretreatment measurement (baseline) and measurement(s) after starting the treatment during first-line therapy.

Abbreviations: CBDCA, carboplatin; PTX, paclitaxel; QoL, quality of life.

The rates of compliance among these 73% of patients were similar in the two arms. Of the 163 patients, 15 patients failed to provide complete information on their QoL prior to first-line therapy (nine patients in the gefitinib arm and six patients in the chemotherapy arm). Seventy-two patients (63%) in the gefitinib arm and 76 patients (69%) in the chemotherapy arm were investigated in this QoL analysis (Fig. 1). Demographics and disease characteristics were found to be well balanced in the two arms and were similar to those for the primary PFS analysis [13] (Table 1). Most patients had an Eastern Cooperative Oncology Group performance status (PS) score of 0 or 1 at the time of enrollment. Toxicity profiles for the patients in the QoL analysis were also similar to those for the patients in the PFS analysis [13].

Before the initiation of treatment, patients in both arms had similar baseline QoL scores on all subscales (Table 2). They had a low burden of physical well-being, but impairment was seen in the anxiety subscale (mean score, 40.5 and 40.8 in the gefitinib and carboplatin plus paclitaxel arms, respectively).

Time to Deterioration in QoL

In terms of the minimal clinically important difference in QoL, previous studies indicated that patients perceived a 5%–7% change in the scores on QoL questionnaires as clinically significant [23, 24]. The NCIC CTG recommends a 10% change as the value for clinical significance [25]. In the primary analysis of QoL in the NEJ 002 trial, deterioration was recognized when the score changed from baseline by one in 11 points (9.1%) or more in a direction indicating worse QoL at any timepoint. This criterion was chosen on the basis of our previous study, which estimated content validity by performing interviews with cancer patients (unpublished results). The times to 9.1% deterioration for pain and shortness of breath, anxiety, and daily functioning are summarized in Figure 2A. Significant differences between treatment arms were observed in deterioration of pain and shortness of breath (HR, 0.34; 95% CI, 0.23–0.50; $p < .0001$) and daily functioning (HR, 0.43; 95% CI, 0.28–0.65; $p < .0001$). There was no significant difference in anxiety between arms (HR, 0.72; 95% CI, 0.46–1.13; p = .14).

Table 1. Characteristics of patients in QoL analysis

Characteristic	Gefitinib (n = 72), n (%)	CBDCA/PTX (n = 76), n (%)	p-value
Gender			
Male	24 (33%)	29 (38%)	.608[a]
Female	48 (67%)	47 (62%)	
Mean age (range), yrs	63.0 (43–75)	62.2 (35–74)	.576[b]
Smoking status			
Never	51 (71%)	46 (61%)	.227[a]
Ever	21 (29%)	30 (39%)	
Performance status score, 0/1/2	40/32/0	43/32/1	.959[c]
Histology, adenocarcinoma/other	67/5	74/2	.495[a]
Stage, IIIB/IV/postoperative	10/52/10	15/52/9	.621[a]
Type of mutation			
Deletion	37 (51%)	36 (47%)	.616[a]
L858R	31 (43%)	36 (47%)	
Other	4 (6%)	4 (6%)	

Characteristics of patients investigated in the QoL analysis had no significant differences between arms.
[a]Fisher's exact test.
[b]t-test.
[c]Wilcoxon test.
Abbreviations: CBDCA, carboplatin; PTX, paclitaxel; QoL, quality of life.

Table 2. Baseline QoL scores

	Gefitinib		CBDCA/PTX	
Measure	Mean points	SD	Mean points	SD
Physical well-being	11.2	13.5	10.4	12.0
Appetite loss	6.8	13.0	5.9	11.5
Constipation	7.5	14.1	8.0	12.3
Pain and shortness of breath	13.5	23.2	10.5	18.5
Mental well-being	27.6	26.2	25.0	20.6
Anxiety	40.8	31.3	40.5	24.6
Irritation	18.3	25.2	14.3	20.4
Depression	23.5	27.9	20.0	24.3
Life well-being	26.4	19.3	22.9	17.1
Daily functioning	31.1	27.0	25.5	22.8
Social functioning	13.4	18.4	10.4	13.8
Subjective QoL	30.5	23.0	29.4	21.2

A 0–10 linear analog rating was changed to 0–100 points. For physical and mental well-being, a score of 100 represents the highest burden of symptoms. For life well-being, a score of 100 represents the worst possible function or QoL by changing the score polarity. There were no significant differences in scale and subscale scores between arms before starting first-line therapies.
Abbreviations: CBDCA, carboplatin; PTX, paclitaxel; QoL, quality of life; SD, standard deviation.

From previous reports [23, 24], a change in QoL score >20%, indicating more severe QoL deterioration, was also investigated. Figure 2B summarizes the time to a 27.3% (three of 11 points) deterioration in pain and shortness of breath, anxiety, and daily functioning. Patients who received gefitinib had a significantly longer time to deterioration than patients who received carboplatin plus paclitaxel for pain and shortness of breath (HR, 0.28; 95% CI, 0.17–0.46; $p < .0001$) and daily functioning (HR, 0.32; 95% CI, 0.17–0.59; $p < .0001$) as well as anxiety (HR, 0.44; 95% CI, 0.22–0.87; $p = .01$), for which a significant difference was not observed in the analysis of a 9.1% deterioration (see above).

Proportion of Patients with Improved, Stable, or Worsened QoL

Table 3 details the QoL responses according to three categories (improved, stable, worse) defined in Methods. The χ^2 test indicated that the QoL subscales of appetite loss ($p = .014$), constipation ($p < .0001$), and pain and shortness of breath ($p < .0001$) favored gefitinib over standard chemotherapy, leading to superiority of the gefitinib group on the physical well-being scale ($p < .0001$). A similar trend was observed for the QoL subscales of daily functioning ($p = .007$), social functioning ($p = .035$), and subjective QoL ($p = .042$), leading to superiority of the gefitinib group on the life well-being scale ($p < .0001$). The subscale of the mental well-being scale did not show any significant difference between the treatment arms ($p = .458$).

Discussion

This QoL analysis clearly demonstrated superior QoL in NSCLC patients with mutated *EGFR* receiving gefitinib, com-

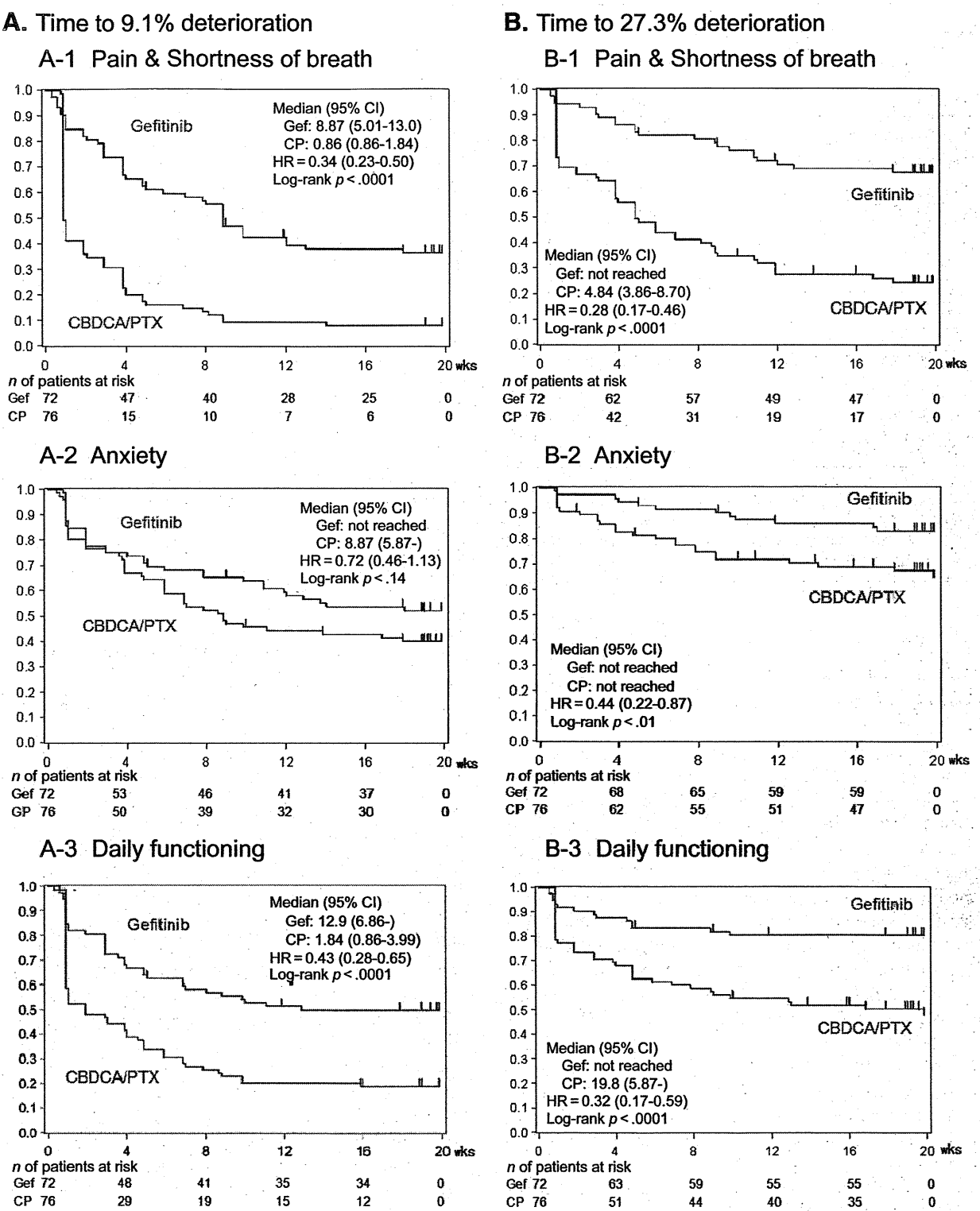

Figure 2. Time to deterioration of QoL. **(A):** Time to a 9.1% QoL deterioration for pain and shortness of breath **(A-1)**, anxiety **(A-2)**, and daily functioning **(A-3)** **(B):** Time to a 27.3% QoL deterioration for pain and shortness of breath **(B-1)**, anxiety **(B-2)**, and daily functioning **(B-3)**.

Abbreviations: CBDCA, carboplatin; CI, confidence interval; CP, carboplatin plus paclitaxel; Gef, gefitinib; HR, hazard ratio; PTX, paclitaxel; QoL, quality of life.

Table 3. QoL response

	Gefitinib, *n*			CBDCA/PTX, *n*			
Measure	**Improved**	**Stable**	**Worse**	**Improved**	**Stable**	**Worse**	***p*-value**
Physical well-being	18	28	26	16	10	50	<.0001
Appetite loss	13	21	38	14	8	54	.014
Constipation	16	24	32	23	6	47	<.0001
Pain and shortness of breath	21	18	33	16	3	57	<.0001
Mental well-being	33	16	23	40	11	25	.458
Anxiety	48	8	16	57	6	13	.535
Irritation	27	18	27	27	11	38	.181
Depression	35	15	22	36	10	30	.346
Life well-being	38	22	12	32	8	36	<.0001
Daily functioning	40	10	22	30	4	42	.007
Social functioning	23	28	21	16	22	38	.035
Subjective QoL	41	15	16	38	8	30	.042

In a secondary analysis of QoL responses, patients were classified as improved (>9.1%), stable (<9.1%, >−9.1%), or worsened (<−9.1%) for all scales and subscales according to the National Cancer Institute of Canada Clinical Trials Group standard QoL analysis framework.
The χ^2 test was used to compare the distributions of these three categories between two treatment arms.
Abbreviations: CBDCA, carboplatin; PTX, paclitaxel; QoL, quality of life.

pared with patients receiving chemotherapy. Better QoL in patients receiving gefitinib further endorses the preference of gefitinib as the first-line therapy for patients with NSCLC with mutated *EGFR* despite a lack of difference in OS outcomes. Accordingly, integration of QoL analyses into a clinical trial should be considered, because maintenance of a good QoL solidifies the clinical efficacy of the treatment being investigated. In addition, this analysis also highlights the importance of QoL endpoints in randomized trials analyzing PFS outcomes, because OS outcomes may be affected by subsequent therapies.

QoL recorded by patients in a self-reported form accurately demonstrated how the patients felt about their QoL during treatment. As soon as chemotherapy with carboplatin plus paclitaxel was started, a striking difference in QoL was observed (Fig. 2A). It seems reasonable that physical well-being deteriorated with chemotherapy in a high proportion of patients, considering that >95% of patients had a PS score of 0–1, a fact that is probably reflected by the low scoring in the baseline scores of physical well-being and daily functioning, with the majority of patients scoring <30. The NCIC CTG recommended matrix (Table 2) also showed that physical well-being was stable or improved in 60% of patients in the gefitinib group. In sharp contrast, scores for physical well-being deteriorated in 75% of patients in the chemotherapy group. This better QoL in the gefitinib group will help patients to maintain social activities, continue to work, and enjoy spending time with their families.

When patients were treated with gefitinib monotherapy in other trials, QoL and symptom improvement were rapid and were correlated with tumor response and survival [26, 27]. In the BR.21 study using unselected patients, another EGFR TKI, erlotinib, also improved tumor-related symptoms and important aspects of QoL such as physical functioning [28]. Post hoc investigations in the IPASS study employing selection by background indicated that QoL was better in the gefitinib group than in the chemotherapy group for patients with *EGFR*-mutated NSCLC [29]. Taken together with our first prospective QoL analysis of patients with *EGFR*-mutated NSCLC, EGFR TKI therapy provides an advantage in terms of improving QoL and symptoms over conventional cytotoxic agents.

The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30) [30] and Functional Assessment of Cancer Therapy (FACT)–General [31] have been used in many clinical trials to assess the QoL of patients worldwide, and we have developed and validated Japanese versions of these tests for use mainly in clinical studies with the original developers [32, 33]. The Care Notebook [20–22] was originally developed in the 1990s for clinical practice and has a notebook-style format to collect valid and reliable QoL information repeatedly. The NEJ 002 study lacked sufficient support from clinical research coordinators, and doctors had to personally administer QoL questionnaires to patients and pick them up after the answers were completed. Therefore, we chose the Care Notebook, which has good results concerning concurrent validity with the EORTC QLQ-C30 and FACT–Spiritual Well-being [22], for QoL investigation on a weekly basis instead of the above gold standard questionnaires. More than 3,000 Care Notebooks were collected during the initial 20 weeks of treatment in this study, and this method might be the first success of a QoL investigation on a weekly basis for advanced cancer patients in a phase III trial.

This study has some limitations. First, compliance with the QoL survey was modest. Missing data in the QoL investigation

were found to be institution dependent. Namely, the doctors in some institutions did not give the Care Notebook to patients or did not pick it up after the answers were completed. However, randomization of the study treatments was stratified by institution, and therefore, the effects of selection bias might not be large. Both arms had similar patient characteristics (Table 1) and similar baseline QoL scores (Table 2). Although compliance was modest, this QoL difference between arms may represent that in the overall population. Secondly, because the primary endpoint of the NEJ 002 study focused on the PFS interval after first-line treatment, the QoL analysis also focused on patients treated during first-line treatment, and, therefore, the investigation period for the primary QoL analyses was relatively short (20 weeks) to reduce the effects of second-line treatment. Finally, the patients in this QoL analysis were a selected population—patients with a PS score of 0–1 whose tumor had *EGFR* mutation—which might potentially influence the QoL outcomes. However, in another study, namely the NEJ 001 study [7], which employed *EGFR* mutation-positive patients with an extremely poor PS, 68% of the patients improved from a PS score ≥3 at baseline to a PS score ≤1 with gefitinib therapy. Although no QoL investigation was conducted in the NEJ 001 study because of the patients being in extremely poor condition, the striking PS score improvement might have been related to improved QoL. This indicates that EGFR TKIs might universally ameliorate the QoL of patients with *EGFR*-mutated NSCLC, irrespective of their PS scores or symptomatic burdens.

Summary

The QoL analysis of the NEJ 002 study clearly demonstrated that gefitinib maintained patient QoL longer than carboplatin plus paclitaxel during first-line treatment. A longer PFS interval with a better QoL during first-line treatment is valuable for advanced NSCLC patients with limited survival times. Although the OS time for patients first treated using gefitinib was not significantly different from that of patients treated using chemotherapy, the first-line use of gefitinib for advanced NSCLC harboring *EGFR* mutations is strongly recommended.

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This study is registered in University Hospital Medical Information (UMIN) Network Clinical Trial Registry (identification number, UMIN C000000376).

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References

1. Lynch TJ, Bell DW, Sordella R et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–2139.

2. Paez JG, Jänne PA, Lee JC et al. EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 2004;304:1497–1500.

3. Pao W, Miller V, Zakowski M et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004; 101:13306–13311.

4. Inoue A, Suzuki T, Fukuhara T et al. Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. J Clin Oncol 2006;24:3340–3346.

5. Asahina H, Yamazaki K, Kinoshita I et al. A phase II trial of gefitinib as first-line therapy for advanced non-small cell lung cancer with epidermal growth factor receptor mutations. Br J Cancer 2006;95:998–1004.

6. Sutani A, Nagai Y, Udagawa K et al. Gefitinib for non-small-cell lung cancer patients with epidermal growth factor receptor gene mutations screened by peptide nucleic acid-locked nucleic acid PCR clamp. Br J Cancer 2006;95:1483–1489.

7. Inoue A, Kobayashi K, Usui K et al. First-line gefitinib for patients with advanced non-small-cell lung cancer harboring epidermal growth factor receptor mutations without indication for chemotherapy. J Clin Oncol 2009;27:1394–1400.

8. Morita S, Okamoto I, Kobayashi K et al. Combined survival analysis of prospective clinical trials of gefitinib for non-small cell lung cancer with EGFR mutations. Clin Cancer Res 2009;15:4493–4498.

9. Kim ES, Hirsh V, Mok T et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): A randomised phase III trial. Lancet 2008;372:1809–1818.

10. Maruyama R, Nishiwaki Y, Tamura T et al. Phase III study, V-15–32, of gefitinib versus docetaxel in previously treated Japanese patients with non-small-cell lung cancer. J Clin Oncol 2008;26:4244–4252.

11. Lee DH, Park K, Kim JH et al. Randomized phase III trial of gefitinib versus docetaxel in non-small cell lung cancer patients who have previously received platinum-based chemotherapy. Clin Cancer Res 2010;16: 1307–1314.

12. Mok TS, Wu YL, Thongprasert S et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–957.

13. Maemondo M, Inoue A, Kobayashi K et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–2388.

14. Mitsudomi T, Morita S, Yatabe Y et al. 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 2010;11: 121–128.

15. Zhou C, Wu YL, Chen G et al. 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 2011;12:735–742.

16. Rosell R, Carcereny E, Gervais R et al. 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 2012;13:239–246.

17. Fukuoka M, Wu YL, Thongprasert S et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011;29:2866–2874.

18. Inoue A, Kobayashi K, Maemondo M et al. Final overall survival results of NEJ 002, a phase III trial comparing gefitinib to carboplatin (CBDCA) plus paclitaxel (PTX) as the first-line treatment for advanced non-small cell lung cancer (NSCLC) with EGFR mutations. J Clin Oncol. 2011;29(15 suppl):480s.

19. Nagai Y, Miyazawa H, Huqun et al. Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. Cancer Res 2005;65: 7276–7282.

20. Care Notebook Center. Care Notebook. Available at http://homepage3.nifty.com/care-notebook/, accessed March 31, 2006.

21. Ando M, Kobayashi K, Kudoh S et al. Using Care Note to measure the level of satisfaction patients feel with their care, in palliative cancer care, as a measure of their quality of life. J Nippon Med Sch 1997;64:538–545.

22. Kobayashi K, Green J, Shimonagayoshi M et al. Validation of the care notebook for measuring physical, mental and life well-being of patients with cancer. Qual Life Res 2005;14:1035–1043.

23. Osoba D, Rodrigues G, Myles J et al. Interpreting the significance of changes in health-related quality-of-life scores. J Clin Oncol 1998;16:139–144.

24. Cella D, Eton DT, Fairclough DL et al. What is a clinically meaningful change on the Functional Assessment of Cancer Therapy-Lung (FACT-L) Questionnaire? Results from Eastern Cooperative Oncology Group (ECOG) Study 5592. J Clin Epidemiol 2002;55: 285–295.

25. Osoba D, Bezjak A, Brundage M et al. Analysis and interpretation of health-related quality-of-life data from clinical trials: Basic approach of The National Cancer Institute of Canada Clinical Trials Group. Eur J Cancer 2005;41:280–287.

26. Kris MG, Natale RB, Herbst RS et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: A randomized trial. JAMA 2003;290:2149–2158.

27. Cella D, Herbst RS, Lynch TJ et al. Clinically meaningful improvement in symptoms and quality of life for patients with non-small-cell lung cancer receiving gefitinib in a randomized controlled trial. J Clin Oncol 2005;23:2946–2954.

28. Bezjak A, Tu D, Seymour L et al. Symptom improvement in lung cancer patients treated with erlotinib: Quality of life analysis of the National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 2006;24:3831–3837.

29. Thongprasert S, Duffield E, Saijo N et al. Health-related quality-of-life in a randomized phase III first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients from Asia with advanced NSCLC (IPASS). J Thorac Oncol 2011;6:1872–1880.

30. Aaronson NK, Ahmedzai S, Bergman B et al. The European Organization for Research and Treatment of Cancer QLQ-C30: A quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst 1993;85:365–376.

31. Cella DF, Tulsky DS, Gray G et al. The Functional Assessment of Cancer Therapy scale: Development and validation of the general measure. J Clin Oncol 1993;11: 570–579.

32. Kobayashi K, Takeda F, Teramukai S et al. A cross-validation of the European Organization for Research and Treatment of Cancer QLQ-C30 (EORTC QLQ-C30) for Japanese with lung cancer. Eur J Cancer 1998;34:810–815.

33. Fumimoto H, Kobayashi K, Chang CH et al. Cross-cultural validation of an international questionnaire, the General Measure of the Functional Assessment of Cancer Therapy Scale (FACT-G), for Japanese. Qual Life Res 2002;10:701–709.

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First-Line Gefitinib in Patients Aged 75 or Older With Advanced Non–Small Cell Lung Cancer Harboring Epidermal Growth Factor Receptor Mutations

NEJ 003 Study

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Introduction: Recent studies have demonstrated that first-line treatment with gefitinib, an epidermal growth factor receptor (EFGR)–targeted tyrosine kinase inhibitor, is significantly superior to standard chemotherapy for advanced non–small-cell lung cancer (NSCLC) harboring EGFR sensitive mutations. Meanwhile, the efficacy of gefitinib therapy among elderly populations diagnosed with EGFR-mutated NSCLC has not yet been elucidated. The purpose of this study was to investigate the efficacy and feasibility of gefitinib for chemotherapy-naive patients aged 75 or older with NSCLC harboring EGFR mutations; generally, these patients have no indication for treatment with platinum doublets.

Methods: Chemotherapy-naive patients aged 75 years or older with performance status 0 to 1 and advanced NSCLC harboring EGFR mutations, as determined by the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method, were enrolled. The enrolled patients received 250 mg/day of gefitinib orally.

Results: Between January 2008 and May 2009, 31 patients were enrolled, all of whom were eligible. The median age was 80 (range, 75–87) years. Twenty-five patients (81%) were women, and 30 patients (97%) had adenocarcinoma. The overall response rate was 74% (95% confidence interval, 58%–91%), and the disease control rate was 90%. The median progression-free survival was 12.3 months. The common adverse events were rash, diarrhea, and liver dysfunction. One treatment-related death because of interstitial lung disease occurred.

Conclusions: This is the first study that verified safety and efficacy of first-line treatment with gefitinib in elderly patients having advanced NSCLC with EGFR mutation. Considering its strong antitumor activity and mild toxicity, first-line gefitinib may be preferable to standard chemotherapy for this population.

Key Words: Non–small cell lung cancer, Epidermal growth factor receptor mutation, Gefitinib

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Non–small-cell lung cancer (NSCLC), which accounts for 80% of lung cancer, remains the major cause of cancer-related death in both Western and Asian countries. With prolongation of life expectancy, both the incidence and mortality of lung cancer in the elderly are rising. In Japan, 48 500 individuals aged 70 years or older were estimated to die of lung cancer in 2009[1]; moreover, the ratio of elderly patients dying from lung cancer increased from 57% in 1989 to 72% in 2009. Treatment strategy in elderly patients with lung cancer has, thus, become an important issue.

About half of the newly diagnosed NSCLC patients have advanced disease, with no indication for local therapy such as surgery and radiotherapy. Chemotherapy for the elderly shows similar efficacy to that observed in younger

patients. However, it is generally more toxic, in terms of both incidence and severity, because of age-related weakening of organ function.[2] Consequently, standard chemotherapy for elderly NSCLC patients, especially those aged 75 years or older, is performed as monotherapy with vinorelbine, gemcitabine, or docetaxel instead of platinum doublets, which are the standard for younger patients.[3–7] Although a recent phase III study suggested that the platinum doublet of monthly carboplatin and weekly paclitaxel may be superior to the gemcitabine or vinorelbine monotherapy in the elderly population, the treatment-related death rate of the doublet group was determined to be 7%.[8] Thus, investigation into safer and more effective treatments for elderly NSCLC patients is required.

Gefitinib, an orally administered tyrosine kinase inhibitor (TKI) of the epidermal growth factor receptor (EGFR), is a key molecularly targeted drug used for the treatment of advanced NSCLC. In May 2004, seminal studies showed that the presence of somatic mutations in the kinase domain of EGFR strongly correlated with increased responsiveness to EGFR TKIs in patients with NSCLC.[9,10] Before this observation, it had been known that subgroups of NSCLC patients, including those of Asian race, female sex, nonsmoking status, and having adenocarcinoma, displayed significant responses to gefitinib.[11,12] These subgroups turned out to have a high incidence of EGFR mutations.[13] Recently, two phase III studies comparing gefitinib treatment with chemotherapy in chemo-naive patients selected on the basis of EGFR mutations were reported from Japan.[14,15] These studies revealed the superiority of gefitinib treatment over standard chemotherapy by demonstrating that first-line gefitinib administration doubled progression-free survival (PFS) as compared with standard chemotherapy. One of two studies we conducted, namely the NEJ002 study, demonstrated that treatment with gefitinib provided patients with a better quality of life as compared with chemotherapy.[16] The eligibility criteria in these studies was limited to patients aged 75 years or younger, as the treatments with platinum doublets were considered to be inappropriate for more elderly populations because of increased toxicity. Moreover, it has been reported in Japan that this more elderly group of patients develop interstitial lung disease (ILD) frequently when treated with gefitinib.[17] In previous studies, we demonstrated that patient selection by EGFR mutation can dramatically improve the risk–benefit balance of gefitinib treatment; however, no study thus far has investigated the efficacy and feasibility of first-line gefitinib treatment in elderly NSCLC patients with EGFR mutation. Thus, the current phase II study was conducted.

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METHODS

Patient Selection

This multicentric phase II study was approved by the institutional review board of each participating institute. The main eligibility criterion was to select chemotherapy-naive patients with NSCLC harboring sensitive EGFR mutations. Namely, patients with exon 19 deletions, L858R, L861Q, G719A, or G719S were included, but those with a resistant T790M mutation were excluded. Patients who were 75 years of age or older with Eastern Cooperative Oncology Group performance status (PS) 0 to 2 were also deemed eligible. Other eligibility requirements were stage IIIB to IV or postoperative recurrent NSCLC, presence of a measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST), adequate organ function including liver function (aspartate transaminase and alanine aminotransferase ≤100 U/liter, total bilirubin <2.0 mg/dL), and written informed consent.

EGFR Mutation

Cytological or histological specimens were examined for EGFR mutation by the peptide nucleic acid-locked nucleic acid polymerase chain reaction (PCR) clamp method.[18] Briefly, genomic DNA fragments containing mutation hot spots of the EGFR gene were amplified via PCR in the presence of a peptide nucleic acid clamp primer synthesized from a peptide nucleic acid with a wild-type sequence. This method leads to preferential amplification of the mutant sequence, which is then detected by a fluorescent primer that incorporates locked nucleic acids to increase the specificity. As a result, the mutant EGFR sequence is detected in specimens that contain 100 to 1000 excess copies of wild-type EGFR sequence. The sensitivity and specificity of the peptide nucleic acid-locked nucleic acid PCR clamp method are 97% and 100%, respectively.

Drug Administration

Gefitinib was administered orally once a day at a dose of 250 mg. Patients continued to receive gefitinib until progression of disease, occurrence of intolerable severe toxicity, or withdrawal of consent. When severe toxicity was observed, patients were allowed to receive a reduced dose of gefitinib in accordance with the protocol.

Treatment Assessment

Complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) were determined based on RECIST version 1.0. The primary end point of this study was overall objective response rate (ORR), which was the rate of patients with CR + PR; secondary end points were PFS, overall survival (OS), and toxicities. Computer

tomography (CT) scans were taken every month until CR or PR was observed. CR and PR required confirmation via reassessment no earlier than 4 weeks after the first assessment meeting the criteria for response. After the confirmation, CT scans were taken every other month until PD was observed. The CT films of all patients were extramurally reviewed for confirmation of response. PFS was defined as the time from the date of randomization to the first observation of disease progression or death. OS was defined as the time from the date of randomization to the date of death or the most recent follow-up. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0.

Statistical Consideration

Sample size was determined using the data as follows. Response rates greater than 70% had been previously observed in nonage-restricted patients with EGFR-mutated NSCLC.[15] Meanwhile, clinical studies with elderly patients that investigated the efficacy of first-line chemotherapies in Japan showed ORR of 28% to 55%.[7,19] Thus, we assumed that an ORR of more than 55% was clinically useful, whereas an ORR of less than 30% was not clinically useful. With $\alpha = 0.05$ and $\beta = 0.1$, the number of patients required was 27. Allowing 10% loss in follow-up, a total of 30 patients were planned for enrollment.

All enrolled patients were evaluated for efficacy of received regimen. All patients treated with gefitinib, even for a short period of time, were entered into safety analysis.

RESULTS

Patient Characteristics

Between January 2008 and May 2009, a total of 31 patients were enrolled. Baseline characteristics are described in Table 1. The median age at the time of enrollment was 80.3 years (range, 75–89 years); 52% of the patients were over the age of 80. Of the 31 patients enrolled, 25 (81%) were women and 2 (6%) had a PS of 2. Histological types were all adenocarcinoma except for one adenosquamous carcinoma. There were 7 patients (23%) with stage IIIB, 22 (71%) with stage IV, and 2 (6%) with postoperative recurrence.

Efficacy

The ORR was 74.2% (95% confidence interval [CI], 57.9%–90.5%); one patient had CR, and 22 patients had PR. Five of the remaining 8 patients (16.1%) had SD, with the resulting disease control rate (CR + PR + SD) reaching 90.3% (Table 2). This result attained the primary end point by a wide margin. The median follow-up period at the time of analysis was 27.5 months. Of all 31 patients enrolled, 15 (48.3%) were alive and free from progression for at least 6 months. The median PFS was 12.1 months (Fig. 1*A*), the 1-year OS was 83.9% (95% CI, 70.2%–97.6%), and 2-year OS was 58.1% (95% CI, 45.2%–70.9%). At the data cutoff point (December 2010), 13 patients (41.9%) had died, and the median OS was 33.8 months (Fig. 1*B*).

TABLE 1. Character

	N = 31	(%)
Sex		
Women	6	19
Men	25	81
Age		
Mean (SD)	80.3	(4.1)
Range	75–89	
Smoking status		
Nonsmoker	23	74
Smoker	8	26
Performance status		
0	16	55
1	13	39
2	2	6
Stage		
IIIB	7	23
IV	22	71
Postop	2	6
Histology		
Adenocarcinoma	30	97
Adenosquamous	1	3

Safety and Toxicity

Toxicity data for all 31 patients are presented in Table 3. Nine patients (29%) had a grade 3 adverse event (AE); 1 had a grade 5 AE ILD, and died of respiratory failure. The most common hematologic AE was elevation of transaminases; grade 3 to 4 elevation occurred in three patients (19%). The most common nonhematologic AEs were rash in 21 patients (71%), diarrhea in 10 patients (32%), and appetite loss in 9 patients (29%). Dose reduction was seen in 14 patients (45%). Incidence and severity of AEs were acceptable and comparable with previous reports.[13–15]

Treatment After Progression of Disease

Patient management after the protocol treatment was retrospectively investigated. Any treatment was allowed after confirmation of PD. Gefitinib was continued in 10 of 20 patients confirmed to have PD. Three patients were treated with monotherapies of cytotoxic agents, including vinorelbine, gemcitabine, or docetaxel, and one patient was given

TABLE 2. Response Rate of Treatment With Gefitinib

Response	N = 31	(%)
CR	1	3
PR	22	71
Stable disease	5	16
Progressive disease	3	10
Overall response rate (CR + PR)	23	74
95% confidence interval		(57.9–90.5)

CR, complete response; PR, partial response.