advanced stages at the time of cancer diagnosis), and a lower likelihood of receiving definitive treatment [9–18]. However, previous studies did not consider these variables and did not clarify the effects of each factor on the associations between marital status and sex-specific survival from NSCLC.

In this prospective study, we investigated the influence of marital status on survival in patients with NSCLC in Japan. We were able to evaluate survival according to each sex and marital status in view of potential confounding factors and to clarify the effects of each modifying factor, such as smoking habits, psychological reactions, delays in seeking treatment, and likelihood of receiving definitive treatment, on the associations between marital status and survival. If several intermediate factors are provided, the physician could suggest possible means of improving the prognosis to their patients.

Methods

Participants

The design of this study, which was included as part of The Lung Cancer Database Project in Japan, has been reported in detail elsewhere [19]. Briefly, consecutive newly diagnosed lung cancer patients were invited to participate in the study, which was conducted at the Thoracic Oncology Division, National Cancer Center Hospital East, Kashiwa, Japan. Patients were included in the database study if they met all of the following criteria: informed of their lung cancer diagnosis; newly diagnosed patients with primary lung cancer; physically capable of completing the questionnaires; absence of cognitive impairment, such as dementia and delirium; ability to provide written consent; and no problems regarding the patients' participation in this project, as judged by their physicians.

In total, the project was explained to 2506 patients, and 2036 (81.3%) patients with newly diagnosed, untreated primary lung cancer were admitted during the project enrolment period. A total of 470 cases were ineligible for the following reasons: could not be contacted (49 cases), lung cancer diagnosis not confirmed at the time of admission (175 cases), non-lung cancer (120 cases), poor physical state (77 cases), refusal to participate in the project (43 cases), treated for lung cancer at another hospital (5 cases), and not yet informed of the diagnosis (1 case). For 40 of the 2036 patients, written informed consent could not be confirmed, and one patient withdrew consent during the follow-up period. Finally, the analyzed cohort consisted of 1995 patients.

As a result, the analytic cohort consisted of 1995 patients who were enrolled in the study between July 1999 and July 2004. The study protocol was approved by the institutional review board of the National Cancer Center, Japan. Each patient was fully informed of the purpose of the study before obtaining written consent and prior to participation in the study.

Exposure data

The patients completed the questionnaires during the waiting period prior to admission, and the questionnaires were collected after the patients were admitted. Questionnaires on demographic data and health habits (excluding the questionnaires on psychological factors) were distributed to all patients who had been registered by July 2004. Questionnaires on psychological factors were distributed only to patients who had registered by July 2003.

Demographic factors (age at cancer diagnosis, sex, education level, marital status, body mass index [BMI], smoking status, past history of cancer) and medical information (histology, clinical stage, PS, and first treatment) were obtained from the self-administered questionnaires and the patients' medical charts. PS was assessed by each attending physician using the Eastern Cooperative Oncology Group criteria [20].

To examine patient characteristics associated with variations in best-treatment practices, we defined, *a priori*, the minimally recommended initial therapies for each cancer stage at the time of diagnosis. As a practical matter, therapy for lung cancer is mainly decided, which take into account not only clinical stage but also age, comorbid illness, organopathy, and physical status. For the purposes of this analysis, the determination of the recommended therapies was based on pertinent information from medical literature published before 2004, including both randomized trials and meta-analyses of randomized trials, as well as the definitions of accepted therapy reflected in the Japan Lung Cancer Society clinical practice guidelines for the treatment of lung cancer, published in 2005 [21]. For tumor-node metastasis system stages I, II, and IIIA N0-1 surgical resection was considered the recommended initial therapy. For stage IIIA N2 patients, combination chemoradiotherapy was defined as the recommended therapy. For stage IIIB patients, combination chemoradiotherapy or chemotherapy alone was defined as the recommended therapy. For patients with stage IV disease, chemotherapy alone was considered the recommended therapy.

Depression symptoms were evaluated using the depression subscale of the Hospital Anxiety and Depression Scale (HADS) [22]. The HADS has

been used as a reliable and valid method of screening for depression in patients with cancer. Each item is rated on a scale of 0–3, with higher scores denoting a greater mood disturbance. The reliability and validity of the Japanese version of this questionnaire has been established in Japanese cancer patients [23]. The present study used a cutoff point of four out of five [23].

Follow-up

In order to follow up the subjects for vital status, confirmation was made by medical records, normal postal mail, and municipality registration data. The survival of subjects was followed from July 1999 to December 2004. The psychological questionnaire was only distributed to the patients who had registered by July 2003. In this study, we analyzed the subject who answered psychological questionnaire. Out of the remaining 1995 patients, 414 patients were excluded from the analysis because of lack of psychological questionnaires. A total of 351 cases were excluded from the analysis for the following reasons: double cancer (188 cases) or SCLC (163 cases). Finally, 1230 patients were included in the subsequent analyses.

The person-months of follow-up were counted for each subject from the date of enrollment in the study until death or the end of the study period (December 2004), whichever occurred first, and a total of 31 508 person-months (median, 24 months; range, 0–67 months) were accrued. During the follow-up period, 716 deaths from all causes were identified.

Statistical analysis

All statistical analyses were performed according to sex. Standard descriptive statistics were used to characterize the marital status. Thus, marital status was categorized into married, widowed, separated/divorced, and single. Intergroup comparisons of categorical and continuous variables were performed using chi-square tests and one-way analyses of variance, respectively. Hazard ratios (HRs) were computed as the number of deaths from all causes among the subjects in each marital status category versus the number of deaths from all causes among the respective reference category (married patients). A Cox proportional-hazards regression analysis was conducted to adjust for age at the time of cancer diagnosis, BMI in kg/m^2 (<18.5, >18.5, or unknown), education level (college/university or higher, or not), PS (0, 1, or 2–4) histological type (adenocarcinoma, squamous carcinoma, large, or other), smoking status (never-smoker, ex-smoker, or current smoker), clinical stage (IA–IIB, IIIA–IIIB, or IV), HADS depression score (<5, $\geqslant 5$, or unknown), and choice of cancer treatment (definitive treatment or non-definitive treatment) using the SAS PHREG procedure included in the SAS version 8.2 statistical software package (Cary, NC, USA). The assumption of proportional hazards was verified graphically. In all the statistical evaluations, p-values of less than or equal to 0.05 were considered to denote a significant difference. All p-values were two-tailed.

In secondary analyses, we also conducted stratified analyses to examine factors that markedly modified the associations between marital status and survival, such as smoking status, clinical stage, HADS-depression, or definitive treatment.

Results

The mean age of the subjects was 63.9 years, and the percentage of men was 70%. The proportions of married, widowed, separated/divorced, and single patients were 84, 9, 4, and 3%, respectively. The mean age differed significantly according to marital status for both male and female patients (Table 1). Moreover, the smoking status also differed significantly according to marital status for both male and female patients. In women, BMI, histology, and definitive treatment differed significantly according to marital status. No significant associations between marital status and any other variables were seen.

According to the univariate Cox proportional-hazards regression analyses, six demographic or clinical variables were significantly associated with increased HRs of lung cancer survival for male and female subjects versus their respective reference categories: BMI (<18.5), smoking status (ex-smoker and current smoker), clinical stage (IIIA–IIIB or IV), PS (1 or 2–4), histology type (squamous cell carcinoma or large cell carcinoma), definitive treatment (non-definitive), and HADS depression score ($\geqslant 5$) (Table 2).

Table 3 shows the HRs for lung cancer survival according to marital status. A univariate Cox proportional-hazards regression analysis showed no significant association between survival and marital status for male and female subjects (Table 3). These findings remained basically unchanged even after multivariate adjustments for age, BMI, education level, PS, histology type, clinical stage, smoking status, choice of definitive treatment, and HADS depression score. For male patients, however, a multivariate Cox proportional-hazards regression analysis showed a significant association between survival and marital status. The multivariable adjusted HRs of widowed, separated/divorced, and single patients versus married patients were 1.7 (95% confidence interval (CI), 1.2–2.5; $p = 0.005$), 1.1 (0.7–1.7; $p = 0.72$), and 0.9 (0.5–1.5; $p = 0.61$), respectively.

Table 1. Demographic, medical, and psychological characteristics in NSCLC patients to marital status

	Male				Female			
	Marital status				Marital status			
	Married	Widowed	Separate/ divorced	Single	Married	Widowed	Separate/ divorced	Single
No. of subjects	774	41	26	24	262	72	19	12
Demographic characteristics								
Mean age in years (SD)	64.3 (8.9)	70.5 (8.3)	62.7 (8.4)	50.0 (10.1)	61.9 (9.3)	69.6 (8.2)	59.6 (8.1)	59.7 (14.6)
Body mass index (kg/m^2) (%)								
<18.5	11	10	12	8	8	3	26	25
≥18.5	88	90	85	92	91	96	74	75
Unknown	1	0	4	0	1	1	0	0
Duration of education (%)								
>15 yr	23	20	27	29	6	4	0	17
≤15 yr	77	78	73	71	94	96	100	83
Unknown	1	2	0	0	0	0	0	0
Smoking status (%)								
Never-smoker	4	0	0	25	76	71	42	58
Ex-smoker	33	44	23	4	7	11	16	8
Current smoker	62	56	77	71	17	18	42	33
Medical characteristics								
Clinical stage[a] (%)								
IA–IIB	44	44	38	25	57	71	53	50
IIIA–IIIB	29	39	42	29	18	10	26	8
IV	27	17	19	46	25	19	21	42
Performance status[b] (%)								
0	39	39	27	21	56	63	47	50
1	55	59	65	79	39	36	42	42
2–4	6	2	8	0	5	1	11	8
Histology type (%)								
Adenocarcinoma	57	49	62	67	86	88	68	75
Squamous cell carcinoma	28	44	35	25	8	6	26	17
Large cell carcinoma	12	7	0	8	6	7	5	0
Other	3	0	4	0	1	0	0	4
Definitive treatment (%)								
Definite	85	85	73	83	91	94	74	83
Non-definitive	15	15	27	17	9	6	26	17
Psychological characteristics								
HADS depression (%)								
<5	42	37	23	54	43	44	47	50
≥5	53	51	69	38	54	51	47	42
Unknown	5	12	8	8	3	4	5	8

[a] Defined by TNM classification: International Union Against Cancer.
[b] Defined by Eastern Cooperative Oncology Group (ECOG).

For female patients, however, a multivariate Cox proportional-hazards regression analysis showed no significant association between survival and marital status. The multivariable HRs of widowed, separated/divorced, and single patients versus married patients were 0.7 (0.5–1.1; $p = 0.15$), 0.5 (0.3–1.1; $p = 0.10$), and 1.2 (0.5–2.7; $p = 0.71$), respectively.

In addition, we conducted an effect modification analysis to assess the effects of clinical stage, smoking status, choice of definitive treatment, and HADS depression score on the relationship between marital status and survival in male widowed patients. All of these factors had no significant effect on the association between male widowed patients and survival ($p > 0.05$ for all variables).

No survival differences were seen between married and unmarried (including widowed, separated/divorced, and single) patients. The multivariable adjusted HR of unmarried patients versus married patients was 1.0 (0.8–1.2; $p = 0.91$).

Discussion

In this prospective study conducted in Japan, a significant association was found between marital status and survival in male patients with NSCLC. Male widowed patients had a higher mortality risk

Table 2. Results of univariate analysis for survival from lung cancer

	Male				Female			
	No. of subjects	Person-months median (range)	Cases	Univariate HR (95% CI)	No. of subjects	Person-months median (range)	Cases	Univariate HR (95% CI)
No. of subjects	865	21.6 (0.6–66.3)	548		365	26.8 (0.5–66.7)	168	
Demographic characteristics								
Age								
<49	58	20.5 (0.9–64.4)	38	1.0 (referent)	30	25.7 (1.8–56.7)	16	1.0 (referent)
50–59	193	21.8 (1.7–65.7)	125	1.0 (0.7–1.5)	95	28.1 (2.9–66.1)	42	0.7 (0.4–1.3)
60–69	350	20.7 (0.8–65.9)	221	1.0 (0.7–1.5)	136	28.2 (1.9–66.7)	63	0.8 (0.4–1.3)
70<	264	22.1 (0.6–66.3)	164	0.9 (0.7–1.4)	104	26.7 (0.5–63.7)	47	0.8 (0.4–1.4)
Body mass index (kg/m^2)								
⩾18.5	765	22.1 (0.6–66.3)	469	1.0 (referent)	330	27.3 (0.5–66.7)	146	1.0 (referent)
<18.5	93	14.4 (0.8–66.3)	73	1.6 (1.2–2.0)	31	18.6 (3.7–62.8)	20	1.9 (1.2–3.0)
Unknown	7	15.7 (5.6–58.6)	6	1.6 (0.7–3.6)	4	25.7 (11.7–30.7)	2	1.4 (0.3–5.5)
Duration of education								
>15 yr	197	20.3 (0.9–65.8)	122	1.0 (referent)	22	29.3 (3.4–45.2)	6	1.0 (referent)
⩽15 yr	661	21.9 (0.8–66.3)	423	0.9 (0.8–1.2)	343	26.7 (0.5–66.7)	162	1.8 (0.8–4.0)
Unknown	7	29.7 (0.6–63.9)	3	0.7 (0.2–2.3)	0	—	0	—
Smoking status								
Never-smoker	39	28.4 (2.4–63.9)	18	1.0 (referent)	265	28.3 (0.9–66.7)	110	10 (referent)
Ex-smoker	283	21.3 (0.8–66.3)	179	1.6 (0.9–2.6)	34	26.7 (0.5–54.8)	15	1.1 (0.7–1.9)
Current smoker	543	20.3 (0.6–66.3)	351	1.7 (1.1–2.8)	66	22.1 (1.8–63.6)	43	2.0 (1.4–2.9)
Medical characteristics								
Clinical stage[a]								
IA, IB, IIA, IIB	371	34.2 (3.1–66.3)	121	1.0 (referent)	216	35.2 (0.9–66.7)	45	1.0 (referent)
IIIA, IIIB	259	16.1 (0.6–65.9)	201	4.1 (3.3–5.2)	61	23.1 (3.2–65.6)	46	6.1 (4.0–9.3)
IV	235	8.0 (0.8–63.8)	226	9.8 (7.8–12.3)	88	11.3 (0.5–62.1)	77	12.0 (8.2–17.7)
Performance status[b] (%)								
0	336	29.7 (3.1–66.3)	140	1.0 (referent)	207	33.9 (0.9–66.7)	51	1.0 (referent)
1	482	15.6 (0.8–66.3)	363	2.8 (2.3–3.4)	141	21.5 (2.4–66.1)	100	4.2 (2.9–5.9)
2–4	47	4.1 (0.6–25.2)	45	12.7 (8.9–17.9)	17	5.7 (0.5–23.2)	17	28.9 (16.1–52.0)
Histology type								
Adenocarcinoma	490	23.0 (0.6–66.3)	306	1.0 (referent)	309	27.9 (0.5–66.7)	130	1.0 (referent)
Squamous cell carcinoma	252	20.7 (0.9–66.3)	157	0.9 (0.8–1.2)	32	24.9 (3.4–60.8)	23	2.2 (1.4–3.4)
Large cell carcinoma	99	14.6 (1.4–65.8)	72	1.4 (1.0–1.8)	21	22.5 (2.9–61.9)	14	1.8 (1.0–3.1)
Other	24	29.0 (2.8–65.6)	13	0.8 (0.4–1.3)	3	29.7 (22.9–57.6)	1	0.7 (0.1–4.8)
Definitive treatment								
Definitive	733	23.0 (0.8–66.3)	445	1.0 (referent)	331	27.9 (0.9–66.7)	140	1.0 (referent)
Non-definitive	132	10.1 (0.6–65.9)	103	1.9 (1.5–2.3)	34	12.9 (0.5–56.7)	28	3.2 (2.1–4.8)
Psychological characteristics								
HADS depression								
<5	452	23.5 (0.9–66.3)	265	1.0 (referent)	189	28.7 (0.9–66.7)	71	1.0 (referent)
⩾5	364	16.7 (0.8–65.9)	251	1.3 (1.1–1.6)	163	25.1 (0.5–66.1)	89	1.7 (1.2–2.4)
Unknown	49	24.4 (0.6–60.6)	32	1.1 (0.8–1.6)	13	38.6 (4.9–60.9)	8	1.6 (0.8–3.4)

[a] Defined by TNM classification: International Union Against Cancer.
[b] Defined by Eastern Cooperative Oncology Group (ECOG)

than male married patients. Our study had some methodological advantages over previous studies in that we were able to take into account differences in sex and marital status as well as potential modifying factors, such as smoking status, psychological variables, choice of definitive treatment, and disease stage at the time of diagnosis. The present study indicates that these potential modifying factors did not participate in association between marital status and survival in male patients with NSCLC. Further examinations are needed to clarify the details of this association.

Of the three studies that examined the association between marital status and lung cancer survival according to sex and subdivided marital status [7,8]. Kravdal [7] followed up SCLC and NSCLC patients (number of patients were not specified) and documented 15 882 deaths in males and 3944 deaths in females. Single female patients had a higher risk of death than married patients. Lastly, Kvikstad *et al.* [8] followed up 333 female married, divorced, and widowed cases of SCLC and NSCLC for 6 years, revealing 268 deaths. No significant associations were found between marital

Table 3. Hazard ratios (HR) of cancer survival according to the marital status

	Male				Female				Total			
	Married	Widowed	Separate/ divorced	Single	Married	Widowed	Separate/ divorced	Single	Married	Widowed	Separate/ divorced	Single
No. of subjects	774	41	26	24	262	72	19	12	1036	113	45	36
Person-months of follow-up	21.8 (0.6–66.3)	17.0 (2.5–57.9)	23.7 (0.9–65.9)	19.1 (3.0–65.1)	26.5 (0.5–66.7)	30.5 (4.4–63.6)	28.1 (5.9–62.2)	23.6 (5.2–50.2)	23.6 (0.5–66.7)	25.9 (2.5–63.6)	27.2 (0.9–65.9)	21.0 (3.0–65.1)
No. of death from all causes	481	31	20	16	121	31	9	7	602	62	29	23
Unadjusted HR	1.0 (referent)	1.4 (0.9–1.9)	1.3 (0.8–2.0)	1.1 (0.7–1.9)	1.0 (referent)	0.9 (0.6–1.3)	0.9 (0.5–1.8)	1.8 (0.9–3.4)	1.0 (referent)	0.9 (0.7–1.1)	1.1 (0.7–1.5)	1.2 (0.8–1.8)
p-Value		0.08	0.26	0.62		0.45	0.84	0.23		0.32	0.77	0.39
Multivariable adjusted HR1	1.0 (referent)	1.4 (0.9–2.1)	1.1 (0.7–1.7)	1.1 (0.7–1.9)	1.0 (referent)	0.8 (0.5–1.3)	0.7 (0.3–1.4)	1.8 (0.8–3.9)	1.0 (referent)	0.9 (0.7–1.3)	0.9 (0.6–1.3)	1.2 (0.8–1.8)
p-Value		0.06	0.81	0.69		0.43	0.27	0.17		0.81	0.57	0.52
Multivariable adjusted HR2	1.0 (referent)	1.7 (1.2–2.5)	1.1 (0.7–1.7)	0.9 (0.5–1.5)	1.0 (referent)	0.7 (0.5–1.1)	0.5 (0.3–1.1)	1.2 (0.5–2.7)	1.0 (referent)	1.1 (0.9–1.5)	0.9 (0.6–1.3)	0.9 (0.6–1.4)
p-Value		0.005	0.72	0.61		0.15	0.10	0.71		0.41	0.42	0.65

HR1: age, BMI, education, PS, and histology type adjusted.
HR2: age, BMI, education, PS, histology type, smoke stage, definitive treatment, and HADS-depression adjusted.

status and survival among female divorced and widowed patients. The present study showed no significant association between marital status and survival when male and female patients were examined as a single group. On the other hand, when the subjects were divided into male and female patients, only the male widowed patients had a higher mortality risk than the male married patients. Having a spouse die significantly increases a person's risk of death in the general population, and this 'widow/widower effect' is especially pronounced in men [3–6]. In the present study, the findings for male patients with NSCLC are consistent with these previous results.

Possible associations between marital status and survival may be mediated by several factors. An unmarried status has been associated with an increased frequency of smoking, depression, advanced disease stage at the time of diagnosis, and a lower likelihood of receiving definitive treatment [9–13,15–18]. Previous studies did not consider possible modifying factors' effects to examine differences in sex and marital status [7,8]. Therefore, it is not clarified why single, separate/divorced, and widowed patients have a higher mortality compared with married patients. This is the first study to examine differences in sex and subdivided marital status as well as the effects of potential modifying factors, such as smoking status, psychological variables, choice of definitive treatment, and disease stage at the time of diagnosis, on the association between marital status and survival from NSCLC. In the present study, smoking status, disease stage at the time of diagnosis, choice of definitive treatment, and the HAD depression score did not have a significant modifying effect on the relationship between male widowed patients and survival. Thus, smoking status, disease stage at the time of diagnosis, choice of definitive treatment, and the HAD depression score might not have a major impact on the association between marital status and survival. However, an unmarried status has been associated with an increased chance of the patient continuing to smoke even after a diagnosis of cancer has been made [12]. The continuation of smoking even after a diagnosis of cancer has been made is known to be significantly associated with survival [12,14]. In this study, we could not evaluate this association because information on smoking continuation after cancer diagnosis was not available.

Our study had several limitations. First, the study was performed at a single National Cancer Center. Whether our results can be generalized to reflect other institutions remains unclear. Thus, further studies performed at multiple institutions are necessary to clarify the prognostic effects of marital status on the survival of lung cancer patients. Second, in this study the subjects were only NSCLC patients. Histological classification of

the lung cancers in our database at the National Cancer Center Hospital East (NCCHE), Japan, revealed that small cell carcinomas were much less common (11%) than NSCLC (89%); other reports have suggested that these cancers account for nearly 80 and 20% of all lung cancers, respectively [24]. Moreover, NSCLC and SCLC differ in terms of their prognosis as well as the therapeutic strategies employed [25]. Therefore, we clarified the association between marital status and survival using a homogeneous group, focusing only on NSCLC patients. Third, data on unhealthy lifestyle behaviors after a cancer diagnosis had been made were unavailable. An unmarried status has been associated with an increased frequency of maladjustment to the cancer diagnosis (especially among subjects who continue to smoke even after they have been diagnosed as having cancer) [12]. There is some possibility that the association between marital status and survival may be mediated by this factor. If data on unhealthy lifestyle behaviors after cancer diagnosis were made available, then the mechanism responsible for the association between marital status and survival could be clarified, and the physician could suggest possible means to improve the prognosis to their cancer patients.

In conclusion, our data indicated that marital status might influence survival among male widowed NSCLC patients in Japan. The present results indicate that potential modifying factors, such as smoking status, disease stage at the time of diagnosis, choice of definitive treatment, and the HAD depression score, did not participate in association between marital status and survival in male patients with NSCLC. Further research on marital status and survival in male patients with NSCLC within the potential modifying factors such as continued smoking and including a large population is needed to clarify the details of this association.

Conflict of interest

None of the authors have any conflict of interest with any aspect of submitting this article for publication.

Acknowledgements

This study was supported by a grant-in-aid for cancer research for the 3rd Term Comprehensive Ten-Year Strategy for Cancer Control (H16-3jigan-010) from the Ministry of Health, Labour and Welfare, Japan. We would like to express special thanks to Toyoko Matsumoto and Fumiko Koh for collecting and filing all the data for this project. The authors wish to thank Y. Kojima, N. Taguchi and R. Katayama of the Psycho-Oncology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Japan, for their research assistance.

References

1. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. *Int J Cancer* 1999;**80**:827–841.
2. Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. *Int J Cancer* 1999;**83**:18–29.
3. Young M, Benjamin B, Wallis C. The mortality of widowers. *Lancet* 1963;**13**:454–456.
4. Parkes CM, Benjamin B, Fitzgerald RG. Broken heart: a statistical study of increased mortality among widowers. *BMJ* 1969;**646**:740–743.
5. Hesling KJ, Szklo M. Mortality after bereavement. *Am J Epidemiol* 1981;**114**:41–52.
6. Johnson NJ, Backlund E, Sorlie PD, Loveless CA. Marital status and mortality: The National Longitudinal Mortality Study. *Ann Epidemiol* 2000;**10**:224–238.
7. Kravdal O. A cancer survival model that takes sociodemographic variations in "normal" mortality into account: comparison with other models. *J Epidemiol Community Health* 2002;**56**:309–318.
8. Kvikstad A, Vatten LJ, Tretli S. Widowhood and divorce in relation to overall survival among middle-aged Norwegian women with cancer. *Br J Cancer* 1995;**71**:1343–1347.
9. Goodwin JS, Hunt WC, Key CR, Samet JM. The effect of marital status on stage, treatment, and survival of cancer patients. *JAMA* 1987;**258**:3125–3130.
10. Greenberg ER, Chute CG, Stukel T *et al.* Social and economic factors in the choice of lung cancer treatment. A population-based study in two rural states. *N Engl J Med* 1988;**318**:612–617.
11. Tammemagi CM, Neslund-Dudas C, Simoff M, Kvale P. Smoking and lung cancer survival: the role of comorbidity and treatment. *Chest* 2004;**125**:27–37.
12. Saito-Nakaya K, Nakaya N, Fujimori M *et al.* Marital status, social support and survival after curative resection in non-small-cell lung cancer. *Cancer Sci* 2006;**97**:206–213.
13. Pomerleau J, Gilmore A, McKee M, Rose R, Haerpfer CW. Determinants of smoking in eight countries of the former Soviet Union: results from the living conditions, lifestyles and health study. *Addiction* 2004;**99**: 1577–1585.
14. Videtic GM, Stitt LW, Dar AR *et al.* Continued cigarette smoking by patients receiving concurrent chemoradiotherapy for limited-stage small-cell lung cancer is associated with decreased survival. *J Clin Oncol* 2003;**21**:1544–1549.
15. Schoenborn CA. Marital status and health: United States, 1999–2002. *Adv Data* 2004;**351**:1–32.
16. Nayeri K, Pitaro G, Feldman JG. Marital status and stage at diagnosis in cancer. *N Y State J Med* 1992;**92**: 8–11.
17. Buccheri G. Depressive reactions to lung cancer are common and often followed by a poor outcome. *Eur Respir J* 1998;**11**:173–178.
18. Cartmel B, Moon TE, Levine N, Rodney S, Alberts D. Predictors of inactivation and reasons for participant inactivation during a skin cancer chemoprevention study. *Cancer Epidemiol Biomarkers Prev* 2000;**9**: 999–1002.
19. Nakaya N, Goto K, Saito-Nakaya K *et al.* The lung cancer database project in the national cancer center, Japan: study design, response rate, and profiles of the cohort subjects. *Jpn J Clin Oncol* 2006;**36**: 280–284.
20. Oken MM, Creech RH, Tormey DC *et al.* Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982;**5**:649–655.

21. The Japan Lung Cancer Society. *Haigann Sinryo Gaidolainn* (2nd edn). Kanehara Shuppan: Tokyo, Japan, 2005.
22. Zigmond AS, Snaith RP. The Hospital and Depression Scale. *Acta Psychiatr Scand* 1983;**67**:361–370.
23. Kugaya A, Akechi T, Okuyama T, Okamura H, Uchitomi Y. Screening for psychological distress in Japanese cancer patients. *Jpn J Clin Oncol* 1998;**28**:333–338.
24. ESMO Guidelines Task Force. ESMO minimum clinical recommendations for diagnosis, treatment and follow-up of non-small-cell lung cancer (NSCLC). *Ann Oncol* 2001;**12**:1049–1050.
25. Nakaya N, Saito-Nakaya K, Akechi T *et al.* Negative psychological aspects and survival in lung cancer patients. *Psycho-Oncology* 2007. DOI: 10.1002/pon.1259.

Lung Cancer (2008) 62, 316–320

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/lungcan

Mutational status of *EGFR* and *KIT* in thymoma and thymic carcinoma

Kiyotaka Yoh[a,*], Yutaka Nishiwaki[a], Genichiro Ishii[b], Koichi Goto[a], Kaoru Kubota[a], Hironobu Ohmatsu[a], Seiji Niho[a], Kanji Nagai[a], Nagahiro Saijo[a]

[a] *Division of Thoracic Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan*
[b] *Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan*

Received 13 February 2008; received in revised form 16 March 2008; accepted 19 March 2008

KEYWORDS
Thymoma;
Thymic carcinoma;
EGFR;
KIT;
Mutation;
Treatment

Summary This study was conducted to evaluate the prevalence of *EGFR* and *KIT* mutations in thymomas and thymic carcinomas as a means of exploring the potential for molecularly targeted therapy with tyrosine kinase inhibitors. Genomic DNA was isolated from 41 paraffin-embedded tumor samples obtained from 24 thymomas and 17 thymic carcinomas. *EGFR* exons 18, 19, and 21, and *KIT* exons 9, 11, 13, and 17, were analyzed for mutations by PCR and direct sequencing. Protein expression of EGFR and KIT was evaluated immunohistochemically. *EGFR* mutations were detected in 2 of 20 thymomas, but not in any of the thymic carcinomas. All of the *EGFR* mutations detected were missense mutations (L858R and G863D) in exon 21. EGFR protein was expressed in 71% of the thymomas and 53% of the thymic carcinomas. The mutational analysis of *KIT* revealed only a missense mutation (L576P) in exon 11 of one thymic carcinoma. KIT protein was expressed in 88% of the thymic carcinomas and 0% of the thymomas. The results of this study indicate that *EGFR* and *KIT* mutations in thymomas and thymic carcinomas are rare, but that many of the tumors express EGFR or KIT protein.

1. Introduction

Thymic epithelial tumors are uncommon neoplasms and there are two major histological types: thymoma and thymic carcinoma [1]. Surgical resection is the preferred treatment option for all subtypes of thymoma and thymic carcinoma. However, thymic carcinomas and some thymomas tend to behave in a malignant manner clinically, and in many cases dissemination or distant metastasis has already occurred at presentation. Patients with metastatic or unresectable tumors are candidates for systemic chemotherapy, but no standard chemotherapy has been established because of the rarity of both tumors [2–5], and alternative therapeutic molecular targets are needed.

* Corresponding author. Tel.: +81 4 7133 1111; fax: +81 4 7131 4724.
E-mail address: kyoh@east.ncc.go.jp (K. Yoh).

0169-5002/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2008.03.013

Receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and KIT, contribute to a number of processes related to the survival and growth activity of many solid tumors, making them promising targets for cancer therapy [6–8]. Recent studies have shown that the presence of kinase domain mutations in the EGFR gene in non-small cell lung cancer (NSCLC) tissue predicts a significant clinical response to small-molecule tyrosine kinase inhibitors (TKIs) of EGFR, such as gefitinib and erlotinib [9], and it is widely known that there is an association between exon 11 mutations of the KIT gene in gastrointestinal stromal tumors (GISTs) and greater responsiveness to imatinib as a small-molecule TKI of KIT [10].

Several immunohistochemical studies have shown overexpression of EGFR protein in both thymoma and thymic carcinoma [11,12], and in thymic carcinoma, immunohistochemical studies have shown a high frequency of KIT overexpression but that thymomas express hardly any KIT [13,14]. Two interesting cases have recently been reported. One was a case of thymic carcinoma with an activating *KIT* mutation that responded to imatinib, reported by Strobel et al. [15], and the other was a case of thymic carcinoma with *EGFR* mutations that was responsive to gefitinib, reported by Yamaguchi et al. [16]. However, because of the rarity of these tumors, information on the mutational status of *EGFR* and *KIT* in thymomas and thymic carcinomas has been limited to only a few reports, and the prevalence of *EGFR* and *KIT* mutations remains unknown.

In this study, we investigated the status of *EGFR* and *KIT* mutations in thymoma and thymic carcinoma patients to explore the potential for molecularly targeted therapy with TKIs. We also investigated the relation between protein expression assessed by immunohistochemistry and the mutational status of *EGFR* and *KIT*.

2. Patients and methods

2.1. Patients

The tumor samples used in this study were obtained from paraffin-embedded surgical specimens from 41 cases of thymoma or thymic carcinoma treated surgically at the National Cancer Center Hospital East between 1993 and 2005. All samples were reviewed to confirm the diagnosis of thymoma or thymic carcinoma. The clinical data of all patients was collected from their medical records. This study was approved by the Institutional Review Board of our institution.

The characteristics of all of the patients are listed in Table 1. Patient age ranged from 21 to 77 years, and their median age was 61 years. The specimens used were from 24 thymomas and 17 thymic carcinomas. According to the World Health Organization (WHO) classification of thymic epithelial tumors, the histological subtype of the thymomas was type A in 7 cases, type AB in 7 cases, type B1 in 6 cases, and type B2 in 4 cases. The histological subtype of the thymic carcinomas was squamous cell carcinoma in 14 cases, and adenocarcinoma, adenosquamous carcinoma, and nonspecified in 1 case each. According to the system described by Masaoka et al. [17], the clinical stage was stage I in 15 patients, stage II in 8 patients, stage III in 9 patients, stage IVa in 1 patient, and stage IVb in 8 patients. All patients had undergone total resection ($n = 36$) or partial resection ($n = 5$) after obtaining their informed consent in accordance with institutional guidelines.

Table 1 Patient characteristics

	Patients ($n = 41$)
Age, years	
Median	61
Range	21–77
Gender	
Female	20
Male	21
Histology	
Thymoma	24
Thymic carcinoma	17
Stage	
I	15
II	8
III	9
IVa	1
IVb	8
Surgical procedure	
Total resection	36
Partial resection	5
Smoking history	
Never	19
Former	11
Current	11

2.2. Mutational analysis of *EGFR* and *KIT*

Tumor genomic DNA was isolated from paraffin-embedded samples of a total of 41 tumors, 24 thymomas and 17 thymic carcinomas. To ensure that tumor-cell-rich areas of tissues were isolated, hematoxylin and eosin stained slides were prepared from each selected paraffin-embedded block. Polymerase chain reaction (PCR) was performed to amplify exons 18, 19, and 21 of *EGFR* and exons 9, 11, 13, and 17 of *KIT* by using previously described primers [9,18], and the PCR products were directly sequenced with an ABI 3100 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). All sequencing reactions were performed in both forward and reverse directions. A series of mutational analyses was performed at Mitsubishi Chemical Safety Institute Ltd.

2.3. Immunohistochemistry

Protein expression of EGFR and KIT was evaluated immunohistochemically in representative paraffin-embedded sections. EGFR staining was performed by using the DAKO (Carpinteria, CA, USA) pharmDX kit for EGFR according to the manufacturer's instructions, and immunostaining for KIT was performed by using a polyclonal rabbit antibody (A 4502; Dako, Glostrup, Denmark) according to the manufacturer's instructions. Staining of both markers was considered posi-

tive if more than 50% of the tumor cells stained. All slides were examined and scored independently by two observers (G.I. and K.Y.).

2.4. Statistical analysis

The variables measured in the study were tested for associations by Fisher's exact test. P values <0.05 were considered statistically significant.

3. Results

3.1. EGFR analysis of thymomas and thymic carcinomas

Sequencing of the *EGFR* tyrosine kinase domain encoded by exons 18, 19, and 21 was successful in 29 of the 41 tumors (Table 2). *EGFR* mutations were detected in 2 of the 20 thymomas, but direct sequencing showed no evidence of mutations in any of the 9 thymic carcinomas. All of the *EGFR* mutations detected were missense mutations in exon 21 (L858R or G863D), and no mutations were detected in exons 18 and 19. Examination of 21 thymomas and 17 thymic carcinomas for EGFR protein expression by immunohistochemistry revealed EGFR expression in 15 (71%) of the 21 thymomas and 9 (53%) of the 17 thymic carcinomas. The difference in EGFR expression between the thymomas and thymic carcinomas was not significant ($P = 0.31$).

3.2. KIT analysis of thymomas and thymic carcinomas

It was possible to analyze the *KIT* mutation status of 22 thymomas and 11 thymic carcinomas by direct sequencing (Table 3). A missense mutation in exon 11 (L576P) was found in only one thymic carcinoma, and direct sequencing of *KIT* exons 9, 13, and 17 revealed no mutations in any of the tumors analyzed. Immunohistochemistry showed KIT protein expression in 15 (88%) of the 17 thymic carcinomas, but no KIT expression in any of the 24 thymomas ($P < 0.0001$).

Table 4 summarizes the data of all patients whose tumors were positive for *EGFR* or *KIT* mutations. Exon 21 mutations in the *EGFR* gene were found in two thymomas (Fig. 1A and B), and an exon 11 mutation was identified in the *KIT* gene of 1 thymic carcinoma (Fig. 1C). Because these mutations were not detected in the normal lung tissues from the same patients, they were considered to be somatic mutations. Both patients whose tumors were positive for *EGFR* mutation were never smokers. All three patients had undergone surgical resection, and they are currently alive and relapse-free.

Table 2 EGFR status of thymomas and thymic carcinomas

EGFR mutation	Thymoma ($n = 20$)	Thymic carcinoma ($n = 9$)	
Exon 18	0	0	
Exon 19	0	0	
Exon 21	2	0	
No mutation	18	9	
EGFR expression	Thymoma ($n = 21$)	Thymic carcinoma ($n = 17$)	P
Positive	15 (71%)	9 (53%)	0.31

Table 3 KIT status of thymomas and thymic carcinomas

KIT mutation	Thymoma ($n = 22$)	Thymic carcinoma ($n = 11$)	
Exon 9	0	0	
Exon 11	0	1	
Exon 13	0	0	
Exon 17	0	0	
No mutation	22	10	
KIT expression	Thymoma ($n = 24$)	Thymic carcinoma ($n = 17$)	P
Positive	0 (0%)	15 (88%)	< 0.0001

4. Discussion

In this study, *EGFR* mutations were observed in the DNA sequences of 2 thymomas of 29 tumors analyzed, and analysis of the *KIT* mutation status of 22 thymomas and 11 thymic carcinomas by direct sequencing revealed a missense mutation in exon 11 in only 1 thymic carcinoma. By contrast, 71% of the thymomas and 53% of the thymic carcinomas expressed EGFR protein, and overexpression of KIT was observed in 88% of the thymic carcinomas and 0% of the thymomas. The results show that the EGFR and KIT protein expression in the thymomas and thymic carcinomas was not associated with *EGFR* or *KIT* mutations.

A review of the medical literature retrieved reports of two studies that investigated *EGFR* mutations in thymomas or thymic carcinomas [19,20] and of one study that tested thymic carcinomas for *KIT* mutations [13]. Suzuki et al. reported that direct sequencing did not reveal any *EGFR* missense mutations in a total of 38 thymoma samples obtained from Japanese patients [19]. Meister et al. reported detecting no mutations in the tyrosine kinase domain of *EGFR* in 20 DNA samples from 17 thymomas and 3 thymic carcinomas analyzed by direct sequencing [20]. Pan et al. performed a mutation analysis of *KIT* by direct DNA sequencing in 21 thymic carcinomas, but found none [13]. To date, *EGFR* mutations (double missense mutations: G719A in exon 18 and L858R in exon 21) have been reported in one case of thymic carcinoma [16], and a *KIT* mutation (V560del in exon 11) in one case of thymic carcinoma [15]. The results of our study and review of the literature suggest that *EGFR* or *KIT* mutations are rare in thymomas and thymic carcinomas but that expression of EGFR and KIT is frequently present. Mutations that activate receptor tyrosine kinases contribute to the development of human carcinomas, and the activation of a mutation in the *KIT* gene is thought to be the most important factor in the pathogenesis of GISTs [7,8]. However, we speculate that *EGFR* or *KIT* mutations may not be implicated in the carcinogenesis of thymomas and thymic

Table 4 Summary of thymoma and thymic carcinoma patients with *EGFR* or *KIT* mutations in their tumors

Clinical characteristics						Mutation				IHC
No.	Age/sex	Smoking status	Masaoka stage	Histology	Clinical outcome	Gene	Exon	Nucleotide change	Amino acid change	
1	65/F	Never	II	Thymoma (type A)	3 years of disease-free survival after complete resection	*EGFR*	21	2573T > G	L858R	EGFR (+)
2	69/F	Never	III	Thymoma (type B1)	5 years of disease-free survival after complete resection	*EGFR*	21	2588G > A	G863D	EGFR (−)
3	59/M	Former (20 pack-years)	I	Thymic carcinoma (Sq)	6 years of disease-free survival after complete resection	*KIT*	11	1748T > C	L576P	KIT (+)

Abbreviations: Sq, squamous cell carcinoma; IHC, immunohistochemistry.

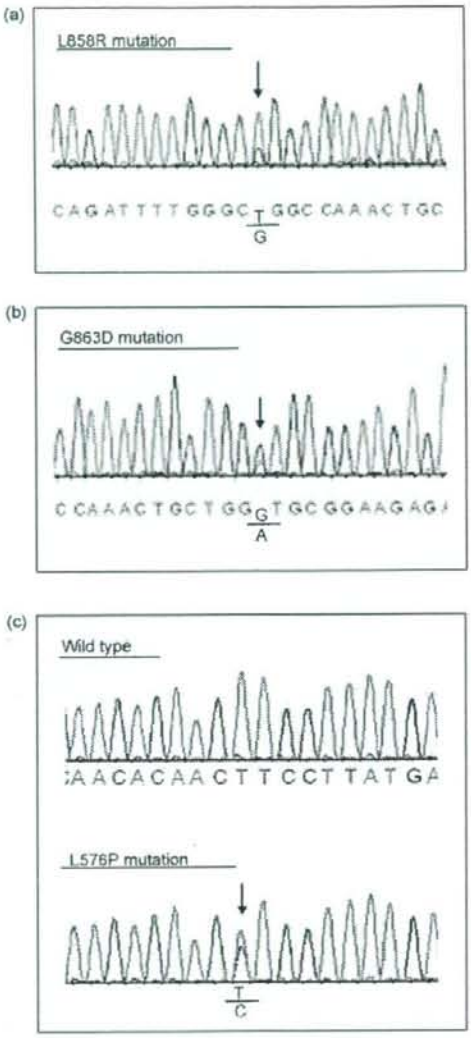

Fig. 1 Electropherograms of the products of direct sequencing of *EGFR* and *KIT*. (a and b) Two thymomas contained a single missense point mutation in exon 21 of *EGFR*. (c) One thymic carcinoma contained a single missense point mutation in exon 11 of *KIT*.

carcinomas because of the low frequency of *EGFR* or *KIT* mutations in these tumors.

Remarkably, the *EGFR* mutations (L858R and G863D, respectively, in exon 21) observed in the 2 thymomas in our study were similar to the active mutations in NSCLC that have been reported to be predictors of a therapeutic response to EGFR-TKI by NSCLCs [9,21]. Moreover, the *KIT* mutation (L576P in exon 11) identified in the 1 thymic carcinoma in our study had previously been described as one of the mutations that predicts a clinical response of GISTs to

imatinib [22]. We therefore speculate that patients whose thymoma or thymic carcinoma harbors *EGFR* or *KIT* mutations may profit from molecularly targeted therapy with a TKI of EGFR or KIT.

In conclusion, our findings indicate that somatic mutations of *EGFR* or *KIT* of the thymomas and thymic carcinomas are presented in a small number of patients. Further investigation is warranted to determine the susceptibility of such tumors to TKI therapy.

Conflict of interest

None declared.

Acknowledgments

We thank Kazuto Nishio and Hideyuki Yokote of Kinki University School of Medicine for their support of the study concept. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan.

References

[1] Strobel P, Marx A, Zettl A, Muller-Hermelink HK. Thymoma and thymic carcinoma: an update of the WHO Classification 2004. Surg Today 2005;35:805–11.

[2] Strobel P, Bauer A, Puppe B, Kraushaar T, Krein A, Toyka K, et al. Tumor recurrence and survival in patients treated for thymomas and thymic squamous cell carcinomas: a retrospective analysis. J Clin Oncol 2004;22:1501–9.

[3] Eng TY, Fuller CD, Jagirdar J, Bains Y, Thomas Jr CR. Thymic carcinoma: state of the art review. Int J Radiat Oncol Biol Phys 2004;59:654–64.

[4] Kondo K, Monden Y. Therapy for thymic epithelial tumors: a clinical study of 1320 patients from Japan. Ann Thorac Surg 2003;76:878–84.

[5] Thomas CR, Wright CD, Loehrer PJ. Thymoma: state of the art. J Clin Oncol 1999;17:2280–9.

[6] Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 2005;5:341–54.

[7] Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995;19:183–232.

[8] Heinrich MC, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. J Clin Oncol 2002;20:1692–703.

[9] Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, 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;350:2129–39.

[10] Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol 2003;21:4342–9.

[11] Henley JD, Cummings OW, Loehrer Sr PJ. Tyrosine kinase receptor expression in thymomas. J Cancer Res Clin Oncol 2004;130:222–4.

[12] Ionescu DN, Sasatomi E, Cieply K, Nola M, Dacic S. Protein expression and gene amplification of epidermal growth factor receptor in thymomas. Cancer 2005;103:630–6.

[13] Pan CC, Chen PC, Chiang H. KIT (CD117) is frequently overexpressed in thymic carcinomas but is absent in thymomas. J Pathol 2004;202:375–81.

[14] Nakagawa K, Matsuno Y, Kunitoh H, Maeshima A, Asamura H, Tsuchiya R. Immunohistochemical KIT (CD117) expression in thymic epithelial tumors. Chest 2005;128:140–4.

[15] Strobel P, Hartmann M, Jakob A, Mikesch K, Brink I, Dirnhofer S, et al. Thymic carcinoma with overexpression of mutated KIT and the response to imatinib. N Engl J Med 2004;350:2625–6.

[16] Yamaguchi H, Soda H, Kitazaki T, Tsukamoto K, Hayashi T, Kohno S. Thymic carcinoma with epidermal growth factor receptor gene mutations. Lung Cancer 2006;52:261–2.

[17] Masaoka A, Monden Y, Nakahara K, Tanioka T. Follow-up study of thymomas with special reference to their clinical stages. Cancer 1981;48:2485–92.

[18] Tarn C, Merkel E, Canutescu AA, Shen W, Skorobogatko Y, Heslin MJ, et al. Analysis of KIT mutations in sporadic and familial gastrointestinal stromal tumors: therapeutic implications through protein modeling. Clin Cancer Res 2005;11:3668–77.

[19] Suzuki E, Sasaki H, Kawano O, Endo K, Haneda H, Yukiue H, et al. Expression and mutation statuses of epidermal growth factor receptor in thymic epithelial tumors. Jpn J Clin Oncol 2006;36:351–6.

[20] Meister M, Schirmacher P, Dienemann H, Mechtersheimer G, Schnabel PA, Kern MA, et al. Mutational status of the epidermal growth factor receptor (EGFR) gene in thymomas and thymic carcinomas. Cancer Lett 2007;248:186–91.

[21] Chou TY, Chiu CH, Li LH, Hsiao CY, Tzen CY, Chang KT, et al. Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer. Clin Cancer Res 2005;11:3750–7.

[22] Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. J Clin Oncol 2004;22:3813–25.

Efficacy and Safety of Erlotinib Monotherapy for Japanese Patients with Advanced Non-small Cell Lung Cancer

A Phase II Study

Kaoru Kubota, MD, PhD, Yutaka Nishiwaki, MD, Tomohide Tamura, MD, Kazuhiko Nakagawa, MD, Kaoru Matsui, MD, Koshiro Watanabe, MD, PhD, Toyoaki Hida, MD, Masaaki Kawahara, MD, Nobuyuki Katakami, MD, Koji Takeda, MD, Akira Yokoyama, MD, Kazumasa Noda, MD, Masahiro Fukuoka, MD, and Nagahiro Saijo, MD, PhD

Introduction: The aim of this study was to evaluate the efficacy and safety of Erlotinib in Japanese patients with previously treated non-small cell lung cancer (NSCLC). Available tumor biopsy samples were analyzed to examine relationships between biomarkers and clinical outcome.

Methods: This open-label phase II trial enrolled stage III/IV NSCLC patients who had progressive disease after at least one prior platinum-based chemotherapy regimen. Erlotinib was administered at a dose of 150 mg/d orally until disease progression or intolerable toxicity. Analysis of epidermal growth factor receptor gene mutations in exon 18–21 by direct sequencing was performed in tumor tissue specimens obtained at the first diagnosis.

Results: Sixty-two patients were enrolled and 60 patients were evaluable for efficacy. Objective response rate and disease control rate were 28.3% and 50.0%; median time to progression and overall survival were 77 days and 14.7 months, respectively. In logistic regression analysis, only smoking history was proved to be a statistically significant predictive factor for response (odds ratio: 0.06, $p < 0.001$). Only 7 patients had samples available for mutation analysis. Three patients who had deletion mutations on exon 19 (del E746-A750 or del S752-I759) exhibited objective response. Common toxicities were rash (98%), dry skin (81%), and diarrhea (74%). Discontinuation due to adverse events occurred in 11 patients (18%). Four patients (6%) experienced interstitial lung disease-like events, one of whom died.

Conclusion: Erlotinib is efficacious in Japanese patients with previously treated NSCLC. The toxicity profile was similar to that in Western patients, except for a somewhat higher incidence of skin disorders and interstitial lung disease. Further studies are needed to determine the relationship between epidermal growth factor receptor mutations and outcomes with Erlotinib in Japanese patients.

National Cancer Center Hospital East, Kashiwa, Chiba, Japan.

Disclosure: Kazuhiko Nakagawa had served as an adviser for pre-approval consulting of this drug. Masahiro Fukuoka was paid an honorarium as the chairman of the meeting and as medical advisor for clinical trial in relation to this drug. Nagahiro Saijo had received research grant in relation to this drug. The other authors declare no conflicts of interest.

Address for correspondence: Kaoru Kubota, MD, PhD, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan. E-mail: kkubota@east.ncc.go.jp

ISSN: 1556-0864/08/0312-1439

Key Words: Non-small cell lung cancer, Erlotinib, Molecular target therapy, EGFR-TKIs, *EGFR* mutation.

(*J Thorac Oncol.* 2008;3: 1439–1445)

Lung cancer affects approximately 1.2 million people annually, and is the leading cause of cancer death in the world.[1] More than 80% of affected patients are diagnosed with non-small cell lung cancer (NSCLC). The standard first-line treatment for metastatic NSCLC is a combination of platinum chemotherapy with a third-generation agent such as docetaxel, paclitaxel, gemcitabine, vinorelbine, and irinotecan.[2,3] Although patients with stage II, IIIA, or IIIB NSCLC receive platinum-based chemotherapy as part of combined modality treatment with thoracic radiotherapy or surgery, many will be candidates for second or third-line chemotherapy. Docetaxel is the only cytotoxic agent with a proven survival advantage over supportive care in patients with disease progression after cisplatin-based chemotherapy for NSCLC.[4] The other agent for which a survival benefit has been demonstrated in this setting is erlotinib,[5] which was approved in Japan for the treatment of relapsed NSCLC in October 2007. Erlotinib is a selective, orally active epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI). In contrast to the experience with the cytotoxic chemotherapeutic agents, response to treatment with EGFR-TKIs has been reported to be influenced by gender, histological type, race or ethnic origin, and smoking status.[5–8]

Tumor molecular markers, including *EGFR* gene mutations and protein expression, have been widely studied in patients with NSCLC, and there is strong evidence that the presence of *EGFR* gene mutations is a predictor of tumor response and resistance.[9–12] However, few prospective studies have evaluated molecular markers as predictors of outcome, and their clinical usefulness is unproven.

This report presents the results of the first phase II study of erlotinib conducted in Japanese patients with NSCLC. The purpose was to evaluate the efficacy and safety of erlotinib in this population. Where available, tumor biopsy samples were analyzed for EFGR-related markers.

PATIENTS AND METHODS

This phase II, multicenter, open-label study recruited patients at 11 hospitals in Japan. The primary end point was the objective response rate (ORR) to erlotinib treatment (150 mg/d). Secondary endpoints were disease control rate (DCR), response duration, time to progression, overall survival (OS), quality of life (QoL), and safety. The protocol was approved by the ethics review boards of all participating institutions, and conducted in accordance with Japanese Good Clinical Practice guidelines.

Patient Selection

Patients with histologically or cytologically documented stage IIIB or IV NSCLC at study entry (not curable with surgery or radiotherapy) that was recurrent or refractory to treatment with one or more chemotherapy regimens (including at least one platinum-containing regimen), were enrolled into this study. Additional eligibility criteria included: the presence of measurable lesions by Response Evaluation Criteria in Solid Tumors (RECIST); age ≥20, <75 years; Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2, and adequate bone marrow, hepatic, and renal function, i.e., aspartate aminotransferase and alanine aminotransferase (ALT) levels ≤2.5 times the upper limit of normal and total bilirubin of ≤1.5 times the upper limit of normal. Patients with existing or previous interstitial lung disease (ILD) were excluded, although a history of radiation pneumonitis (limited to the field of radiation treatment) was permitted. Concomitant anticancer treatment and prophylactic medication for adverse events (AEs) were not permitted, nor was prior use of anti-EGFR or anti human epidermal growth factor receptor (HER2) agents (small molecules and monoclonal antibodies). Written informed consent was obtained from all patients.

Treatment Procedure

After completion of the baseline assessments (see below), all patients received erlotinib (150 mg orally) each morning, 1 hour before breakfast, until the occurrence of progressive disease (PD) or unacceptable toxicity (all AEs were graded using the National Cancer Institute Common Toxicity Criteria Version 2.0). In the event of treatment-related toxicity, 2 dose reductions of 50 mg were permitted per patient, and dosing could also be interrupted for up to 14 days. For grade 3 or intolerable grade 2 rash, treatment was withheld until the rash improved to grade 2 or less, when a lower dose of erlotinib was initiated. For grade 3 diarrhea, treatment was withheld until the diarrhea was grade 1 or less, when a lower dose was started. For ILD of any grade, or any grade 4 toxicity, treatment was immediately and permanently discontinued.

Evaluation of Efficacy

Objective tumor response was assessed in accordance with RECIST.[13] Tumor assessments were performed at baseline, then every 4 weeks until week 16, and then every 8 weeks thereafter. Confirmation of complete or partial responses (PR) was required, by means of a second assessment conducted 28 days or more after the initial assessment. Stable disease (SD) was defined as disease control (absence of progression) maintained for at least 6 weeks. An independent response evaluation committee consisting of 2 oncologists and a radiologist reviewed images of patients with complete response, PR, and SD. Individual survival times were determined from the survival status of each patient during the study period and at the post study follow-up survey conducted in June-July 2005 and May-July 2006. OS was defined as the time from first administrated to death.

Quality of Life Evaluation

The Functional Assessment of Cancer Therapy-Lung (FACT-L) questionnaire (Version 4-A)[14] was used to assess QoL. The full FACT-L questionnaire was administered at baseline and then every 28 days. In addition, the Lung Cancer Subscale (LCS), an independently validated component of FACT-L, was administered weekly during the treatment period. Best responses on the LCS were analyzed for all patients with a baseline LCS score of 24 or less (out of a possible 28 points) and symptomatic improvement was defined as an increase from the baseline score of 2 or more points, sustained for at least 4 weeks.

Evaluation of Safety

Baseline assessment included a full patient history, physical examination, standard laboratory tests, electrocardiography, chest radiography, pregnancy test, and ophthalmologic tests (vision test and slit-lamp examination). Every week until week 8 and every 2 weeks thereafter, vital signs and ECOG PS were monitored and blood samples were taken for hematology and blood chemistry tests. A radiograph examination to assess pulmonary toxicity was conducted weekly until week 4 and every 2 weeks thereafter. Ophthalmologic examinations were repeated at week 8 and at the end of the study. Observation and evaluation of AEs was conducted as appropriate throughout the study period. All AEs were graded using National Cancer Institute Common Toxicity Criteria Version 2.0. For all ILD-like events, the data safety monitoring board (which consisted of oncologists and pneumonologists) reviewed the clinical data and images; the images were also examined by a review committee of radiologists with expertise in drug-induced pulmonary disorders.

Biomarker Analysis

EGFR mutations and EGFR and HER2 protein expression were assessed in patients with suitable tumor tissue specimens at first diagnosis or surgery; these assessments were done only with separate written consent. Tumor samples were obtained from each center as formalin-fixed and paraffin-embedded blocks, or as thinly sliced tissue sections mounted on glass microscope slides. For the mutation analysis, the tissue was microdissected by Targos Molecular Pathology (Kassel, Germany) and direct sequencing was conducted at the Roche Centre of Medical Genomics (Basel, Switzerland), using a nested polymerase chain reaction of exon 18–21. EGFR protein expression was analyzed by Lab Corp (Mechelen, Belgium). EGFR expression analysis was conducted by immunohistochemistry using Dako EGFR PharmDx™ kits (Dako, Carpinteria, CA). A positive test was

defined as membranous staining in ≥10% of the tumor cells. HER2 protein expression was measured using HercepTest™ (Dako, Carpinteria, CA), and a score of 1+ or above (possible scores were: 0, 1+, 2+, 3+) was regarded as positive.

Statistical Analysis

Given an expected ORR of 20%, a Fisher's exact test was performed (one-sided $\alpha = 2.5\%$). Based on 50 patients, the power to test the null hypothesis (ORR = 5%) was 89.66%. The target sample size of 60 patients was chosen on the expectation that a proportion of patients would prove to be ineligible for the study. The main analysis of efficacy was conducted on the full analysis set (FAS), which was produced by omitting ineligible patients. The 95% confidence interval (CI) for ORR, DCR, and symptom improvement rate was calculated by the Clopper-Pearson method. The time-to-event variables were estimated by the Kaplan-Meier method. Logistic regression and Cox proportional hazards regression analysis was conducted on best response and survival time, respectively. In both cases, univariate and multivariate analyses were used to evaluate the effects of 11 factors relating to patient and disease characteristics, and previous treatment.

RESULTS

Patient Characteristics

A total of 62 patients were enrolled between December 2003 and January 2005. All were evaluable for safety and 60 were evaluable for efficacy (FAS). Two patients did not have a measurable lesion according to RECIST. The baseline characteristics of the patients, including their treatment history, are shown in Table 1. The median age was 60.5 years (range: 28–74 years), and 71% of patients were male. Fifty-seven patients (92%) had adenocarcinoma, and 20 (32%) were never-smokers. Twenty-seven patients (44%) had received only one previous chemotherapy regimen.

Efficacy

Tumor response rates in the FAS (as assessed by extrainstitutional review) are shown in Table 2. Seventeen patients were assessed as having a PR and 13 as having SD. The ORR was 28.3% (95% CI: 17.5–41.4%) and the DCR was 50% (95% CI: 36.8–63.2%). In three patients, objective response could not be adequately confirmed, because each discontinued treatment early in the study due to AEs. The median duration of response was 278 days (95% CI: 203–422 days), and time to progression was 77 days (95% CI: 55–166 days). OS was determined based on information collected until the follow-up survey conducted in May–July 2006. The median survival time was 14.72 months (95% CI: 11.07–20.57 months; 19 censored cases) and the 1-year survival rate was 56.5% (95% CI: 43.9–69.1%) (Figure 1). The median OS of patients with PD was 9.95 months. The symptom improvement rate measured using the LCS was 42.1% (24/57; 95% CI: 29.1–55.9%).

The overall response rate was higher in women (58.8%; 10/17) than in men (16.3%; 7/43, χ^2 test: $p = 0.0029$), and in never-smokers (63.2%; 12/19) than in current or former smokers (12.2%; 5/41, $p = 0.0002$). There was no statistically significant difference between the response rate in patients with adenocarcinoma (28.6%; 16/56) and nonadenocarcinoma histology (25.0%; 1/4, $p = 1.0000$). The response

TABLE 1. Summary of Baseline Patient Characteristics and Demographics

Patient and Disease characteristics	No. of Patients ($n = 62$)	%
Age (yr)		
Median	60.5	
Range	28–74	
Sex		
Female	18	29
Male	44	71
Performance status		
0	20	32
1	41	66
2	1	2
Histology		
Adenocarcinoma	57	92
Squamous cell	4	6
Unclassified	1	2
Stage		
IIIB	8	13
IV	54	87
Smoking history		
Never smoked	20	32
Current- or former smoker	42	68
Time since initial diagnosis (d)		
Median	304.0	
Range	2–2353	
Prior chemotherapy regimens		
1	27	44
2	23	37
≥3	12	19
Prior taxanes		
No	10	16
Yes	52	84
Time since last regimen (d)		
Median	80.0	
Range	29–528	

TABLE 2. Response Assessment

Parameter	n	(%)
Partial response	17	28.3
Stable disease	13	21.7
Progressive disease	27	45.0
Not assessable	3	5.0
Response rate (%) (95% CI)	28.3 (17.5–41.4)	
Disease control rate (%) (95% CI)	50.0 (36.8–63.2)	
Duration of response (median: days)* (95% CI)	278 (203.0–422.0)	
Time to progression (median: days)* (95% CI)	77 (55–166)	

* Kaplan–Meier method.
CI, confidence intervals.

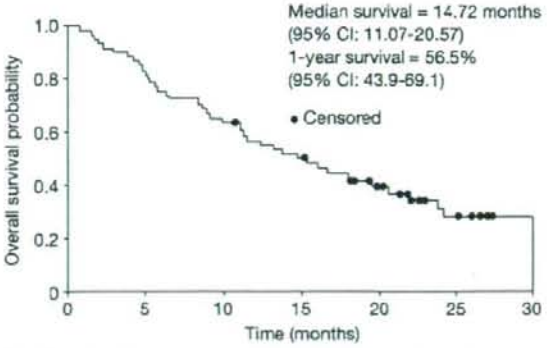

FIGURE 1. Kaplan-Meier plot showing overall survival.

rate was not affected by the number of previous chemotherapy regimens, however, being 27% for patients with one previous regimen (7/26) and 29% for those with 2 or more regimens (10/34). No statistically significant differences were found between other patient subgroups. In a multivariate logistic regression analysis, only smoking history was found to be a statistically significant predictor of response. A multivariate Cox regression analysis showed that both smoking history and ECOG PS were significant predictors for OS (Table 3).

Safety

All 62 patients who received erlotinib were assessed for safety. Treatment-related AEs were observed in all patients, and there were 24 serious AEs in 18 patients (29%). AEs led to discontinuation of erlotinib in 11 patients (18%), including 3 due to ILD-like events, 2 due to ALT elevation, and one each due to rash, paronychia, punctate keratitis, dyspnea/hypoxia, pneumonia and fever/inflammatory neck swelling, and to dose interruptions in 30 patients (48.4%). While the main reasons for the dose interruptions were rash (n = 15; 24.2%) and diarrhea (n = 4; 6.5%), only one patient with rash

TABLE 3. Logistic and Cox Regression Analysis

	Odds Ratio[b]	(95% CI)	p
Logistic regression analysis of response			
Univariate analysis			
Sex (female vs male)	0.14	0.04–0.48	0.002
Age (<65 vs ≥65)	1.26	0.38–4.13	0.704
Histology (non-AD vs AD)	1.20	0.12–12.41	0.878
Smoking history (never vs current or former)	0.08	0.02–0.30	<0.001
Performance status (0 vs ≥1)	0.62	0.19–1.98	0.420
Prior regimens (1 vs ≥2)	1.13	0.36–3.53	0.832
Stage (IIIB vs IV)	0.99	0.17–5.65	0.988
KL-6 (baseline) (<median [496.5 U/ml[a]] vs ≥median)	1.64	0.53–5.12	0.392
Best response to previous chemotherapy (non-PR vs PR)	0.90	0.24–3.33	0.869
Prior taxanes (no vs yes)	0.43	0.10–1.84	0.253
Time since initial diagnosis (≤12 mo vs >12 mo)	1.02	0.31–3.30	0.976
Multivariate analysis			
Smoking history (never vs current or former)	0.06	0.02–0.28	<0.001
Time since initial diagnosis (<12 mo vs ≥12 mo)	2.22	0.49–10.20	0.304
Cox regression analysis of survival			
Univariate analysis			
Sex (female vs male)	1.76	0.85–3.61	0.126
Age (<65 vs ≥65)	0.86	0.44–1.71	0.675
Histology (non-AD vs AD)	0.55	0.19–1.55	0.255
Smoking history (never vs current or former)	1.90	0.93–3.90	0.079
Performance status (0 vs ≥1)	2.31	1.12–4.73	0.023
Prior regimens (1 vs ≥2)	0.93	0.50–1.75	0.833
Stage (IIIB vs IV)	1.38	0.49–3.89	0.542
KL-6 (baseline) (<median [496.5 U/ml[a]] vs ≥median)	1.64	0.87–3.06	0.125
Best response to previous chemotherapy (non-PR vs PR)	0.66	0.31–1.44	0.300
Prior taxanes (no vs yes)	2.09	0.74–5.90	0.163
Time since initial diagnosis (≤12 mo vs >12 mo)	0.76	0.40–1.47	0.418
Multivariate analysis			
Smoking history (never vs current or former)	2.20	1.06–4.56	0.035
Performance status (0 vs ≥1)	2.59	1.25–5.37	0.011

[a] Or 629 ng/ml.
[b] Left site of 'vs' indicates reference group.
PR, partial response; AD, adenocarcinoma; CI, confidence interval.

TABLE 4. Major Treatment-Related Adverse Events and Interstitial Lung Disease-Like Events

			NCI-CTC Grade (*n*)			
Event[a]	*n*	%	1	2	3	>4
Rash	61	98.4	18	41	2	0
Dry skin	50	80.6	44	6	—	—
Diarrhea	46	74.2	33	10	3	0
Pruritus	45	72.6	38	7	0	—
Stomatitis	24	38.7	19	4	1	0
Fatigue	21	33.9	15	6	0	0
Anorexia	19	30.6	11	6	2	0
Paronychia	18	29.0	12	5	1	0
C-reactive protein increased	15	24.2	8	7	0	0
Alanine aminotransferase increased	15	24.2	11	2	2	0
Total bilirubin increased	15	24.2	8	7	0	0
Weight loss	13	21.0	13	0	0	—
ILD-like events	4	6.5	1	0	2	1[b]

Case	Sex	Age	Smoking History	Brinkman Index	Performance Status	Histology	Onset (day)	Outcome	Relation to Erlotinib[c]
1	Male	75	Former	640	1	Adenocarcinoma	52	Recovery	Probable
2	Male	67	Never	—	1	Adenocarcinoma	103	Death (145)	Possible
3	Female	39	Never	—	0	Adenocarcinoma	85	Recovery	Probable
4	Male	69	Former	1000	1	Adenocarcinoma	13	Recovery	Unlikely

[a] Categorized by MedDra Ver.7.1 (except for event).
[b] Grade 5.
[c] Judged by ILD review committee.
NCI-CTC, National Cancer Institute Common Toxicity Criteria; ILD, interstitial lung disease.

had to discontinue treatment, and no patients had to discontinue because of diarrhea or any other digestive toxicity. Fourteen patients (23%) had dose reductions due to AEs, mostly due to rash ($n = 9$; 15%). Treatment-related AEs with an incidence of 20% or more are shown in Table 4; the main events were rash (98%), dry skin (81%), and diarrhea (74%). Elevated laboratory test values related to liver function were found in some patients (total bilirubin: 24%, ALT: 24%), and grade 3 ALT elevation led to treatment discontinuation in 2 patients. Four patients had ILD-like events, including worsening of radiation pneumonitis in one patient, and one died (Table 4). All four (three men; one woman) had an ECOG PS of 0–1 and 2 were former smokers. The patient who died was a 67-year-old man with adenocarcinoma and no history of smoking who discontinued treatment on day 84 due to PD. He developed interstitial pneumonia on day 103 and received 3 days of palliative thoracic irradiation from day 99, after completing the study (3 Gy × 3 days). A computed tomography scan showed characteristic features of ILD (cryptogenic organizing pneumonia-like pattern), and the ILD review committee decided that use of erlotinib could not be excluded as the cause. For the patient with worsening of radiation pneumonitis (case 4), the committee concluded that there was a possible influence of previous radiation therapy, and that this could be seen in the computed tomography scan on day 1. There was, therefore, little reason to suspect that the use of erlotinib had been the cause. Rather, it appeared that the radiation pneumonitis had worsened according to the normal course of illness.

Biomarker Analysis

Tissue samples for measurement of *EGFR* mutations were available for 16 of the 60 patients evaluated for efficacy. For 7 patients, all base sequences were successfully identified in the 4 segments of exons 18–21. All seven (three men, four women) had adenocarcinoma; three were never-smokers, three former smokers and one a current smoker. Three had PR, two SD and two PD. Five of the seven patients had *EGFR* gene mutations and, in all, seven different mutations were detected. The 3 patients with PR all had deletion mutations in exon 19 (del E746-A750 or del S752-I759). One of the 2 patients with PD had no mutations and the other had 2 substitution mutations: L858R in exon 21 and the resistance mutation T790M in exon 20 (Table 5).

Paraffin-embedded tissue samples for immunohistochemistry were available from 12 patients, among whom, 11 had successful determinations of immunohistochemical staining (including 3 patients with PR). Six of the 11 were found to be EGFR-positive and 4 were HER2-positive. However, there were no notable relationships between the EGFR and HER2 expression status and either tumor response or patient characteristics such as sex, histological type or smoking history (data not shown).

DISCUSSION

The present study was conducted on the basis of results from a phase I study of erlotinib in Japanese patients with solid tumors,[15] which showed erlotinib to be well tolerated at

TABLE 5. *EGFR* Mutation Analysis

Response	TTP (d)	Survival (d)	Sex	Histology	Smoking history	Mutation status	Exon	Type of Mutation
PR	222	546	Female	Adenocarcinoma	Never	+	19	del E746–A750
PR	230	811+	Male	Adenocarcinoma	Current	+	19	del S752 –I759 and T751N
PR	278+	911	Female	Adenocarcinoma	Never	+	19	V786M, del E746–A750
SD	224	649+	Male	Adenocarcinoma	Former	+	21	del V834-
SD	77	737	Female	Adenocarcinoma	Former	−	—	—
PD	60	604+	Female	Adenocarcinoma	Never	+	20, 21	L858R, T790M
PD	19	347	Male	Adenocarcinoma	Former	−	—	—

TTP, time to progression; PR, partial response; SD, stable disease; PD, progressive disease.

a dose of 150 mg/d, as well as a phase II study of erlotinib in NSCLC conducted in the United States.[16] In this study, erlotinib achieved an ORR of 28.3%, which was higher than expected, and a DCR of 50%. The response rate was higher than that determined in the above-mentioned phase II study[16] and in keeping with the rate seen in the Japanese subgroup in the phase II study of gefitinib (IDEAL1; 27.5%).[6] Assessment of QoL using the LCS demonstrated a clinically meaningful rate of symptom improvement of 42.1%.

The characteristics of the patients in this study were generally similar to those of NSCLC patients as a whole, in terms of their demographics and disease and treatment history, with the exception of a particularly high proportion of patients with adenocarcinoma (92%). The possibility of enrollment bias on the basis of histological type cannot be ruled out, in part because enrollment coincided with the emergence of reports that the efficacy of EGFR-TKI therapy was greater in patients with adenocarcinoma.[17] However, we also observed one PR and two SDs among three patients with squamous cell carcinoma (FAS population), and our results do not rule out the efficacy of erlotinib in any patient subtype. A multivariate logistic regression analysis showed that smoking status was significantly associated with tumor response, in agreement with previous studies of predictive factors for response to EGFR-TKIs.[5,18,19]

The median survival time with erlotinib was an encouraging 14.7 months. One of the reasons for this long survival may be the high proportion of never-smokers and patients with adenocarcinoma compared with those of other studies, particularly the multinational phase III erlotinib study (BR.21).[5] On the other hand, the presence of *EGFR* gene mutations is currently regarded as an important determinant of treatment response to EGFR-TKIs[20,21] and may be the most important factor in relation to the favorable results seen in the present study. However, it is important to recognize that the potential prognostic effect of mutation status cannot be excluded. The sample size of this and previous trials limits the interpretation of this effect, which will be adequately assessed only by means of appropriately powered trials specifically designed to examine these factors.

Assessment of the presence or absence of *EGFR* gene mutation was possible in only seven patients in the present study. Despite this, the results were consistent with the results of some previous studies. All three of the patients who had a PR (including a male current smoker) had an in-frame deletion in exon 19, which is considered to be the most frequent mutation site in the EGFR-TK domain.[22] One of the 2 patients with PD had a point substitution mutation (L858R) in exon 21, the second most frequent mutation site,[22] and a point mutation (T790M) in exon 20, which is suggested to be involved in tolerance to EGFR-TKI.[12,23,24] It would be valuable to conduct further prospective randomized studies on the association between these markers and survival during treatment with erlotinib in Japanese patients.

Rash and diarrhea were the main AEs reported by patients on erlotinib treatment, as reported in previous studies.[5,15,16] Rash was observed in almost all patients, and was the main reason for treatment interruptions or dose reductions. Although the protocol allowed treatment to be interrupted for grade 3 rash (or intolerable grade 2 rash), grade 3 rash only occurred in 2 patients, leading to discontinuation of treatment in one. Most cases of rash responded to symptomatic treatment and either interruption or dose reduction of erlotinib. Despite suggestions in some reports that the presence of erlotinib-related rash is associated with treatment efficacy and can be used to predict response,[25] no supportive evidence was found in the present study.

The incidence of ILD, which is the most clinically problematic AE associated with erlotinib, tended to be higher than that reported in other clinical studies of erlotinib.[5,26] This is in keeping with this class of agent, and is not unexpected in the Japanese population.

We would recommend that careful screening of patients for ILD risk factors, particularly signs of interstitial pneumonia and pulmonary fibrosis, is done before erlotinib therapy is initiated. Individuals with any previous history of ILD were excluded from this study.

In conclusion, erlotinib (150 mg/d) was shown to have promising antitumor efficacy in Japanese patients with previously treated NSCLC, leading to clinically meaningful improvements in symptoms and an encouraging median survival time. Despite, as expected, a high rate of rash and diarrhea, erlotinib was well tolerated at a dose of 150 mg/d by the majority of patients.

REFERENCES

1. Parkin MD. Global cancer statistics in the year 2000. *Lancet Oncol* 2001;2:533–543.
2. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small cell lung cancer. *N Engl J Med* 2002;346:92–98.

3. Ohe Y, Ohashi Y, Kubota K, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: four-arm cooperative study in Japan. *Ann Oncol* 2007;18: 317–323.
4. Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small cell lung cancer previously treated with platinum-based chemotherapy. *J Clin Oncol* 2000;18:2095–2103.
5. Shepherd F, Rodrigues J, Ciuleanu T, et al. Erlotinib in previously treated non-small cell lung cancer. *N Engl J Med* 2005;353:123–132.
6. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2003;21:2237–2246.
7. Chang A, Parikh P, Thongprasert S, et al. Gefitinib (IRESSA) in patients of Asian origin with refractory advanced non-small cell lung cancer: subset analysis from the ISEL study. *J Thorac Oncol* 2006;1:847–855.
8. Miller VA, Kris MG, Shah N, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:1103–1109.
9. 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.
10. 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.
11. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;352:786–792.
12. Pao W, Miller V, Zakovski 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 USA* 2004;101:13306–13311.
13. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205–216.
14. Cella DF, Bonomi AE, Lloyd SR, et al. Reliability and validity of the functional assessment of cancer therapy-lung (FACT-L) quality of life instrument. *Lung Cancer* 1995;12:199–220.
15. Yamamoto N, Horiike A, Fujisaka Y, et al. Phase I dose-finding and pharmacokinetic study of the oral epidermal growth factor receptor tyrosine kinase inhibitor Ro50–8231 (erlotinib) in Japanese patients with solid tumors. *Cancer Chemother Pharmacol* 2008;61:489–496.
16. Pérez-Soler RS, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non-small-cell lung cancer. *J Clin Oncol* 2004;22:3238–3247.
17. Kaneda H, Tamura K, Kurata T, et al. Retrospective analysis of the predictive factors associated with the response and survival benefit of gefitinib in patients with advanced non-small-cell lung cancer. *Lung Cancer* 2004;46:247–254.
18. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005;366: 1527–1537.
19. Clark GM, Zborowski DM, Santabárbara P, et al. Smoking history and epidermal growth factor receptor expression as predictors of survival benefit from erlotinib for patients with non-small-cell lung cancer in the National Cancer Institute of Canada Clinical Trials Group study BR. 21. *Clin Lung Cancer* 2006;7:389–394.
20. Toyooka S, Matsuo K, Shigematsu H, et al. The impact of sex and smoking status on the mutational spectrum of epidermal growth factor receptor gene in non small cell lung cancer. *Clin Cancer Res* 2007;13: 5763–5768.
21. Mitsudomi T, Kosaka T, Endoh H, et al. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small cell lung cancer with postoperative recurrence. *J Clin Oncol* 2005;23:2513–2520.
22. Sequist LV, Bell DW, Lynch TJ, et al. Molecular predictors of response to epidermal growth factor receptor antagonists in non-small-cell lung cancer. *J Clin Oncol* 2007;25:587–595.
23. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005;2:e73.
24. Tokumo M, Toyooka S, Ichihara S, et al. Double mutation and gene copy number of EGFR in gefitinib refractory non-small cell lung cancer. *Lung Cancer* 2006;53:117–121.
25. Wacker B, Nagrani T, Weinberg J, et al. Correlation between development of rash and efficacy in patients treated with the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib in two large phase III studies. *Clin Cancer Res* 2007;13:3913–3921.
26. Tsuboi M, Le Chevalier T. Interstitial lung disease in patients with non-small cell lung cancer treated with epidermal growth factor receptor inhibitors. *Med Oncol* 2006;23:161–170.

VOLUME 26 · NUMBER 26 · SEPTEMBER 10 2008

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Phase III Study, V-15-32, of Gefitinib Versus Docetaxel in Previously Treated Japanese Patients With Non–Small-Cell Lung Cancer

Riichiroh Maruyama, Yutaka Nishiwaki, Tomohide Tamura, Nobuyuki Yamamoto, Masahiro Tsuboi, Kazuhiko Nakagawa, Tetsu Shinkai, Shunichi Negoro, Fumio Imamura, Kenji Eguchi, Koji Takeda, Akira Inoue, Keisuke Tomii, Masao Harada, Noriyuki Masuda, Haiyi Jiang, Yohji Itoh, Yukito Ichinose, Nagahiro Saijo, and Masahiro Fukuoka

From the National Kyushu Cancer Center, Fukuoka; National Cancer Center Hospital East, Chiba; National Cancer Center Hospital, Tokyo Medical University Hospital, Tokyo; Shizuoka Cancer Center, Shizuoka; Kinki University School of Medicine, Osaka Medical Center for Cancer and Cardiovascular Diseases; Osaka City General Hospital; AstraZeneca KK, Osaka; Shikoku Cancer Center, Ehime; Hyogo Medical Center for Adults; Kobe City General Hospital, Hyogo; Tokai University Hospital, Kanagawa; Tohoku University Hospital, Miyagi; Hokkaido Cancer Center, Hokkaido; and Kitasato University School of Medicine, Kanagawa, Japan.

Submitted November 5, 2007; accepted March 14, 2008.

Supported by AstraZeneca.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Yukito Ichinose, MD, Department of Thoracic Oncology, National Kyushu Cancer Center, 3-1-1 Notame Minami-ku, Fukuoka, 811-1395, Japan; e-mail: yichinos@nk-cc.go.jp.

0732-183X/08/2626-4244/$20.00

DOI: 10.1200/JCO.2007.15.0185

ABSTRACT

Purpose
This phase III study (V-15-32) compared gefitinib (250 mg/d) with docetaxel (60 mg/m^2) in patients (N = 489) with advanced/metastatic non–small-cell lung cancer (NSCLC) who had failed one or two chemotherapy regimens.

Methods
The primary objective was to compare overall survival to demonstrate noninferiority for gefitinib relative to docetaxel. An unadjusted Cox regression model was used for the primary analysis.

Results
Noninferiority in overall survival was not achieved (hazard ratio [HR], 1.12; 95.24% CI, 0.89 to 1.40) according to the predefined criterion (upper CI limit for HR ≤ 1.25); however, no significant difference in overall survival (P = .330) was apparent between treatments. Poststudy, 36% of gefitinib-treated patients received subsequent docetaxel, and 53% of docetaxel-treated patients received subsequent gefitinib. Gefitinib significantly improved objective response rate and quality of life versus docetaxel; progression-free survival, disease control rates, and symptom improvement were similar for the two treatments. Grades 3 to 4 adverse events occurred in 40.6% (gefitinib) and 81.6% (docetaxel) of patients. Incidence of interstitial lung disease was 5.7% (gefitinib) and 2.9% (docetaxel). Four deaths occurred due to adverse events in the gefitinib arm (three deaths as a result of interstitial lung disease, judged to be treatment related; one as a result of pneumonia, not treatment related), and none occurred in the docetaxel arm.

Conclusion
Noninferiority in overall survival between gefitinib and docetaxel was not demonstrated according to predefined criteria; however, there was no statistically significant difference in overall survival. Secondary end points showed similar or superior efficacy for gefitinib compared with docetaxel. Gefitinib remains an effective treatment option for previously treated Japanese patients with NSCLC.

J Clin Oncol 26:4244-4252. © 2008 by American Society of Clinical Oncology

INTRODUCTION

In Japan, patients with advanced non–small-cell lung cancer (NSCLC) who fail first-line platinum-based therapy often receive second-line docetaxel.[1,2] However, docetaxel has been associated with significant levels of toxicity, especially grades 3 to 4 neutropenia (40% to 67% and 63% to 73% for docetaxel 75 mg/m^2 and 60 mg/m^2, respectively).[1-4] In North America and in European countries, docetaxel,[3,4] pemetrexed,[2] and erlotinib[5] are approved second-line treatments for NSCLC.[3,6]

In phase II trials (IDEAL 1 and 2), the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib (Iressa; AstraZeneca, London, United Kingdom) 250 mg/d showed response rates of 12% to 18% and median survival of 7.0 to 7.6 months in patients who had pretreated advanced NSCLC.[7,8] A subset of Japanese patients in IDEAL 1 demonstrated a higher response rate (27.5%) and longer median survival (13.8 months) compared with the overall population.[9] A phase III study (Iressa Survival Evaluation in Lung Cancer) in patients who had previously treated refractory NSCLC