

**Table 2.** Internal consistency and item-total correlation

Items to subscale	Cronbach's alpha coefficients	I-T correlation	Mean	SD	Min	Max	Response rate
Physical well being	0.789		65.45	11.17	30	80	87
Fatigue		0.737	6.81	2.35	0	10	100
Appetite		0.761	8.64	2.05	0	10	100
Aches/pain		0.695	8.11	2.09	2	10	100
Sleep		0.781	7.98	2.44	0	10	100
Constipation		0.615	8.24	2.55	0	10	100
Nausea		0.650	9.45	1.60	0	10	100
Menstrual chg/fertility		0.523	8.64	2.72	0	10	87
Overall physical		0.489	6.95	2.36	0	10	100
Psychological well being	0.890		121.46	28.65	36	180	100
Coping		0.517	8.04	2.05	1	10	100
QOL item		0.467	7.78	1.78	2	10	100
Happiness		0.432	7.90	2.02	2	10	100
Control		0.404	7.58	2.21	1	10	100
Satisfaction		0.566	7.80	1.77	3	10	100
Concentration/memory		0.489	6.55	1.92	1	10	100
Usefulness		0.369	7.14	2.38	0	10	100
Appearance		0.448	7.62	2.75	0	10	100
Self concept		0.431	6.89	2.74	0	10	100
Initial dx distress		0.546	3.77	3.31	0	10	100
Ca treatment distress		0.606	5.19	3.53	0	10	100
Time since tx distress		0.660	7.50	2.28	0	10	100
Anxiety		0.766	6.44	2.89	0	10	100
Depression		0.792	7.87	2.40	1	10	100
Fear future test		0.749	7.04	3.03	0	10	100
Fear second ca		0.779	5.21	3.32	0	10	100
Fear recurrent ca		0.784	5.44	3.48	0	10	100
Fear spread ca		0.756	5.69	3.49	0	10	100
Social well being	0.684		59.34	11.55	18	80	100
Family distress		0.384	3.10	2.98	0	10	100
Support/others		0.063	8.12	2.61	0	10	100
Personal relationship		0.689	8.94	1.86	0	10	100
Sexuality		0.614	8.28	2.85	0	10	100
Employment		0.679	7.72	2.91	0	10	100
Home activity		0.714	7.46	2.41	1	10	100
Feel isolate		0.748	8.19	2.41	0	10	100
Financial burden		0.691	7.53	2.49	0	10	100
Spiritual well being	0.652		33.76	11.28	8	70	100
Import relig. activ		0.574	1.88	2.78	0	10	100
Import spiritual activ		0.568	1.90	2.64	0	10	100
Spiritual change		0.537	5.15	2.91	0	10	100
Uncertainty		0.144	6.02	2.78	0	10	100
Positive change		0.685	4.76	3.31	0	10	100
Life purpose		0.748	7.04	2.65	0	10	100
Hopefulness		0.734	7.02	2.69	0	10	100
Overall QOL	0.904						

0.70. Most items indicated a strong to moderate correlation with the subscale. However, items 15 (usefulness), 27 (family distress), 28 (amount of social support received), and 38 (uncertainty about the future) demonstrated a low consistency with

the subscale (psychological;  $r = 0.37$ , social;  $r = 0.38$  and  $r = 0.06$ , and spiritual;  $r = 0.14$ , respectively). The Cronbach's  $\alpha$  coefficients of all subscales were more than 0.70, when these items were excluded from each subscale.

### Validity

To confirm the concurrent validity, the Pearson's correlation coefficients between the subscales of SF-36, the fighting spirit and the helplessness/hopelessness subscales of the MAC, and the subscales of the QOL-CS-J were calculated (Table 3). There were moderate correlations between associated subscales including QOL-CS-J physical to SF-36 bodily pain ( $r = 0.45$ ,  $p < 0.01$ ) and vitality ( $r = 0.52$ ,  $p < 0.01$ ); QOL-CS-J psychological to SF-36 mental health ( $r = 0.55$ ,  $p < 0.01$ ); QOL-CS-J social to SF-36 general health perception ( $r = 0.31$ ,  $p < 0.01$ ) and mental health ( $r = 0.47$ ,  $p < 0.01$ ); QOL-CS-J spiritual to MAC fighting spirit ( $r = 0.33$ ,  $p < 0.01$ ) and helplessness/hopelessness ( $r = -0.32$ ,  $p < 0.01$ ); and the QOL-CS-J total to each subscale of SF-36 ( $r = 0.25-0.64$ ,  $p < 0.05$ ).

To test discriminant validity,  $t$ -tests or  $\chi^2$  tests were conducted between grade 0 and more than 1 of performance status, with and without pain, and with and without dyspnoea. Each score of the QOL-CS-J physical and social subscales of patients with good performance status, without pain, and without dyspnoea was significantly higher than each score of patients with poor performance status, with pain, and with dyspnoea.

### Discussion

The feasibility was reasonably good because 93% of the participants who received the questionnaire

responded, and there were no missing data except for one item. However, 13.3% of the participants apparently refused to respond to the item regarding menstrual changes or fertility. The participants who did not respond to this item were significantly older than the participants who did respond. There are two possible reasons for this: those participants who failed to respond may have already been post menopausal; or the Japanese, especially the elderly, are not accustomed to talk with other people about extremely personal matters such as menstruation or fertility.

A strong to moderate correlation value was indicated for the individual items to the subscale, except for four items; usefulness, family distress, the amount of received social support, and uncertainty about the future. When these items were excluded from each subscale, the internal consistencies of all subscales and total scores were good. There are three possible reasons that these four items showed low associations with each subscale: the participants' characteristics differed from the original QOL-CS validation study, in which 43% of the participants were breast cancer patients and 81% were female [1]; in the current study, the content validity was insufficient, that is, the content of the items was not confirmed by cancer patients; or the cultural differences, that is, the family-centered model of decision making and Buddhism and/or Shintoism as the religious beliefs in Japan. However, these four items had also been

Table 3. Concurrent validity

	QOL-CS				
	Physical	Psychological	Social	Spiritual	Total
QOL-Psychological	0.596**				
QOL-Social	0.569**	0.696**			
QOL-Spiritual	0.101**	0.191*	0.160		
QOL-Total	0.737**	0.934**	0.797**	0.407**	
SF-Physical functioning	0.339**	0.256**	0.273**	0.164	0.315**
SF-Role-Physical	0.267**	0.251**	0.253**	0.129	0.251*
SF-Bodily Pain	0.454**	0.272**	0.208*	0.163	0.384**
SF-General health perception	0.420**	0.554**	0.310**	0.162	0.533**
SF-Vitality	0.520**	0.414**	0.288**	0.235*	0.508**
SF-Social functioning	0.302**	0.265**	0.295**	0.095	0.326**
SF-Role-Emotional	0.440**	0.296**	0.295**	0.180	0.349**
SF-Mental health	0.511**	0.548**	0.474**	0.200*	0.635**
MAC-Fighting spirit	0.161	0.179	0.145	0.329**	0.253*
MAC-Helplessness/hopelessness	-0.356**	-0.418**	-0.328**	-0.321**	-0.473**

\* $p < 0.05$ , \*\* $p < 0.01$ .

suggested to have a weak association with the subscale in the original study [1].

Regarding the concurrent validity, there was correlation between the physical related subscales, the psychological related subscales, and the social related subscales of the QOL-CS-J and the SF-36, and the spiritual well being subscale of the QOL-CS-J and the fighting spirit and helplessness/hopelessness subscales of the MAC. Regarding the discriminant validity, the participants with poor performance status, pain, and dyspnoea demonstrated low scores in the physical and social well being subscale of the QOL-CS-J. These results imply that the validity of the QOL-CS-J is good.

This study had two limitations. First, this study examined subjects' responses at only one point in of time. A test-retest reliability needs to be conducted to examine fully the stability of the QOL-CS-J. Second, participants in this study were the survivors of only NSCLC. Further study on cancer survivors of other types and sites needs to be conducted.

#### Acknowledgements

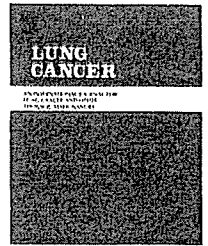
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## Eg5 expression is closely correlated with the response of advanced non-small cell lung cancer to antimitotic agents combined with platinum chemotherapy

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### KEYWORDS

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Cyclin B1

### Summary

**Background:** Eg5 is a microtubule motor protein that functions in bipolar spindle assembly. We investigated the relationship between Eg5 expression and the response to chemotherapy of patients with advanced non-small cell lung cancer (NSCLC).

**Patients and methods:** Eg5 expression was investigated immunohistochemically in 122 formalin-fixed tumor samples from untreated stage IIIB or IV NSCLC patients. We also investigated cyclin B1 expression, which is involved in the G2/M transition. All patients received antimitotic agents combined with platinum chemotherapy. The response to chemotherapy was compared in relation to Eg5 and cyclin B1 expression and in relation to clinicopathological factors.

**Results:** The response rate to chemotherapy of patients with Eg5-positive tumors was 37%, as opposed to 10% for patients with Eg5-negative tumors, and Eg5 expression was significantly associated with the response to chemotherapy ( $P=0.002$ ). The response rate of patients with cyclin B1-positive tumors (53%) was higher than that of patients with cyclin B1-negative tumors (23%) ( $P=0.009$ ), and Eg5 expression was significantly correlated with cyclin B1 expression ( $P=0.005$ ). A multivariate analysis confirmed Eg5 status to be an independent variable related to response to chemotherapy ( $P=0.008$ ).

**Conclusions:** Eg5 expression can predict a response to antimitotic agents combined with platinum chemotherapy among patients with advanced NSCLC.

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## 1. Introduction

Lung cancer is a major cause of death from cancer worldwide, and non-small cell lung cancer (NSCLC) accounts for ~85% of all cases of lung cancer. More than half of patients with NSCLC have advanced stage IIIB or IV disease at presentation, and patients with advanced NSCLC are candidates for systemic chemotherapy [1]. Meta-analyses have demonstrated that cisplatin-based chemotherapy for metastatic NSCLC statistically improves patient survival, compared with supportive care alone [2]. However, the response rate to chemotherapy has been poor, and very few patients survive for 5 years [3]. During the 1990s, five new drugs became available for the treatment of metastatic NSCLC: paclitaxel, docetaxel, vinorelbine, gemcitabine, and irinotecan. Each of these drugs has since been evaluated in combination regimens with cisplatin or carboplatin and has produced responses in 20–30% of patients [1]. Unfortunately, despite the increasing number of active chemotherapeutic agents, none of these chemotherapeutic regimens has offered a significant advantage over the others in the treatment of advanced NSCLC in randomized studies [4,5], and advanced NSCLC patients still have a median survival time of <1 year. Several reasons have been offered to explain the response to chemotherapy, such as the presence of drug-resistant tumor cells [6] and the redistribution of tumor cells within the cell cycle after chemotherapy. However, the molecular basis of the response to chemotherapy remains to be explored.

A network of microtubular filaments forms the cytoplasmic matrix, giving rise to the concept of the cytoskeleton, which comprises microtubules, actin, and intermediate filaments. Microtubules display a remarkable versatility of function and are involved in multiple biologic phenomena, including mitosis, cell shape determination, cell locomotion, and the movement of intracellular organelles [7]. Microtubule-polymerizing agents, including paclitaxel and docetaxel, and microtubule-depolymerizing agents, including vinorelbine, target preliminary tubulin and can induce disrupting kinetic stabilization of microtubules' polymerization–depolymerization, thus blocking the cell cycle in the mitotic phase [8].

Microtubule motors bind to and move unidirectionally on microtubules, and they have been proposed to generate the force required for spindle assembly and maintenance, attachment of the chromosomes to the spindle, and movement of chromosomes toward opposite poles. The microtubule motor proteins, which are members of the kinesin, dynein, or myosin families, can account for many of the movements of the spindle and chromosomes in dividing cells. Kinesin motors have been shown to be necessary to establish spindle bipolarity, position chromosomes on the metaphase plate, and maintain forces in the spindle [9]. Evidence that kinesin motors facilitate microtubule depolymerization also exists, raising the possibility that the motors modulate microtubule dynamics during mitosis. Eg5, which is a part of the kinesin-5 molecule (a member of the kinesin superfamily), is a microtubule motor protein. Eg5 accounts for many of the movements of the spindle and chromosomes in dividing cells and localizes to the spindle in mitotically dividing cells. It has been implicated in spindle function by both its cellular localization and the effects of mutations. Eg5 function in centrosome or spindle pole body sep-

aration is necessary for bipolar spindle assembly [10]. The latest antimitotic agent, named monastrol, is an inhibitor of mitotic kinesin Eg5 [11,12]. Monastrol arrests mitosis by reversibly inhibiting mitotic kinesin Eg5 and impairing bipolar mitotic spindle formation. Prolonged mitotic arrest leads to apoptosis in tumor cells and to senescence or apoptosis in primary cells, and the inhibition of mitotic kinesin Eg5 results in the formation of monoaster spindles leading to mitotic arrest [13].

Cyclin and cyclin-dependent kinase complexes play an important role in the control of the cell cycle [14], and the cyclin B1/cdc2 complex has a role as a maturation/mitosis-promoting factor in the G2–M phase transition during the cell cycle [15]. Thus, lack of regulation of cyclin B1 expression may be involved in uncontrolled cell growth and malignant transformation. Overexpression of cyclin B1 has been reported in various malignant tumors and has been shown to predict a poor outcome in NSCLC, esophageal carcinoma, and head and neck cancer [16–18].

In this retrospective study, we investigated the level of expression of Eg5, in addition to cyclin B1—a molecule involved in the G2/M transition, in clinical samples from patients with advanced NSCLC who were subsequently treated with antimitotic agents and investigated whether its expression predicts response to chemotherapy and outcome.

## 2. Materials and methods

### 2.1. Subjects

A total of 122 stage IIIB or IV NSCLC patients received platinum-based combination chemotherapy combined with docetaxel, paclitaxel or vinorelbine at the National Cancer Center Hospital East between August 1997 and July 2004 because of PS 0 or 1 on the Eastern Cooperative Oncology Group scale. Adequate tumor biopsy specimens were obtained from all 122 of these patients before chemotherapy and were analyzed in this study. All of the tumor specimens were obtained before chemotherapy, by bronchoscopy in 83 patients, by percutaneous needle biopsy in 31 patients, by thoracotomy in five patients, and by mediastinoscopy in three patients. The histological classification was based on the third edition of the WHO classification. Clinical staging was based on an initial evaluation consisting of a clinical assessment, chest radiography, computed tomography of the chest and abdomen, computed tomography or magnetic resonance imaging of the brain, and bone scintigraphy. The current international staging system was used for clinical disease staging [19]. The clinicopathological characteristics of all the patients are listed in Table 1. Their median age at diagnosis was 62 years (range, 42–78 years). Seven of the 43 stage IIIB patients were women, and 32 of the 79 stage IV patients were women. All of the patients were treated with antimitotic agents combined with platinum chemotherapeutic regimens in what were considered standard regimens for patients with metastatic NSCLC [20]. Nine of the 43 stage IIIB patients received thoracic radiotherapy after the completion of chemotherapy; three of these patients were women. The median follow-up time of the 122 patients was 26 months (range, 18–54 months).

**Table 1** Characteristics of 122 patients with advanced NSCLC

Characteristics	No. of patients
Total no. of patients	122
Gender	
Male	83
Female	39
Age (years)	
Median	62
Range	42–78
Histology	
Adenocarcinoma	80
Squamous cell carcinoma	28
Large cell carcinoma	13
Others	1
Stage	
IIIB	43
IV	79
Performance status	
0	32
1	90
Chemotherapeutic regimen	
Cisplatin + vinorelbine	76
Cisplatin + docetaxel	20
Carboplatin + paclitaxel	26
Smoking history	
Positive	91
Negative	31

NSCLC: non-small cell lung cancer.

After obtaining informed consent in accordance with our institution's guidelines, all of the patients underwent a tumor biopsy and chemotherapy.

## 2.2. Chemotherapy

The platinum-based regimens were vinorelbine (25 mg/m<sup>2</sup>) on days 1 and 8 plus cisplatin (80 mg/m<sup>2</sup>) on day 1 of a 21-day cycle (76 patients), docetaxel (60 mg/m<sup>2</sup>) on day 1 plus cisplatin (80 mg/m<sup>2</sup>) on day 1 of a 21-day cycle (20 patients), and paclitaxel (200 mg/m<sup>2</sup> administered over 3 h) on day 1 plus carboplatin (dosed with an area under the curve of 6) on day 1 of a 21-day cycle (26 patients). All of the patients received two or more courses of chemotherapy before the appearance of progressive disease. We used the RECIST guidelines [21] to evaluate the response to chemotherapy. A complete response was defined as the disappearance of all clinically detectable lesions for at least 4 weeks. A partial response required a minimum of a 30% reduction in the greatest diameter of all of the measurable lesions for a minimum of 4 weeks. Progressive disease was defined as the appearance of new lesions or an increase in disease of >20% measured in the same manner as for partial response. All other results were classified as "no change". The response rate was defined as the total of the complete response cases and partial response cases expressed as a percentage of all

cases. PFS (progression-free survival) was measured from the start of chemotherapy until the documentation of progressive disease or death.

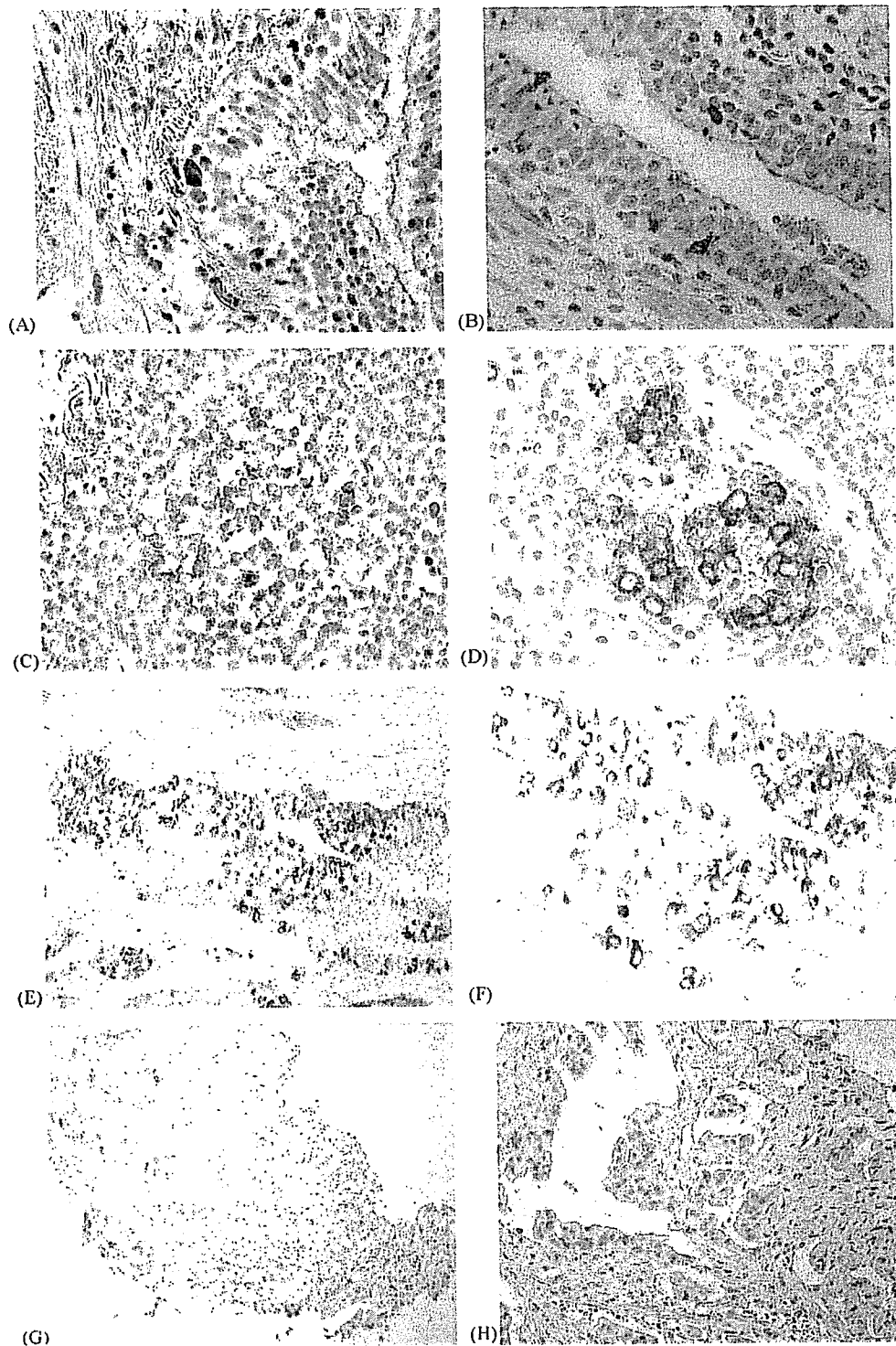
## 2.3. Immunohistochemistry

Immunostaining was performed on 4- $\mu$ m formalin-fixed, paraffin-embedded tissue sections. The slides were deparaffinized in xylene and dehydrated in a graded ethanol series. For antigen retrieval, the slides for cyclin B1 were immersed in 10 mM citric buffer solution (pH 6.0) and the slides for Eg5 were immersed in 1 mM EDTA retrieval fluid (pH 8.0). All of the slides were heated to 95 °C by exposure to microwave irradiation for 20 min. The slides were then cooled for 1 h at room temperature and washed in water and PBS. Endogenous peroxidase was blocked with 0.3% H<sub>2</sub>O<sub>2</sub> in methanol for 15 min. Non-specific binding was blocked by preincubation with 2% BSA plus 0.1% NaN<sub>3</sub> for 30 min; after draining off the blocking serum, the slides were incubated overnight at 4 °C with anti-Eg5 monoclonal antibody (Clone, 20; Dilution, 1:50; BD Biosciences, NJ, USA) or with anti-cyclin B1 monoclonal antibody (Clone, 7A9; Dilution, 1:20; Novocastrol Laboratories, Newcastle upon Tyne, UK). The slides were then washed three times in PBS and incubated with a labeled polymer Envision+ (DAKO, Glostrup, Denmark) for 60 min. The chromogen used was 2% 3,3'-diaminobenzidine in 50 mM Tris buffer (pH 7.6) containing 0.3% hydrogen. Slides were counterstained with hematoxylin [22,23]. Normal human lung tissue was used as a positive control.

Eg5 staining was considered positive if the cytoplasm of >10% of the tumor cells stained positive. Cyclin B1 staining was considered positive if the nuclei of >10% of the tumor cells stained positive, because the cyclin B1/cdc2 complex translocates from the cytoplasm into the nucleus during the G2/M transition [24–26]. Thus, the criteria for cyclin B1 positivity used in the present report differed from those used in other reports on non-small cell lung cancer, esophageal carcinoma and head and neck cancer. All of the slides were examined and scored independently by two observers (T.S. and G.I.) who had no knowledge of the patients' clinical data. When the antibody evaluations differed between the observers, the observers discussed the results, with or without re-evaluating the slides, until an agreement was reached.

## 2.4. Statistical analysis

The correlations between immunohistochemical expression and the clinical variables and response to chemotherapy were evaluated by the  $\chi^2$ -test or Fisher exact test, as appropriate. PFS was used as a clinical marker for duration of response to chemotherapy. Overall survival was measured from the start of chemotherapy to the date of death from any cause or the date the patient was last known to be alive. Survival curves were estimated using the Kaplan–Meier method, and any differences in PFS and survival between the subgroups were compared by using the log-rank test. The Cox proportional hazards model was used for a multivariate analysis. A multivariate analysis examining the correlation between variables and response to chemotherapy was performed by using logistic regression. *P* values <0.05 were



**Fig. 1** (A–D) Immunohistochemical staining of Eg5 in normal lung tissue (A), Eg5 is present in part of the basal layer of the bronchial epithelium in this frozen section of normal lung tissue (400 $\times$ ). (B) Eg5 is also present in parts of the basal layer of the bronchial epithelium in this formalin-fixed, paraffin-embedded section of normal lung tissue (400 $\times$ ). (C) Eg5 expression is visible in germinal center lymphocytes giving rise to follicular hyperplasia in this frozen section of normal lung tissue (400 $\times$ ). (D) Eg5 expression is also visible in germinal center lymphocytes giving rise to follicular hyperplasia in this formalin-fixed, paraffin-embedded section of normal lung tissue (400 $\times$ ). (E–H) Immunohistochemical staining of Eg5 in NSCLC (E), low magnification (100 $\times$ ) of squamous cell carcinoma of the lung showing Eg5 immunoreactivity (F), high magnification (200 $\times$ ) of squamous cell carcinoma of the lung showing Eg5 immunoreactivity (G), Eg5 staining was considered to be negative in this adenocarcinoma of the lung: the cytoplasm of <10% of the tumor cells were stained (low magnification; 100 $\times$ ). (H) Eg5 staining was considered to be negative in this adenocarcinoma of the lung: the cytoplasm of <10% of the tumor cells were stained (high magnification; 200 $\times$ ).

considered significant. Two-sided statistical tests were used in all of the analyses. Statistical analysis software (StatView-J Ver. 5.0, Windows) was used for the analyses.

### 3. Results

#### 3.1. Expression of Eg5 in normal lung tissue

To investigate the validation of immunostaining in the present experiment, we first evaluated Eg5 immunostaining in frozen sections and paraffin-embedded tissue sections of surgical specimens and confirmed that the staining intensity and specificity in the paraffin-embedded tissue sections were almost the same as in the frozen sections. Next, to choose the criteria for immunohistochemical positivity, normal lung tissue was used for Eg5 immunohistochemical staining. Representative immunohistochemical Eg5 staining in normal lung tissue is shown in Fig. 1A–D. In normal lung tissue, Eg5 expression was observed in some of the cells in the basal layer of the bronchial epithelium (Fig. 1A and B) and in germinal center lymphocytes exhibiting follicular hyperplasia (Fig. 1C and D). The frequency of positivity for bronchial epithelial cells and lymphoid germinal center lymphocytes were roughly more than 50% and 90%, respectively. We used these tissues as positive controls. Eg5 immunoreactivity was not detected in the pulmonary parenchyma.

#### 3.2. Expression of Eg5 in NSCLC

The tumors of 82 (67%) of the 122 patients were Eg5 positive. Cytoplasmic staining was observed in most of the Eg5-positive tumors, but some tumors also showed nuclear staining. The median of the percentage staining of the lung cancer cells for Eg5 was 35% (range, 0–100%). Representa-

tive immunohistochemical Eg5 staining in NSCLC is shown in Fig. 1E–H. Fig. 1E and F shows the staining results for an Eg5-positive squamous cell carcinoma of the lung. The cytoplasm of almost 80% of the cancer cells stained positive for Eg5. Fig. 1G and H shows an Eg5-negative adenocarcinoma of the lung; this adenocarcinoma of the lung was judged to be negative for Eg5 because the cytoplasm of <10% of the tumor cells showed evidence of staining.

The relationships between the expression of Eg5 and clinical variables are shown in Table 2. Eg5 expression was significantly higher in males than in females ( $P=0.03$ ), in squamous cell carcinoma than in non-squamous cell carcinoma ( $P=0.02$ ), and in current and former smokers than in non-smokers ( $P=0.03$ ).

The tumors of 18 (95%) of the 19 patients with cyclin B1-positive tumors were Eg5 positive, and the tumors of 39 (98%) of the 40 patients with Eg5-negative tumors were cyclin B1-negative (data not shown). Eg5 expression was significantly correlated with cyclin B1 expression ( $P=0.005$ ; data not shown).

#### 3.3. Expression of Eg5 and clinical outcome

All 122 patients were assessed for response to chemotherapy and survival. The relationships between clinical variables, Eg5 expression, and cyclin B1 expression, and the response to chemotherapy and survival in this study are shown in Table 3.

The chemotherapy response rate of patients with Eg5-positive tumors was 37%, as opposed to 10% for patients with Eg5-negative tumors. Eg5 expression was significantly associated with response to chemotherapy ( $P=0.002$ ). The chemotherapy response rate of patients with cyclin B1-positive tumors was 53%, as opposed to 23% for patients

**Table 2** Relationship between clinical variables and expression of primary antibodies

	<i>n</i>	Eg5-positive (%) patients	Cyclin B1-positive (%) patients
Total	122	82 (67)	19 (16)
Gender			
Male	83	61 (73)*	15 (18)
Female	39	21 (54)	4 (10)
Histology			
Sq	28	24 (86)**	6 (21)
Non-sq	94	58 (62)	13 (14)
Stage			
IIIB	43	30 (70)	8 (19)
IV	79	52 (66)	11 (14)
PS			
0	32	20 (63)	1 (3)
1	90	62 (69)	18 (20)**
Smoking history			
Positive	91	66 (73)*	17 (19)
Negative	31	16 (52)	2 (6)

Sq: squamous; PS: performance status.

\*  $P=0.03$ .

\*\*  $P=0.02$ .



**Table 3** Summary of the relationships between clinical variables and response to chemotherapy and survival

	<i>n</i>	Response rate (%)	<i>P</i>	PFS (months)	<i>P</i>	MST (months)	<i>P</i>
Total	122	28		5.0		12.0	
Gender							
Male	83	28	0.95	5.0	0.43	10.0	0.046
Female	39	28		7.0		15.0	
Histology							
Sq	28	32	0.57	5.0	0.72	9.0	0.64
Non-sq	94	27		5.0		13.0	
Stage							
IIIB	43	33	0.39	6.0	0.01	17.0	0.07
IV	79	25		5.0		11.0	
PS							
0	32	25	0.67	5.0	0.21	14.0	0.16
1	90	29		5.0		10.0	
Smoking history							
Positive	91	27	0.87	5.0	0.23	10.0	0.035
Negative	31	29		6.0		15.0	
Eg5							
Positive	82	37	0.002	5.0	0.08	10.0	0.006
Negative	40	10		6.0		13.0	
Cyclin B1							
Positive	19	53	0.009	5.0	0.77	8.0	0.31
Negative	103	23		5.0		13.0	

PFS: progression-free survival; MST: median survival time.

with cyclin B1-negative tumors, and cyclin B1 expression was also significantly associated with response to chemotherapy ( $P=0.009$ ).

The each of PFS and overall survival curves calculated using the Kaplan–Meier method according to Eg5 expression was shown in Fig. 2. The median PFS time for the Eg5-negative group was 6.0 months, as opposed to 5.0 months for the Eg5-positive group (Fig. 2A). The median survival time for the Eg5-negative group was 13.0 months, as opposed to 10.0 months for the Eg5-positive group (Fig. 2B). According to the overall survival data, the Eg5-positive group had a significantly poorer outcome than the Eg5-negative group ( $P=0.006$ ).

The median PFS time in both the cyclin B1-negative and the cyclin B1-positive group was 5.0 months (Fig. 2C). The median survival time in the cyclin B1-negative group was 13.0 months, as opposed to 8.0 months in the cyclin B1-positive group (Fig. 2D). Cyclin B1 expression was not associated with PFS or overall survival. Among the clinical variables, gender and smoking history were significantly associated with overall survival, and disease stage was significantly associated with PFS, also.

### 3.4. Multivariate analysis for response to chemotherapy, PFS, and overall survival

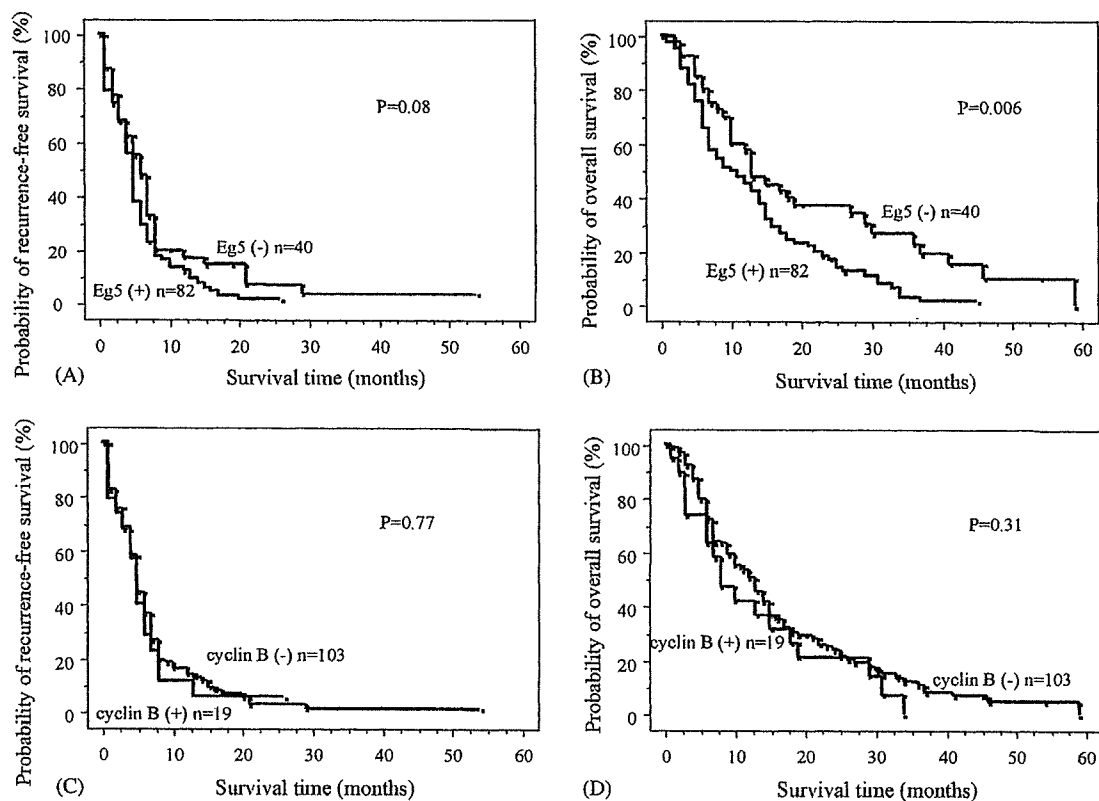
Following the univariate analyses for response to chemotherapy, PFS, and overall survival, we performed

multivariate analyses. Table 4 shows the results of the multivariate analysis for response to chemotherapy, PFS, and overall survival. The multivariate analysis for response to chemotherapy was performed using logistic regression to determine the prognostic value of Eg5 when other prognostic factors were considered. A multivariate analysis that included gender, histology, stage, PS, smoking history, Eg5 expression and cyclin B1 expression, showed that Eg5 expression was the only significant independent variable correlated with response to chemotherapy ( $P=0.008$ ).

A multivariate analysis using the Cox proportional hazards model for PFS and overall survival was performed, using gender, histology, stage, PS, smoking history, Eg5 expression and cyclin B1 expression, as variables. No correlation between variables and PFS was found in the multivariate analysis. Stage was the only independent variable significantly correlated with overall survival ( $P=0.036$ ).

## 4. Discussion

This is the study to investigate the relationship between the level of expression of Eg5 and the clinical response to chemotherapy and outcome of previously untreated patients with advanced NSCLC. Eg5, a kinesin motor, accounts for many of the movements of the spindle and chromosomes in dividing cells. It localizes to the spindle in mitotically dividing cells and has been implicated in spindle function by both its cellular localization and the effects of mutations.



**Fig. 2** (A) Progression-free survival curves of 122 patients with advanced non-small cell lung cancer, according to Eg5 expression. The median progression-free survival periods of Eg5-negative and -positive patients were 6.0 and 5.0 months, respectively. (B) Overall survival curves for 122 patients with advanced non-small cell lung cancer, according to Eg5 expression. The median survival periods for Eg5-negative and -positive patients were 13.0 and 10.0 months, respectively. (C) Progression-free survival curves of 122 patients with advanced non-small cell lung cancer, according to cyclin B expression. The median progression-free survival periods of Eg5-negative and -positive patients were 5.0 and 5.0 months, respectively. (D) Overall survival curves for 122 patients with advanced non-small cell lung cancer, according to cyclin B1 expression. The median survival periods for cyclin B1-negative and -positive patients were 13.0 and 8.0 months, respectively.

Eg5 function in centrosome or spindle pole body separation is necessary for bipolar spindle assembly [10].

In normal lung tissue, Eg5 expression was found to be present in some of the cells in the basal bronchial layer of the bronchial epithelium, but its expression in this region was not as strong as in lung cancer tissue. The overexpression of cyclin B1 has been reported in various malignant tumors and has been shown to predict a poor outcome in patients with NSCLC, esophageal carcinoma, and head and neck cancer [16–18]. It has been postulated that the overexpression of cyclin B1 is involved in uncontrolled cell growth and the malignant potential of carcinoma cells. Since the expression of Eg5 in lung cancer tissue has been found to be correlated with the expression of cyclin B1, lung cancer tissue that overexpresses Eg5 in comparison with normal lung tissue is assumed to have greater malignant potential than lung cancer tissue that does not.

Eg5 expression before chemotherapy was correlated with response to chemotherapy and Eg5 status was found to be an independent prognostic factor of response to chemotherapy in a multivariate analysis. Further investigation showed that Eg5 expression was correlated with the response to each type of regimen: the taxan regimens (CDDP + docetaxel:  $n = 20$ ; CBDCA + paclitaxel:  $n = 26$ ;  $P = 0.046$ ), and the vinca

alcaroid regimen (CDDP + vinorelbine:  $n = 76$ ;  $P = 0.02$ ) (data not shown). The mechanisms by which Eg5 overexpression affects chemotherapy have not been fully elucidated; nevertheless, Marcus et al. [27] recently reported that mitotic kinesin Eg5 inhibitors induce mitotic arrest and cell death in both paclitaxel-resistant and paclitaxel-sensitive cancer cells and that Eg5 was required for paclitaxel-induced microtubule aster formation (multi-polar spindle configuration) in an *in vitro* assay. They suggested that Eg5 functionality is necessary for paclitaxel-induced mitotic arrest and cell death. These findings may explain our result that Eg5 overexpression before chemotherapy was significantly correlated with response to chemotherapy. The results for docetaxel can be explained in the same manner as for paclitaxel because their modes of action are the same. On the other hand, vinorelbine inhibits the polymerization of tubulin. We suspect that some unknown interaction between tubulin and Eg5 may be modified by vinca alkaloids.

Although Eg5 expression was significantly correlated with response to chemotherapy, the Eg5-positive cases tended to have a poorer outcome in terms of overall survival than the Eg5-negative cases. The reason why the Eg5-positive cases had a poorer outcome remains unclear; despite their higher response to antimetabolic agents, Eg5-positive cells may have

**Table 4** Multivariate analysis

Variables	Category	Risk ratio	95% CI	P
<b>Multivariate analysis for response of advanced NSCLC patients</b>				
Gender	Male vs. female	0.77	0.245–2.42	0.66
Histology	Sq vs. non-sq	0.89	0.31–2.57	0.83
Stage	IIIB vs. IV	0.64	0.25–1.65	0.35
PS	0 vs. 1	0.98	0.34–2.82	0.97
Smoking history	(–) vs. (+)	0.59	0.18–1.95	0.39
Eg5	(–) vs. (+)	5.16	1.54–17.29	0.008
Cyclin B1	(–) vs. (+)	2.82	0.94–8.45	0.06
<b>Multivariate analysis for PFS of advanced NSCLC patients</b>				
Gender	Male vs. female	0.90	0.56–1.45	0.67
Histology	Sq vs. non-sq	0.89	0.55–1.43	0.63
Stage	IIIB vs. IV	0.60	0.39–0.93	0.02
PS	0 vs. 1	0.92	0.59–1.45	0.72
Smoking history	(–) vs. (+)	0.84	0.51–1.39	0.50
Eg5	(–) vs. (+)	0.77	0.50–1.19	0.24
Cyclin B1	(–) vs. (+)	1.09	0.62–1.89	0.77
<b>Multivariate analysis for OS of advanced NSCLC patients</b>				
Gender	Male vs. female	0.74	0.44–1.26	0.27
Histology	Sq vs. non-sq	1.03	0.63–1.67	0.92
Stage	IIIB vs. IV	0.63	0.41–0.98	0.04
PS	0 vs. 1	0.76	0.47–1.22	0.25
Smoking history	(–) vs. (+)	0.74	0.43–1.30	0.30
Eg5	(–) vs. (+)	0.62	0.39–0.97	0.04
Cyclin B1	(–) vs. (+)	1.03	0.59–1.78	0.93

PFS: progression-free survival; NSCLC: non-small cell lung cancer; PS: performance status; CI: confidence interval; OS: overall survival.

a higher malignant potential, contributing to a poor clinical outcome. This appears to be consistent with the expression of Eg5 being significantly correlated with the expression of cyclin B1, which may be involved in uncontrolled cell growth and the malignant potential of cancer cells.

The inhibition of Eg5 has recently been exploited as an aid to cancer treatment [12–14,27–32], and small cell-permeable molecules that inhibit mitotic kinesin Eg5 and do not target tubulin arrest cells in mitosis with monoastrial spindles. Chromosomes in Eg5 inhibitor-treated cells frequently have both sister kinetochores attached to microtubules extending to the center of the monoaster. The mitotic kinesin Eg5 inhibitor also induces apoptosis and is effective in inhibiting the proliferation of cancer cells through mitotic arrest. The first small molecule inhibitor of Eg5 was monastrol [11,12], and second-generation Eg5 inhibitors like CK0106023 [29] and HR22C16 [27], which are specific allosteric inhibitors of Eg5 and exhibit anti-tumor activity *in vivo* or *in vitro*, have been discovered by drug screens. Therapeutic intervention with Eg5-specific inhibitors has also been reported, and SB-715992 has been shown to be a potent inhibitor of mitotic kinesin Eg5. Eg5 inhibitors may be used as new antimetabolic agents to treat advanced NSCLC in the future.

In conclusion, our findings indicated that the expression of the mitotic kinesin Eg5 can predict a response to antimetabolic agents combined with platinum chemotherapy among patients with advanced NSCLC. Our results have important implications for the treatment of NSCLC because Eg5

inhibitors, which cause tumor cell apoptosis, may be effective in patients with advanced NSCLC.

## Acknowledgements

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# Is surgical resection indicated for a solitary non–small cell lung cancer recurrence?

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Dr T. Hishida

**Objectives:** Some investigators have reported long-term survival after surgical resection of a solitary non–small cell lung cancer recurrence in various sites. However, the role and indications of the second operation remain unclear.

**Methods:** We reviewed 28 patients with a solitary recurrence after successful initial resection of primary non–small cell lung cancer who underwent resection of the recurrent lesion. The clinicopathologic factors associated with outcome were analyzed.

**Results:** There were 17 men and 11 women. Recurrence resection was performed for the following sites: 16 in the lung, 5 in the brain, 2 in the adrenal gland, and 1 each in the chest wall, stomach, skin, pelvic lymph node, and malar bone. The median survival time was 25 months, and the 1-, 2-, and 5-year survival rates after recurrence were 89%, 59%, and 32%, respectively. Advanced p-stage (p-stage II and III, n = 14) of the primary tumor was the significant negative prognostic factor. Patients with p-stage II or III had survival equivalent to that of those who had multiple recurrences or were unfit for further surgical intervention.

**Conclusions:** Resection of a solitary non–small cell lung cancer recurrence might provide long-term survival in highly selected patients. However, surgical resection might be contraindicated if the primary tumor is stage II or III.

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Five-year survival rates of patients with non–small cell lung cancer (NSCLC) have been disappointing, even after successful complete resection, with about 50% of patients eventually experiencing recurrence and death from the disease.<sup>1</sup> Recurrent lesions are generally multiple and disseminated, and additional surgical intervention is usually not indicated. Some investigators have reported long-term survivals after solitary recurrence resection of the brain, adrenal gland, spleen, liver, and bone.<sup>2-10</sup> However, the role and indication of surgical intervention remain unclear. The aim of this study is to investigate clinicopathologic characteristics of patients with NSCLC who underwent resection of a solitary recurrent lesion and to identify prognostic factors.

## Patients and Methods

### Patients

We retrospectively reviewed the clinical and pathologic files of 1698 consecutive patients with NSCLC who had undergone complete surgical resection at the National Cancer Center Hospital East from 1989 through 2002. Data collection and analyses were approved, and the need for obtaining informed consent from each patient was waived by the institutional review board in January 2004. Patients with synchronous metastasis (M1) were excluded. Among them, we identified 592 (35%) patients with locoregional or distant recurrence in 2003 or earlier. We excluded patients with second pulmonary lesions that were not clearly distinguished from metachronous second primary NSCLC on the basis of the criteria of Martini and Melamed.<sup>11</sup> Our follow-up procedures included physical examination, chest roentgenogra-

**Abbreviations and Acronyms**

FDG	= $^{18}\text{F}$ fluorodeoxyglucose
NSCLC	= non-small cell lung cancer
PET	= positron emission tomography
RFI	= recurrence-free interval

phy, and blood testing, including tumor markers, 1 month after the initial operation, every 3 to 6 months during the first 3 years, and every half year to 1 year thereafter. If any abnormality was found, we performed computed tomographic scans. We did not routinely perform bone scanning and brain examinations for asymptomatic patients. When a lesion suggesting locoregional or distant recurrence was detected, we scrutinized the whole body radiologically.

Thirty of the 592 patients underwent resection of a solitary recurrent lesion. Among them, 2 patients who had recurrence-free intervals (RFIs) of 1 and 4 months, respectively, were eliminated from this study because they possibly had undetectable "missed" M1 disease at the initial operation. Nine of the 28 patients were symptomatic at the time of recurrence detection. All but 1 patient, who had intrapulmonary recurrence, were asymptomatic. The median period from recurrence detection to the second operation was 2.6 months (range, 0.2-24 months). The follow-up protocols were the same before and after recurrence resection. The median follow-up period after recurrence resection was 33 months, ranging from 11 to 128 months.

For the remaining 562 patients, surgical intervention was not indicated because recurrences were multiple, patients were unfit for further surgical intervention, or both. They underwent palliative chemotherapy, radiotherapy, or best supportive care. Patients with multiple recurrences who underwent palliative operations for symptomatic sites were included in this group.

**Prognostic Evaluation**

We attempted to identify prognostic factors associated with subsequent survival after resection of a solitary recurrent lesion. We evaluated the following factors: clinical characteristics at recurrence (sex, age, carcinoembryonic antigen level, time from initial resection to recurrence detection [RFI], symptoms at the time of recurrence, site of recurrence, and mode of recurrence [locoregional or distant]) and pathologic findings of the primary lung cancer (histology, tumor size, lymph node status, p-stage, and lymphatic and vascular permeations). We defined locoregional recurrence as recurrence within the ipsilateral thorax and distant recurrence as all other recurrences. Each pathologic specimen was reviewed by a board-certified pathologist who was blinded to the clinical outcome. Histology was specified on the basis of the World Health Organization classification for cell types.<sup>12</sup> Pathologic stages were determined on the basis of the TNM classification of the International Union Against Cancer.<sup>13</sup>

**Survival Analysis**

Survivals were calculated by using the Kaplan-Meier method and were compared with the log-rank test. Zero time was the date of recurrence identification, and the terminal event was defined as death from any cause. An observation was censored at the last

**TABLE 1. Clinicopathologic characteristics of 28 patients with NSCLC who underwent resection of a solitary recurrent lesion**

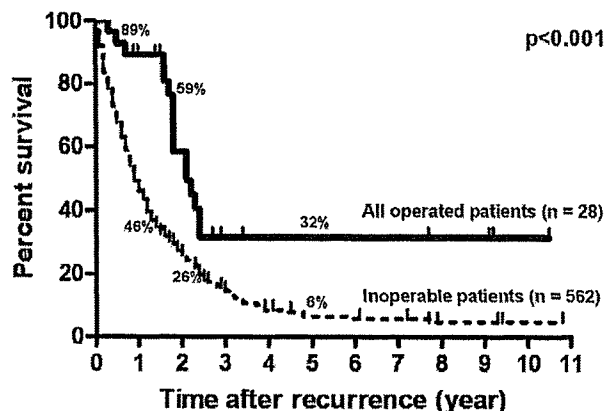
Characteristics	Value	No.
Clinical characteristics at recurrence		
Age at recurrence	Median	65
resection (y)	Range	39-73
Sex	Male	17
	Female	11
RFI (mo)	Median	23
	Range	6-82
CEA level (ng/mL)	Median	3.5
	Range	0.5-3286
Recurrent site	Ipsilateral lung	8
	Contralateral lung	8
	Brain	5
	Adrenal gland	2
	Chest wall	1
	Stomach	1
	Skin	1
	Abdominal lymph node	1
	Bone (malar bone)	1
Pathologic characteristics of primary tumor		
Histology	Adenocarcinoma	21
	Squamous cell carcinoma	5
	Adenosquamous carcinoma	1
	Pleomorphic carcinoma	1
Size of primary tumor (cm)	Mean $\pm$ SD	4.1 $\pm$ 1.7
p-Stage of primary tumor	IA/IB	4/10
	IIA/IIIB	2/6
	IIIA/IIIB	4/2
Nodal status of primary tumor	N0/N1/N2	18/7/3

RFI, Recurrence-free interval; CEA, carcinoembryonic antigen; SD, standard deviation.

follow-up when the patient was alive or lost to follow-up. Factors with a *P* value of less than .15 were entered into the multivariate analysis by using the Cox proportional hazards stepwise model. All statistical analyses were performed with a software package (JMP, release 5.0; SAS Institute Inc, Cary, NC).

**Results****Patient Characteristics**

Clinicopathologic characteristics of 28 patients who underwent resection of a solitary recurrent lesion are shown in Table 1. There were 17 men and 11 women, with a median age of 65 years (range, 39-73 years) at the time of resection of the recurrent lesion. At the initial operation, 26 of 28 patients underwent lobectomy and systemic mediastinal lymph node dissection. Two patients underwent limited



Patients at risk

All operated patients	28	24	13	4	1
Inoperable patients	365	154	63	9	1

**Figure 1. Comparative survival curves among 28 resected patients and 562 patients without resection. The difference in survival probability after recurrence is significant (1-, 2-, and 5-year survivals after recurrence: 89%, 59%, and 32% vs 46%, 26%, and 6%;  $P < .001$ ).**

lung resection because of insufficient pulmonary reserve. Neoadjuvant platinum-based chemotherapy was administered to 1 patient because of clinical N2 status. All patients achieved macroscopically complete surgical removal of their primary NSCLC tumor, but the resection margin was pathologically positive in 1 patient. The patient had recurrence in the adrenal gland. RFI was almost 2 years (median, 23 months; range, 6-82 months). The lung (n = 16) was the most frequent site of recurrence. The mode of resection for intrapulmonary recurrences included 3 completion pneumonectomies, 1 lobectomy, and 12 limited resections. Distal gastrectomy was performed for the patient who had gastric recurrence with severe progressive anemia, and open lymph node resection was performed for the patient with pelvic lymph node recurrence. Complete removal of the recurrence was accomplished in all patients. There was no complication after resection of the recurrent lesion. One of 5 patients with brain recurrence received whole-brain irradiation postoperatively. No patients underwent systemic chemotherapy after resection of the recurrent lesion.

**Survival and Prognostic Factors After Resection of the Solitary Recurrent Lesion**

Figure 1 shows comparative survival curves after recurrence among 28 patients who underwent resection of the solitary recurrent lesion and 562 patients in whom an additional operation was not indicated. Overall 1-, 2-, and 5-year survivals after recurrence were significantly better in pa-

tients who underwent resection of a solitary recurrent lesion than in those who did not undergo resection (89%, 59%, and 32% vs 46%, 26%, and 6%;  $P < .001$ ). The median survival times after recurrence were 25 and 11 months, respectively.

Table 2 shows the relationship between survival after resection of the recurrent lesion and the clinicopathologic characteristics of the 28 patients. Multivariate analysis demonstrated that advanced p-stage (stage II-III) of the primary lung cancer was the significant negative prognostic factor associated with survival after recurrence detection (hazard ratio, 6.15; 95% confidential interval, 1.09-30.8;  $P = .04$ ). As shown in Figure 2, the patients with p-stage II or III disease demonstrated survival statistically equivalent to that of patients not undergoing resection after recurrence detection ( $P = .11$ ). In 14 patients with p-stage I disease, 10 and 3 patients survived for more than 2 and 5 years, respectively, after recurrence detection. One with recurrence in the malar bone is surviving for 7 years without a distant failure. In contrast, 3 and 1 of 14 patients with p-stage II or III disease survived for more than 2 and 5 years, respectively, but with a distant failure.

**Discussion**

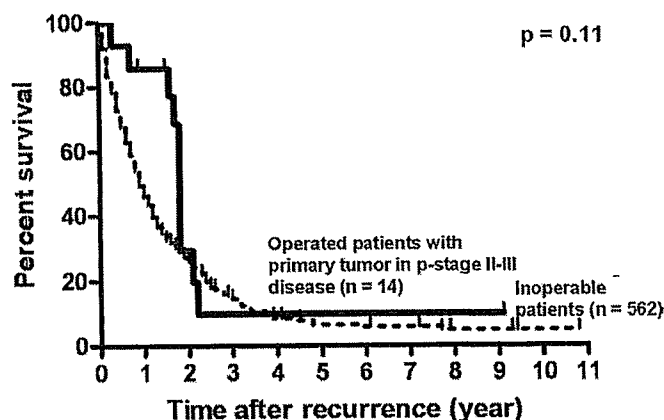
Most recurrences after primary NSCLC resection are multiple and disseminated and are usually treated with systemic chemotherapy when patients can tolerate it. Although many studies have shown that systemic chemotherapy prolongs survival in unresectable stage IV NSCLC, there have been no large-scale, randomized prospective trials addressing whether chemotherapy improves survival of patients with recurrence.<sup>14</sup> In an effort to improve long-term tumor control and subsequent survival, attempts have been made to incorporate surgical intervention in selected cases of solitary NSCLC recurrence. Evidence that a solitary recurrent lesion can be effectively treated with surgical intervention exists for malignancies other than lung cancer. For colorectal cancer, melanoma, and thyroid cancer, resection of recurrent lesions can offer prolonged survival.<sup>15-17</sup> For lung cancer, some investigators have reported acceptable survival after resection of the recurrent lesion, but others have contradicted these conclusions. Abrahams and coworkers<sup>4</sup> demonstrated a satisfactory outcome in brain recurrence, with a median survival time of 18 months and a 5-year survival rate of 28.9%. In contrast, Saitoh and associates<sup>2</sup> conducted 24 brain resections, with a 5-year survival rate of only 8.3%. Prognostic factors for survival after resection of the recurrent lesion have not been clarified.

Although our patient population was heterogeneous, with a variety of recurrence sites, the overall survival after resection of the recurrent lesion was acceptable by current standards, with a median survival time of 25 months and a 5-year survival rate of 32%. The patients with a solitary NSCLC recurrence arising from an advanced primary tumor

**TABLE 2. Relationship between patient clinicopathologic characteristics and survival after resection of a solitary recurrent lesion**

	Factors	No.	MST (mo)	2-y survival (%)	5-y survival (%)	Univariate analysis, P value	Multivariate analysis, P value
Age at recurrence resection (y)	≥65	14	22	43	26	.41	—
	<65	14	27	75	38		
Sex	Male	17	26	53	31	.48	—
	Female	11	28	67	33		
RFI (y)	≥2	13	26	68	45	.25	—
	<2	15	27	51	22		
CEA level at recurrence (ng/mL)	>5	9	22	30	Not reached	.13	.80
	≤5	19	30	70	38		
Symptoms at recurrence	+	9	26	53	18	.33	—
	-	19	28	60	36		
Site of recurrence	Intrapulmonary	16	30	71	40	.15	.50
	Extrapulmonary	12	22	42	21		
Mode of recurrence	Locoregional	10	26	57	46	.41	—
	Distant	18	27	61	23		
Histology	Ad	21	26	55	31	.73	—
	Non-Ad	7	30	67	33		
Size of primary tumor (cm)	≥4	14	26	51	17	.18	—
	<4	14	28	66	47		
Nodal status of primary tumor	N0 (N-)	18	30	67	40	.08	.40
	N1/N2 (N+)	10	22	41	14		
p-Stage of primary tumor	I	14	—	76	51	.0045	.04
	II/III	14	19	10	10		
Ly in primary tumor	+	10	21	44	Not reached	.32	—
	-	18	27	67	33		
V in primary tumor	+	17	27	57	29	.97	—
	-	11	26	61	36		

MST, Median survival time; RFI, recurrence-free interval; CEA, carcinoembryonic antigen; Ad, adenocarcinoma; Ly, lymphatic permeation; V, vascular permeation.



**Figure 2. Comparative survival curves among 14 resected patients with p-stage II or III primary non-small cell lung cancer and 562 patients without resection. There is no significant difference in survival probability after recurrence ( $P = .11$ ).**

with p-stage II or III disease, however, had a poor outcome equivalent to that seen in patients with recurrent NSCLC in whom surgical intervention was not indicated. This suggests that advanced stage (ie, II or III) in the primary tumor is a contraindication for surgical intervention in patients with a solitary recurrence. Consistent with our result, Yoshino and coworkers<sup>18</sup> described a strong relationship between pathologic stage and clinical courses after recurrence in patients with NSCLC. They reported that the mean postrecurrent survival time was 590 days in pathologic stage I disease, 381 days in stage II disease, 257 days in stage IIIA disease, and 180 days in stage IIIB disease, with a significant difference being observed between stages I and IIIA ( $P = .0215$ ). In patients with advanced and biologically aggressive NSCLC, a solitary recurrence might be just the beginning of progressive-disseminated disease.

In our series patients who underwent resection of the intrapulmonary recurrent lesion showed slightly but not significantly better survival than the extrapulmonary recurrence group ( $P = .15$ ). This might be because some in-



trapulmonary lesions were actually metachronous second primary lung cancers. We can expect better prognosis for metachronous lung cancer compared with intrapulmonary recurrence.<sup>19,20</sup> It can often be hard to discriminate a solitary pulmonary recurrence from a metachronous second primary lung cancer if the 2 lesions are of the same histologic type.<sup>21</sup> Therefore aggressive surgical resection for an intrapulmonary lesion might be justified for patients with adequate pulmonary reserve, regardless of the primary tumor pathology.

Although we did not perform positron emission tomography (PET) with <sup>18</sup>F fluorodeoxyglucose (FDG) for the patients in this study, FDG-PET has been reported to be a helpful adjunct in screening for distant metastases but not for brain metastases.<sup>22</sup> Several investigators have reported that FDG-PET could detect unexpected metastatic lesions in 10% to 20% of patients with newly diagnosed NSCLC.<sup>23,24</sup> PET imaging might also be helpful in avoiding surgical intervention in patients who have multiple recurrent lesions.<sup>25</sup> However, it is well known that FDG is not tumor specific and is also taken up in benign lesions.<sup>22</sup> Lardinois and colleagues<sup>26</sup> have reported that 46% of solitary extrapulmonary lesions detected by means of integrated PET and computed tomography were unrelated to lung cancer metastases. Further studies will be needed to clarify whether PET imaging is useful in identifying more clearly the population that benefits from additional surgical intervention and in prolonging subsequent survival.

The limitation of the current study is that the number of enrolled patients, especially in the surgical resection group, was obviously small. Therefore a multi-institutional study would be required to confirm our findings.

In conclusion, long-term survival can be achieved by means of resection of a solitary recurrent lesion in highly selected patients. However, surgical resection might be contraindicated if the primary NSCLC stage is II or III, especially when the recurrent lesion is extrapulmonary.

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## Once-Weekly Epoetin-Beta Improves Hemoglobin Levels in Cancer Patients with Chemotherapy-Induced Anemia: A Randomized, Double-Blind, Dose-Finding Study

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**Objective:** To determine a recommended dose of once-weekly epoetin-beta administration for anemic cancer patients receiving myelosuppressive chemotherapy, we conducted a multicenter, randomized, double-blind trial.

**Methods:** A total of 86 patients with malignant lymphoma or lung cancer who received chemotherapy containing platinum, taxanes or anthracyclines were enrolled in the study. Patients were randomly assigned into groups that received three dose levels of epoetin-beta (9000, 18 000 or 36 000 IU) administered subcutaneously once a week for 12 weeks. The primary endpoint was change in hemoglobin, while the secondary endpoints were quality of life (QOL) assessed by Functional Assessment of Cancer Therapy-Anemia (FACT-An) questionnaire and transfusion requirements.

**Results:** Among the 69 patients (per protocol set population) assessable for efficacy, hemoglobin level change in the 36 000 IU group was significantly greater than that in the 9000 IU group ( $1.75 \pm 2.15$  versus  $0.04 \pm 1.98$  g/dl;  $P = 0.009$ ), and a significant dose-response relationship was observed for the change in hemoglobin level ( $P = 0.003$ ). Although changes in FACT-An Total Fatigue subscale (Fatigue subscale) scores were similar for the three dosage groups, there was a statistically significant correlation ( $r = 0.435$ ,  $P < 0.001$ ) between the change in hemoglobin levels and the change in Fatigue subscale scores. The proportion of transfused patients was significantly smaller in the 36 000 IU group compared with that in the 9000 IU group ( $P = 0.022$ , not adjusted for pre-study transfusions). The incidence of adverse events was similar in the three dosage groups.

**Conclusions:** Once-weekly epoetin-beta 36 000 IU for 12 weeks was well tolerated and significantly increased hemoglobin levels in anemic cancer patients receiving chemotherapy.

*Key words:* chemotherapy-induced anemia – erythropoietin – lung cancer – malignant lymphoma – quality of life

## INTRODUCTION

Erythropoietin (EPO) is a glycoprotein (MW 30 000) which is the hematologic growth factor produced primarily in the kidney. EPO interacts with erythroid progenitor cells in the bone marrow to increase the peripheral red blood cells (1). Epoetin-beta is recombinant human erythropoietin (rhEPO) (2), which was introduced clinically in the 1990s for the treatment of anemia associated with chronic renal failure, especially in patients receiving hemodialysis.

Cancer patients treated with chemotherapy often suffer from anemia, which is a major contributing factor to fatigue leading to compromised quality of life (QOL) (3,4). In addition, the presence of anemia is associated with shorter survival of patients with malignancies (5). Red blood cell transfusion is the traditional and quickest method of alleviating symptoms of cancer-related anemia. However, the side effects of transfusion such as viral infections have not been completely resolved. Patients tend to decline transfusions, and physicians do not prescribe them in most cases until the hemoglobin levels become <8.0 g/dl. The administration of rhEPO is another choice for the treatment of chemotherapy-induced anemia. Numerous studies on anemic cancer patients receiving chemotherapy have demonstrated that rhEPO increased hemoglobin levels and reduced the need for transfusions, and some studies reported improvements in QOL as well (6–11). The schedule of rhEPO administration in most trials was three-times per week. This schedule is inconvenient for outpatients receiving chemotherapy. Gabrilove et al. (10) studied a weekly fixed-dose schedule using 40 000–60 000 IU of epoetin-alfa in cancer patients with anemia. The efficacy was comparable with data on the historical regimen of 10 000 IU three-times weekly. Cazzola et al. (12) compared the efficacy and tolerability of epoetin-beta 30 000 IU once-weekly with that of a 10 000 IU three-times weekly regimen in patients with lymphoproliferative malignancies. Their study showed that the once-weekly regimen was as effective as the three-times weekly one in increasing hemoglobin levels and reducing transfusion requirements.

We therefore conducted a multicenter, randomized, double-blind, dose-finding trial of once-weekly epoetin-beta treatment of malignant lymphoma and lung cancer patients receiving platinum-, taxane- or anthracycline-containing chemotherapy. These chemotherapy regimens are the most active and frequently used for the treatment of these malignancies and also produce relatively high incidences of anemia (4). According to the results of this trial, a recommended dose of epoetin-beta was determined for the subsequent randomized placebo-controlled phase III trial in Japan.

## PATIENTS AND METHODS

### PATIENT ELIGIBILITY

Patients with histologically or cytologically confirmed malignant lymphoma or lung cancer fulfilling the following criteria were enrolled in the study. (i) Age 20–79 years; (ii) Either

platinum-, taxane- or anthracycline-based chemotherapy was administered, and more than 2 courses of chemotherapy were scheduled during the study (radiotherapy during the study period was permitted); (iii) Hemoglobin  $\leq$  11 g/dl after chemotherapy administered within 6 weeks before the study, without iron-deficiencies; (iv) Adequate hepatic and renal function (serum total bilirubin  $\leq$  2.0 mg/dl; serum AST  $\leq$  80 IU/l; serum ALT  $\leq$  80 IU/l; serum creatinine < 2.0 mg/dl); (v) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; (vi) Life expectancy of at least 12 weeks. Exclusion criteria included uncontrolled hypertension, gastrointestinal bleeding, and a known history of myocardial infarction, cerebral infarction or pulmonary embolism. Patients with known hypersensitivity to rhEPO and previous treatment with rhEPO within 4 weeks before the study were also excluded. Female patients who were pregnant were not eligible. Written informed consent was obtained from all patients before entry into the study.

### STUDY DESIGN AND TREATMENT SCHEDULE

This study was a multicenter, randomized, double-blind, parallel-group comparative trial. The study protocol was approved by the institutional review board for each of the 11 participating centers in Japan. Epoetin-beta was supplied by Chugai Pharmaceutical Co., LTD (Tokyo, Japan).

Enrolled patients were randomly assigned to receive one of the three dose levels of epoetin-beta (9000, 18 000, or 36 000 IU). Randomization was prospectively stratified according to age, PS, disease (lung cancer or malignant lymphoma) and institution. Subcutaneous injection of epoetin-beta was started at the beginning of the subsequent chemotherapy course and continued, thereafter, once a week for 12 weeks. If the hemoglobin level increased to more than 14 g/dl, epoetin-beta was discontinued until the hemoglobin level decreased to <12 g/dl, and then re-administered at the same dose. An oral iron supplementation (200 mg/day) was taken daily during the study period. No specific guidelines for transfusion use were defined.

### ASSESSMENT OF EFFICACY AND SAFETY

The primary end point was change in hemoglobin level, and the secondary end points were QOL and red blood cell transfusion requirements. The change in hemoglobin between the baseline and 12 weeks of administration or the last observation was evaluated. If chemotherapy was discontinued within the 12-week period, the change in hemoglobin was evaluated at the last observation; 4 weeks after the beginning of a final-course of chemotherapy. The QOL instrument used in the study was the Functional Assessment of Cancer Therapy-Anemia (FACT-An) questionnaire (13). The Total Fatigue subscale (Fatigue subscale), which consists of 13 items from FACT-An, was mainly analyzed (scores range from 0 to 52). QOL was measured at the baseline, at 7–11 weeks; at the beginning of a chemotherapy course and at 12 weeks after the initiation of epoetin-beta administration.

Adverse events were graded according to the NCI-Common Toxicity Criteria version 2.0 (Japanese edition; Japan Clinical Oncology Group version 1).

#### STATISTICAL ANALYSIS

Of the enrolled patients, those who received epoetin-beta at least once were included in the safety analysis. For efficacy analysis, the per protocol set (PPS) population was defined as eligible patients who received epoetin-beta without protocol violation. Differences in mean changes in hemoglobin between the groups were assessed by Dunnett's multivariate comparison test (14). Changes in the Fatigue subscale scores were compared by using a *t*-test. Pearson's correlation coefficient was calculated to assess the relationship between change in hemoglobin and change in the Fatigue subscale scores. The potential factors influencing the change in the Fatigue subscale scores were examined by multiple regression analysis.

To determine the required number of patients, Dunnett's multiple comparison test was conducted with the 9000 IU group as the control arm. At 2.0 g/dl of the change in hemoglobin from baseline and with a 1.8 g/dl standard deviation between the 9000 and 36 000 IU groups, the required number of patients was calculated to be 21 per group; this means that 63 in total (two-tailed significance level: 5.0%; power: 90%). In the study, it was planned to use the PPS as the main analysis for efficacy; therefore, the target number of subjects was established as 84 to allow for patient dropout.

## RESULTS

#### PATIENT CHARACTERISTICS

A total of 86 patients were enrolled between April 2002 and January 2003, and 83 patients were administered epoetin-beta. All of these 83 patients were eligible for the assessment of safety. For efficacy analysis, 14 patients were then excluded; 13 patients received <7 doses of epoetin-beta with or without <2 courses of chemotherapy mainly due to progression of the disease; and one patient lacked the baseline hemoglobin data. So 69 patients comprised the PPS population evaluated for efficacy. Baseline characteristics of the patients in the PPS population were generally well balanced among the three dosage groups (Table 1), except for transfusion requirements within 4 weeks before the study; in the 9000 IU group, more patients had required transfusions ( $P = 0.130$ ). Table 2 shows the distribution of chemotherapy regimens used during the study.

#### HEMOGLOBIN RESPONSE

Figure 1 shows the mean weekly hemoglobin levels over the 12 weeks of the study for the patients in the PPS population. In the 36 000 IU group, the mean hemoglobin level increased significantly starting from 6 weeks. In contrast, in the 9000 IU group, the mean hemoglobin levels changed little during the study period, despite a higher transfusion rate. The mean changes in hemoglobin level from baseline to last observation for the three dosage groups were summarized in Fig. 2. In

36 000 IU group, a significantly greater increase in the hemoglobin level was observed compared with that in 9000 IU group ( $P = 0.009$ ); however, there was no significant difference between the 18 000 and 9000 IU groups ( $P = 0.154$ ). A significant dose-response relationship for the change in hemoglobin level was observed ( $P = 0.003$ ). As an additional evaluation of efficacy, the proportion of patients who achieved a  $\geq 2$  g/dl increase in hemoglobin level during the study was determined. The results were 40.9% (9/22), 66.7% (16/24) and 78.3% (18/23) in the 9000 IU group, 18 000 IU group and 36 000 IU group, respectively. Epoetin-beta was withheld from 16 patients (one patient in 9000 IU, 8 in 18 000 IU and 7 in 36 000 IU) during the study period, whose hemoglobin levels exceeded 14 g/dl.

#### RED BLOOD CELL TRANSFUSION REQUIREMENTS

Five of 22 patients (22.7%) were transfused in the 9000 IU group, 4 of 24 patients (16.7%) in the 18 000 IU group and none of 23 patients in the 36 000 IU group. The proportion of transfused patients was significantly smaller in the 36 000 IU group compared with that in the 9000 IU group ( $P = 0.022$ ). When patients who had received transfusions within 4 weeks before the study were excluded from the analysis; however, there was no significant difference between the three dosage groups.

#### QOL

Of the PPS population, 69 patients (100%) at baseline, 62 (89.9%) at 7–11 weeks and 61 (88.4%) at 12 weeks were evaluated for QOL scores. No significant mean change in Fatigue subscale scores was observed in any group at 7–11 weeks and 12 weeks. The relationship between change in hemoglobin level and change in the Fatigue subscale score was examined by correlation analysis. There was a statistically significant correlation ( $r = 0.435$ ,  $P < 0.001$ ) between change in hemoglobin levels and change in the Fatigue subscale scores at 7–11 weeks (Fig. 3). Multiple regression analysis was then performed to assess the potential factors contributing to the change in the Fatigue subscale score at 7–11 weeks. The Fatigue subscale score at baseline and change in hemoglobin level were significantly associated with the change in the Fatigue subscale score ( $P = 0.001$ ). Association with other factors such as the weekly dose of epoetin-beta and chemotherapy regimens were not significantly associated. Patients who achieved an increase in hemoglobin of  $\geq 2$  g/dl at 7–11 weeks had significant improvements in their Fatigue subscale scores ( $P = 0.012$ ) (Fig. 4).

#### SAFETY

The incidence of adverse events was generally similar between the three dosage groups (Table 3). As hematological adverse events, most common were leukocytopenia, neutropenia and thrombocytopenia. As non-hematological adverse events, nausea and appetite loss were commonly observed. One patient