pharmacodynamics in elderly patients. Altered pharmacokinetics, increased pharmacodynamic sensitivity, or both can theoretically cause increased toxicity. It is important, therefore, to elucidate the pharmacokinetics and pharmacodynamics of anticancer agents in elderly patients in comparison to those of younger patients in terms of their increased toxicities.

Previous reports have stressed that in the elderly, physiologic age is more important than chronological age, and that age by itself is not a contraindication to cancer chemotherapy.[19,20] Some retrospective studies of chemotherapy failed to demonstrate an increased risk of toxicity among elderly patients; it has been claimed that elderly patients can tolerate chemotherapy as well as younger patients when they fulfill eligibility criteria for clinical studies of cancer chemotherapy, such as good performance status and normal organ functions.[21-24] However, in a feasibility study of chemotherapy for elderly patients with lung cancer, 71% of patients aged 75 years or older were excluded from the study because of comorbidity or poor performance status; furthermore, severe myelotoxicity was observed, even in patients who fulfilled the eligibility criteria.[25] Therefore, we believe that doses of anticancer agents for elderly patients should be determined by phase I studies, specifically conducted in such patients.

When we determined recommended doses of cisplatin and docetaxel administered weekly for 3 consecutive weeks in patients with non–small-cell lung cancer, we conducted two individual phase I studies for elderly patients aged 75 years or older and for non-elderly patients younger than 75 years.[26] The only difference in eligibility criteria for these two phase I studies was age. The recommended dose of cisplatin was 25 mg/m^2 for both patient groups, but doses of docetaxel were different for elderly (20 mg/m^2) and non-elderly (35 mg/m^2) patients. Based on this information, two separate phase II studies against non–small-cell lung cancer were conducted in elderly patients and non-elderly patients, using the different recommended doses.[27,28] Eligibility criteria for the phase II studies were the same as those for phase I studies, except that a measurable disease for response evaluation was required for the phase II studies. To elucidate mechanisms of the difference in recommended doses of docetaxel for elderly and non-elderly patients, we investigated the pharmacokinetics and pharmacodynamics of docetaxel and cisplatin in the two phase II studies and compared them between elderly and non-elderly patients.

PATIENTS AND METHODS

Patient Selection

Eligibility criteria for the two phase II studies were identical except for age: 75 years or older for elderly patients and 20 to 74 years for non-elderly patients. Other eligibility criteria included histologically and/or cytologically confirmed non–small-cell lung cancer, stage IV or IIIB without an indication for curative radiotherapy, Eastern Cooperative Oncology Group performance status 0 or 1, no prior chemotherapy, the presence of measurable lesions, adequate hematologic function (WBC 4,000 to 12,000/μL; absolute neutrophil count $\geq$ 2,000/μL; platelet count $\geq$ 100,000/μL; hemoglobin $\geq$ 9.0 g/dL), adequate hepatic function (total bilirubin $<$ 1.1 mg/dL; AST and ALT $<$ 60 U/L), and adequate renal function (creatinine $<$ 1.2 mg/dL; creatinine clearance $>$ 60 mL/min). Exclusion criteria were active infection, severe heart disease, uncontrolled hypertension or diabetes mellitus, active concomitant malignancy, pleural and/or pericardial effusion requiring drainage, and pregnant/nursing women. In addition to written informed consent to the phase II studies with docetaxel and cisplatin, written informed consent to the pharmacologic study was required before patients were enrolled onto this study. These studies were approved by the institutional review board at the National Cancer Center (Tokyo, Japan).

Treatment and Follow-Up

After premedication with intravenous dexamethasone (16 mg) and granisetron (3 mg), docetaxel was infused over 30 minutes. Cisplatin was given as a 15-minute infusion 90 minutes after completion of the docetaxel infusion, and a total volume of 1,500 mL saline was infused on the day of chemotherapy for diuresis. The dose of docetaxel was 20 mg/m^2 for elderly patients and 35 mg/m^2 for non-elderly patients. All patients received cisplatin at a dose of 25 mg/m^2. These were the recommended doses determined by the phase I studies. Docetaxel and cisplatin was administered weekly for 3 consecutive weeks followed by 1 week of rest. This 4-week course was repeated until there was evidence of disease progression or unacceptable toxicity. Treatment with docetaxel and cisplatin was not given if WBC was less than 2,000/μL and/or platelet count was less than 50,000/μL on the day of chemotherapy.

Physical examination and toxicity assessment included complete blood cell counts with differential counts as well as platelet counts, blood chemistry, and urinalysis. These were performed before treatment and repeated at least weekly during the chemotherapy. Toxicity was graded according to the Japan Clinical Oncology Group criteria,[29] which are basically the same as the National Cancer Institute Common Toxicity Criteria.

Antitumor response was evaluated in lesions with a diameter $\geq$ 2 cm by carrying out a computed tomography scan according to WHO criteria.[30]

Pharmacokinetic Analysis

Blood sampling for pharmacokinetic analysis was performed after the first administration of the first course as follows: (1) blood samples for the measurement of docetaxel concentrations were obtained at the end of a docetaxel infusion, and 0.17, 1, 1.75, 3.25, 5.75, and 24 hours after the docetaxel infusion; (2) for analysis of the pharmacokinetics of cisplatin, blood was drawn at the end of a cisplatin infusion, and 0.25, 0.75, 1.5, 4, and 22.25 hours after the cisplatin infusion. Blood was immediately centrifuged and an aliquot of plasma was ultrafiltered using UFC3GC membranes (Japan Millipore, Tokyo, Japan). Plasma and ultrafiltrate samples were frozen at -80°C until analyzed.

The concentration of docetaxel in plasma was determined by using a previously reported high-performance liquid chromatography (HPLC) method,[31] and the concentration of unchanged cisplatin in the ultrafiltrate was measured according to

a HPLC method with on-line postcolumn derivatization, as reported previously.[32,33]

Because concentrations in plasma at the terminal phase could not be measured in some patients, pharmacokinetic parameters for individuals were calculated by Bayesian estimation after population pharmacokinetic parameters were estimated in the entire population. These calculations were performed using the NONMEM program (version V, level 1.1). A three-compartment open model with zero-order administration and first-order elimination (ADVAN 11 and TRANS 4) was used to describe the plasma concentration-time course for docetaxel in the entire population, and a one-compartment open model (ADVAN 1 and TRANS 2) was used for unchanged cisplatin in the ultrafiltrate. Assuming a log-normal distribution for inter-individual variability in pharmacokinetic parameters, the inter-individual variability was modeled as (eg, for clearance) $CL_j = \hat{CL} \exp(\eta_{jCL})$, where CL_j and $\hat{CL}$ are the estimated values in an individual j and the population mean for clearance, respectively, and η_{jCL} is the individual random perturbation from the population mean. Intrapatient residual variability was also described by a log-normal distribution model. Similarly inter- and intra-individual variability was modeled for the volume of the third compartment (docetaxel) or the central compartment (cisplatin). The area under the concentration-time curve (AUC) was calculated as dose divided by clearance in each patient.

Pharmacodynamic Analysis

Pharmacodynamic analysis was conducted using the AUC for docetaxel and unchanged cisplatin in individual patients. Neutrophil counts were monitored at least weekly and the nadir count during the first course was recorded. The percent change in neutrophil counts (dANC) was defined as:

$$\text{dANC} = \frac{\text{Pretreatment count} - \text{Nadir count}}{\text{Pretreatment count}} \times 100$$

and the relationship between dANC and the AUC of docetaxel or unchanged cisplatin was investigated using a sigmoid Emax model:

$$\text{dANC} = \frac{\text{Emax} \times \text{AUC}^r}{\text{AUC}^r + \text{EC}_{50}{}^r}$$

The Emax represents the maximal effect, and EC_{50} is the AUC value at which the effect is 50% of the maximum effect. The exponent r is a shape factor that determines the steepness of the response curve. These values were determined by using the computer program, WINNonlin (version 4.01, Scientific Consultant, Apex, NC).

Statistical Methods

Continuous variables, including pharmacokinetic parameters, were compared between elderly (75 years or older) and non-elderly patients (74 years or younger), using the Mann-Whitney U test. Differences in distribution of patient characteristics between the two groups were evaluated with the χ^2 test or Fisher's exact test, where appropriate. *P* values less than .05 were regarded as statistically significant, and all reported *P* values are two-tailed.

RESULTS

Of 33 elderly and 36 non-elderly patients who received docetaxel and cisplatin in the phase II studies, the pharmacokinetic study was performed in 25 and 27 patients, respectively (Table 1). There were no differences between the two groups in the distribution by sex, performance status, or the proportion of patients who had been treated with radiotherapy before entry into the study. Elderly patients had slightly lower levels of total protein, albumin and α_1-acid glycoprotein, and neutrophil counts than non-elderly patients, but the differences were small. Patients with hepatic or renal dysfunction were excluded from the phase II studies and there were no differences between groups in these functions except for ALT.

Because of technical problems with blood sampling or with HPLC systems, pharmacokinetic data for docetaxel and cisplatin could not be obtained in two non-elderly patients and one elderly patient, respectively. Therefore,

Table 1. Patient Characteristics

Characteristics	Non-Elderly Patients	Elderly Patients	*P*
No. of patients	27	25	
Age, years			< .001
Median	56	76	
Range	39-73	75-86	
Sex			.74
Female	5	6	
Male	22	19	
Performance status			.70
0	5	3	
1	22	22	
Prior radiotherapy			.50
No	20	21	
Yes	7	4	
Total protein, g/dL			.021
Mean	6.2	5.9	
SD	0.4	0.5	
Albumin, g/dL			.008
Mean	3.4	3.2	
SD	0.4	0.3	
α_1-acid glycoprotein, mg/dL			.018
Mean	121	97	
SD	33	34	
AST, U/L			.11
Mean	22.7	20.2	
SD	7.6	9.0	
ALT, U/L			.001
Mean	23.4	15.2	
SD	10.3	8.1	
Creatinine, mg/dL			.10
Mean	0.69	0.80	
SD	0.11	0.22	
Creatinine clearance, mL/min			.48
Mean	87.4	93.3	
SD	20.6	24.7	
Neutrophil counts, μL			.03
Mean	5,230	4,355	
SD	1,696	1,450	

Abbreviation: SD, standard deviation.

Table 2. Pharmacokinetic Parameters

	Non-Elderly Patients	Elderly Patients	*P*
Docetaxel			
No. of patients	25	25	
Clearance, L/hour			.86
Mean	45.9	45.6	
SD	17.1	16.5	
Volume of distribution, L			.11
Mean	350	273	
SD	216	215	
AUC, μg/mL × hour			< .001
Mean	1.40	0.79	
SD	0.64	0.34	
Cisplatin			
No. of patients	27	24	
Clearance, mL/min			.13
Mean	443	417	
SD	50	65	
Volume of distribution, L			.38
Mean	13.8	14.7	
SD	2.2	3.3	
AUC, μg/mL × min			.49
Mean	91.8	94.3	
SD	11.5	12.6	

Abbreviations: SD, standard deviation; AUC, area under the curve.

pharmacokinetic parameters for docetaxel in 25 elderly patients and 25 non-elderly patients and those for unchanged cisplatin in 24 elderly patients and 27 non-elderly patients were compared (Table 2). There was no difference in the clearance or volume of distribution of docetaxel between the elderly and non-elderly patients. Similarly, the clearance and volume of distribution of unchanged cisplatin were similar in both patient groups. The elderly and non-elderly patients were treated with different doses of docetaxel (20 and 35 mg/m^2, respectively), though the clearance of docetaxel was the same for both populations. Therefore, the AUC of docetaxel in the non-elderly patients was greater than that in the elderly patients.

Despite the fact that the AUC of docetaxel was higher in the non-elderly patients than in the elderly patients, the neutropenia observed was similar for the two groups of patients, with regard to toxicity grades and actual nadir counts (Table 3). Although administrations of docetaxel and cisplatin were omitted on day 8 or 15 of the first course in one elderly patient and in seven non-elderly patients, there was no difference in age between the eight patients who did not receive the treatment on day 8 or 15 and the other 44 patients who were administered chemotherapy three times (63.4 ± 9.9 years *v* 67.4 ± 12.8 years; $P = .41$). When the AUC of cisplatin and docetaxel was compared between patients who did or did not receive all administrations, the AUC of docetaxel was significantly higher for patients who missed a dose than patients who received all

Table 3. Neutropenia in the First Course

	Non-Elderly Patients	Elderly Patients	*P*
Neutropenia, No. of patients			.76
Grade			
0	19	17	
1	4	3	
2	2	4	
3	1	1	
4	1	0	
Nadir neutrophil counts, μL			.72
Mean	2,707	2,867	
SD	1,268	1,404	
Percent change in neutrophil counts, %			.12
Mean	46.0	34.5	
SD	23.3	25.6	
Frequency of measurements of neutrophil counts (per week)			.55
Mean	1.6	1.7	
SD	0.4	0.4	

Abbreviation: SD, standard deviation.

administrations (1.57 ± 0.88 *v* 1.03 ± 0.53 μg/mL × hour; $P = .03$), while the AUC of cisplatin was similar (90.6 ± 15.2 *v* 93.4 ± 11.5 μg/mL × min; $P = .54$).

The relationship between the AUC of docetaxel or cisplatin and percent changes in neutrophil counts was evaluated using a sigmoid Emax model in the elderly or non-elderly patients. The AUC of cisplatin was not correlated with the percent change in neutrophil counts in either elderly or non-elderly patients (Fig 1). On the other hand, the AUC of docetaxel was positively correlated with the percent change in neutrophil counts (dANC) in the non-elderly patients (Fig 2), and the relationship was described as:

$$\text{dANC} = \frac{59 \times \text{AUC}^{3.2}}{\text{AUC}^{3.2} + 0.86^{3.2}} \times 100$$

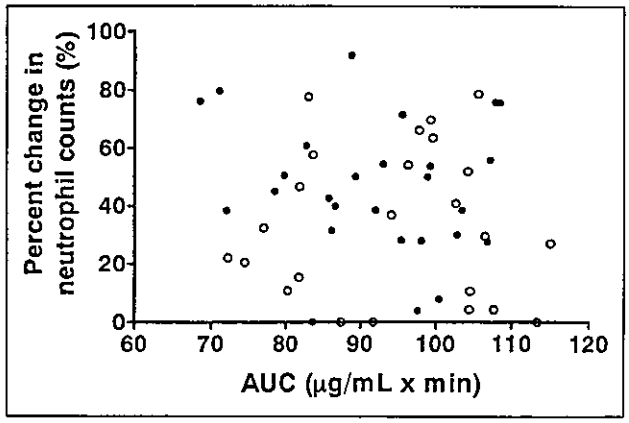

Fig 1. Relationship between the area under the curve (AUC) of cisplatin and percent changes in neutrophil counts in the elderly (○) and the non-elderly (●) patients.

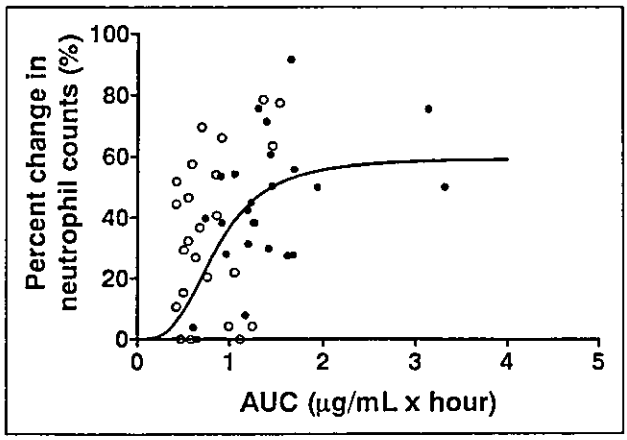

Fig 2. Relationship between the area under the curve (AUC) of docetaxel and percent changes in neutrophil counts in the elderly (○) and the non-elderly (●) patients. The solid line represents predictions by a sigmoid Emax model in the non-elderly patients.

Because the distribution range of the docetaxel AUC in the elderly patients was narrow, a sigmoid relationship between the AUC of docetaxel and the percent change in neutrophil counts was not apparent (Fig 2), and parameters in the sigmoid Emax model could not be calculated in the elderly group.

To investigate whether the pharmacodynamic relationship between the AUC of docetaxel and neutropenia for the elderly patients was different from that of the non-elderly patients, percent changes in neutrophil counts were predicted in the elderly patients. This was done using the sigmoid Emax model developed in the non-elderly patients, and residuals of the prediction (predicted value − observed value) were calculated. The neutropenia observed in the elderly patients was greater than that predicted by the model with a mean of residual of −11.2% (95% CI, −21.8% to −0.5%), while the model predicted neutropenia without bias in the non-elderly patients with a mean residual of 0.21% (95% CI, −7.4% to 7.8%), as expected. Elderly patients had a lower docetaxel AUC than non-elderly patients, and there were two non-elderly patients with a high docetaxel AUC who seemed to be outliers. Therefore, we analyzed the data after excluding non-elderly patients with AUC > 1.53 μg/mL × hour (the maximum value in elderly patients) or after excluding the two outliers. Both reanalyzed models also underestimated neutropenia in the elderly patients: −13.5% (range, −26.2% to −0.8%) and −12.5% (range, −23.7% to −1.3%), respectively.

Partial responses were observed in eight of 27 non-elderly patients, and among 25 elderly patients, a complete response and partial responses were documented in one and 12 patients, respectively. When the AUC of docetaxel and unchanged cisplatin was compared between responders and nonresponders, no differences were observed. The AUC values for docetaxel in responders and nonresponders were 1.02 ± 0.39 and 1.14 ± 0.70 μg/mL × hour, respectively, and the AUC values for unchanged cisplatin were 91.5 ± 12.8 and 94.0 ± 11.5 μg/mL × min, respectively.

DISCUSSION

The purpose of the pharmacologic study was to elucidate mechanisms of the difference in recommended doses of docetaxel in combination with cisplatin in elderly patients and non-elderly patients. We investigated the pharmacokinetics and pharmacodynamics of docetaxel and unchanged cisplatin in two subsequently conducted phase II studies.[27,28] For both docetaxel and cisplatin, the pharmacokinetics did not differ between elderly patients and non-elderly patients. While exposure to cisplatin was not correlated to the extent of neutropenia, there was a sigmoidal relationship between the AUC of docetaxel and neutropenia in the non-elderly patients. However, the relationship between the AUC of docetaxel and neutropenia in the elderly patients was different from that in the non-elderly patients. Although elderly patients had smaller AUC values than non-elderly patients, the same extent of neutropenia was observed in both patient groups (Table 3), and nonhematologic toxicities were mild and similar in both groups.[27,28] These observations suggest that elderly patients were more sensitive to the exposure of docetaxel than non-elderly patients.

There was no difference in docetaxel clearance between elderly and non-elderly patients (Table 2). This conclusion was not changed after the clearance of docetaxel was adjusted for body-surface area (29.6 and 28.2 L/h/m^2, for elderly and non-elderly patients, respectively). These values fall within the range of docetaxel clearance values previously published.[34-37] Furthermore, docetaxel clearance was not correlated to age as a continuous variable, and age was not a significant covariate in the population pharmacokinetic model. These observations seem to be inconsistent with those of a previous report, which found that age was inversely correlated to the clearance of docetaxel in a population pharmacokinetic model.[38] Although the exact reasons for this discrepancy are not clear, ethnic difference or coadministration of cisplatin might explain it. However, the estimated coefficient of age in the population model was small in the previous report. A difference of 20 years in age (the difference in the median ages of the elderly and the non-elderly groups in our study) would yield less than a 10% difference in the clearance of docetaxel. The previous population model was developed by using data from 547 patients, while in our study, data from 52 patients were used. It was possible that the smaller number of patients in our study precluded the detection of a small difference in docetaxel clearance between elderly and non-elderly patients. However, the difference in the dose of docetaxel between elderly patients (20 mg/m^2) and non-elderly pa-

tients (35 mg/m^2) did not seem to be explained by a less than 10% difference in docetaxel clearance values.

Although the concentration of ultrafiltrable platinum was measured in most of the pharmacokinetic studies with cisplatin, measuring the concentration of unchanged cisplatin is clinically more relevant because ultrafiltrable platinum contains inactive low molecular-weight metabolites.[39] The pharmacokinetics of unchanged cisplatin were not different between elderly and non-elderly patients, and there was no correlation between age and the clearance of cisplatin. The clearances of unchanged cisplatin for elderly and non-elderly patients in our study were similar to those reported previously.[40-44]

In the pharmacodynamic analysis in the present study, exposure to docetaxel was correlated to the extent of neutropenia in the non-elderly patients, but the relationship between docetaxel exposure and neutropenia was unclear in the elderly patients. Therefore, for comparison of pharmacodynamics between the elderly and non-elderly patients, we applied the pharmacodynamic model developed in the non-elderly patients to the data from the elderly patients. The residuals of prediction by the model were less than zero in the elderly patients, indicating that the model underestimated the extent of neutropenia in the elderly patients. Although this analysis might be exploratory because uncertainty in the estimates of model parameters was not considered, the results suggest that elderly patients are more sensitive to neutropenia induced by docetaxel than non-elderly patients. This is further supported by observations that the elderly patients and non-elderly patients experienced neutropenia to the same extent, despite the fact that the AUC of docetaxel was greater in the non-elderly patients than the elderly patients.

We used a sigmoid Emax model for pharmacodynamic analysis. Since it is a nonlinear model, parameter estimation may depend on the distribution of variables. Because elderly patients had lower docetaxel AUC than non-elderly patients, and because there were two outliers in the non-elderly patients, we reanalyzed the data after excluding data of non-elderly patients with AUC greater than the maximum for elderly patients, or excluding the two outliers. The results of these reanalyses were the same and confirmed that elderly patients are more sensitive to neutropenia induced by docetaxel. Another approach would be modeling the all data simultaneously and investigating interaction between age and parameters in the model. However, incorporation of age into a sensitivity parameter (EC_{50}) or a shape parameter (r) did not improve model performance (data not shown).

These findings are in agreement with clinical observations in many previous reports; elderly patients experienced more profound myelotoxicity and had greater risk of chemotherapy-related death than younger patients in various cancers.[10,13,14,45-48] We showed that the greater risk of hematologic toxicity in the elderly patients was related to the greater sensitivity of bone marrow function to combination chemotherapy of docetaxel and cisplatin using a weekly schedule without altered pharmacokinetics. The greater sensitivity of myeloid cells to chemotherapeutic agents in the elderly was also in agreement with our previous pharmacodynamic analysis of leukopenia.[49] In that study, we developed a novel pharmacodynamic model relating the entire time course of leukopenia to the time course of drug concentration. A parameter corresponding to the sensitivity of myeloid cells to chemotherapeutic agents showed a significant correlation with age, and myeloid cells of elderly patients showed greater sensitivity than those of younger patients without altered pharmacokinetics of anticancer agents.[49,50] Furthermore, in a pharmacologic analysis of etoposide, elderly patients had greater sensitivity with regard to neutropenia than younger patients at the same level of drug exposure.[18] These observations were in accordance with those made in the current study.

The exact reason why bone marrow function of elderly patients showed greater sensitivity to chemotherapeutic agents than that of younger patients is not clear. Factors stimulating neutrophil production, such as granulocytopoietic cytokines, should be increased during the neutropenic period after chemotherapy. However, the production of these cytokines is reduced in the elderly,[51] and a decreased response to granulocytopoietic stimuli in infection has been reported in aged mice and humans.[52-54] These factors may explain the greater sensitivity of elderly patients to chemotherapeutic agents, although kinetics of cytokines after chemotherapy would also need to be investigated.

Potential drawbacks of this study may be the small number of patients and low incidence of significant neutropenic events, which might be explained by divided doses of docetaxel and restriction of eligibility to patients with a good performance status. It is unclear whether difference in the sensitivity to neutropenia could fully explain the difference in the dose of docetaxel between the elderly patients and the non-elderly patients, considering that the observed neutropenia was moderate. However, nonhematologic toxicities were mild and similar in both groups[26] despite the fact that the AUC of docetaxel was greater in the non-elderly patients than in the elderly patients. These observations suggest that elderly patients are more sensitive to toxicities than non-elderly patients.

It is notable that a high response rate was observed in elderly patients, though a reduced dose of docetaxel was used, compared to non-elderly patients. Further studies of chemotherapy in elderly patients with non–small-cell lung cancer are warranted.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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Dominant Papillary Subtype Is a Significant Predictor of the Response to Gefitinib in Adenocarcinoma of the Lung

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ABSTRACT

Purpose: **Gefitinib (IRESSA; AstraZeneca, Osaka, Japan) shows excellent antitumor activity against advanced non–small-cell lung cancer, especially for the treatment of adenocarcinoma. However, the predictive factors for the response to gefitinib are still controversial. The aim of this study was to identify the clinicopathological and immunohistochemical features that are favorable to the use of gefitinib in adenocarcinoma patients.**

Experimental Design: **Between June 2002 and October 2003, 36 adenocarcinoma patients who experienced a relapse after surgical resection were treated with gefitinib at our hospital. The histologic patterns of the tumors were divided into four distinctive subtypes according to the revised World Health Organization histologic classification, and the dominant histologic subtype for the maximum cut surface of each resected specimen was documented. Association between the response to gefitinib and the clinicopathological features or immunohistochemical expression of epidermal growth factor receptor (EGFR), phosphorylated EGFR, or c-erbB-2 were then investigated.**

Results: **A significant association between the response to gefitinib and dominant papillary subtype findings was observed ($P = 0.0021$); the survival time of papillary subtype patients was also significantly prolonged compared with that of non-papillary subtype patients ($P = 0.03$). No other clinicopathological features or the expression of EGFR, phosphorylated EGFR, or c-erbB-2 were associated with the response to gefitinib.**

Conclusions: **The results of the present study indicate that dominant papillary subtype findings of lung adenocarcinomas can be an important predictor of the response to gefitinib. Thus, this type of adenocarcinoma might be susceptible to postoperative adjuvant treatment with gefitinib.**

INTRODUCTION

Lung cancer is the leading cause of cancer deaths in many countries, and the global incidence is rising at a rate of 0.5% per annum (1, 2). Platinum-based combination chemotherapy has been shown to improve survival and quality-of-life in patients with advanced non–small-cell lung cancer (NSCLC; ref 3 and 4), which accounts for approximately 80% of all lung cancers. New chemotherapeutic agents, like gemcitabine, vinorelbine, docetaxel, paclitaxel, and irinotecan, were developed in the 1990s. However, chemotherapy for advanced NSCLC has been of limited benefit, with response rates of approximately 30% and a median survival period of about 8 months, and it seems to have reached a plateau (1, 5, 6). It is clear that additional treatment strategies are necessary.

Epidermal growth factor receptor (EGFR) has been shown to play an important role in the growth of many solid tumors and is overexpressed in approximately 40 to 80% of NSCLCs (7–9). Furthermore, the overexpression of EGFR has been associated with a poor prognosis in several studies on lung cancer (10, 11). EGFR activation occurs when ligands, such as epidermal growth factor, transforming growth factor-α, or amphiregulin, bind to its extracellular domain, resulting in cell proliferation, angiogenesis, metastasis, and antiapoptosis (8, 9). Gefitinib (IRESSA; AstraZeneca, Osaka, Japan) is an orally active, selective EGFR tyrosine kinase inhibitor that blocks downstream of the EGFR signal transduction pathway (12). In this context, gefitinib has a quite different profile from chemotherapeutic agents that have ever been used.

After phase I studies, two multicenter, randomized, double-blind phase II studies (IDEAL1, ref. 13; IDEAL2, ref. 14) were carried out to evaluate the tolerability and efficacy of gefitinib in patients with advanced NSCLC who had been treated previously with platinum-based combination chemotherapy. In total, 426 patients were enrolled in the two studies, and all of the patients had been treated previously with platinum-based combination chemotherapy. In both studies, the administration of two different gefitinib dosages (250 mg/day and 500 mg/day) were compared. No significant differences in efficacy were seen between the two dosages, but the 250 mg/day treatment was better tolerated than the 500 mg/day treatment in both studies. For the 250 mg/day gefitinib arms, the response rates were 18.4% and 12.0% in IDEAL1 and IDEAL2, respectively.

The results of IDEAL1 showed that gefitinib was significantly more effective for the treatment of adenocarcinomas than for other histologies (odds ratio, 3.45) and was also more effective in females than in males (odds ratio, 2.65). These

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findings were unexpected, given the mechanism of the drug, because squamous cell carcinomas are known to overexpress EGFR, the target of gefitinib, to a greater degree than adenocarcinomas. On the other hand, gefitinib-induced severe acute interstitial pneumonia resulting in a high mortality rate is a serious social problem in Japan (15). Although the precise mechanism of gefitinib's action is not yet completely understood, clinically it is more important to identify favorable characteristics in the treatment with gefitinib.

Gefitinib has been confirmed to be significantly effective for the treatment of adenocarcinomas, and some investigators have reported that gefitinib is especially effective in adenocarcinomas with bronchiolo-alveolar features (16, 17). To the best of our knowledge, however, these studies only examined biopsy specimens, and no studies examining surgical specimens have been done. Lung cancer is generally characterized by histologic heterogeneity; thus, studies examining histologic features should be done using surgically resected specimens.

In this study, we investigated the clinicopathological and immunohistochemical features of surgically resected adenocarcinoma specimens from patients who were subsequently treated with gefitinib after relapse to identify any characteristics that were associated with a favorable response to gefitinib.

PATIENTS AND METHODS

Patients. Between June 2002 and October 2003, 253 consecutive patients were treated with a 250 mg daily dosage of

Table 1 Patient characteristics (N = 36)

Patient no.	Age (year)	Gender (M/F)	PS	Smoking	Recurrent site		Previous treatment		Response	Current status
					Lung	Others		CT regimen (response)		
1	72	F	1	−	+	+	CT, RT	CDDP/VNR (PR)	PR	Continued
2	57	M	1	+	+	+	CT, bone RT	CDDP/VNR (SD)	PR	Continued
3	62	M	1	+	+	+	CT, bone RT	CDDP/VNR (PR)	PR	Ceased
4	60	M	1	+	+	−	CT	CDDP/VNR (SD)	PR	Ceased
5	61	F	1	−	+	−	CT	①CBDCA/PTX (CR) ②GEM/VNR (SD)	PR	Continued
6	64	M	2	−	+	+	brain RT		PR	Dead
7	66	M	1	+	+	+	CT	CDDP/VNR (SD)	PR	Continued
8	69	F	1	−	+	−	CT, OP	CDDP/VNR (PD)	PR	Continued
9	55	F	0	−	+	−	None		PR	Continued
10	66	F	1	−	+	−	CT	①GEM/VNR (SD) ②CBDCA (SD)	PR	Continued
11	61	F	0	−	+	−	CT	CDDP/VNR (SD)	PR	Ceased
12	69	M	0	+	+	−	None		PR	Continued
13	62	F	1	−	+	+	CT, OP, brain RT	CDDP/VNR (PR)	PR	Continued
14	67	M	0	+	+	−	None		PR	Continued
15	47	F	1	−	+	−	CT	CDDP/VNR (SD)	PR	Ceased
16	43	F	1	−	+	−	None		PR	Continued
17	73	F	1	−	−	+	None		PR	Continued
18	45	F	1	−	+	−	CT	CDDP/VNR (SD)	SD	Dead
19	45	F	0	−	+	−	None		SD	Ceased
20	59	M	0	+	+	−	CT	CDDP/VNR (SD)	SD	Continued
21	66	F	2	−	+	−	CT, RT, OP	CDDP/VDS+MMC (SD)	SD	Ceased
22	62	F	0	+	+	-	CT	GEM/VNR (SD)	SD	Ceased
23	60	M	1	+	+	+	CT, RT	①CDDP/GEM/VNR (SD) ②DTX (PD)	SD	Ceased
24	55	M	1	−	+	−	CT	CBDCA/PTX (SD)	SD	Ceased
25	71	F	2	−	−	+	bone RT		SD	Dead
26	82	F	1	−	+	−	OP		SD	Ceased
27	72	M	1	+	+	−	OP		SD	Unknown
28	67	F	1	+	+	−	None		SD	Dead
29	71	M	1	+	+	−	None		SD	Continued
30	57	M	1	+	+	−	None		SD	Ceased
31	74	M	1	+	+	−	None		SD	Dead
32	65	M	2	+	−	+	CT, brain RT	CDDP/VNR (SD)	SD	Continued
33	73	F	1	−	+	−	None		SD	Continued
34	72	F	1	−	+	+	RT		PD	Dead
35	67	M	1	+	+	+	CT	CDDP/VNR (SD)	PD	Dead
36	60	M	1	−	+	−	OP		PD	Unknown
N = 36	70 (43–82)	M/F: 17/19	0–1/2: 32/4	−/+: 20/16	33	12	Previous CT (+): 19	Number of regimen 1 regimen: 16 2 regimen: 3	OR: 47%	

Abbreviations: F, female; M, male; PS, performance status (ECOG, Eastern Cooperative Oncology Group); CT, chemotherapy; RT, radiation therapy; OP, operation; CDDP, cisplatin; CBDCA, carboplatin; VNR, vinorelbine; PTX, paclitaxel; GEM, gemcitabine; VDS, vindesine, MMC, mitomycine C; DTX, docetaxel; PR, partial response; SD, stable disease; PD, progressive disease; OR, overall response.

gefitinib at our hospital. To select fully treated patients, we defined assessable patients as follows. Response evaluation using chest computed tomography was done after receiving gefitinib at least for > 4 weeks. Among 253 patients, 222 satisfied these criteria. Of these 222 patients, 48 patients (36 patients with adenocarcinomas, 7 patients with squamous cell carcinomas, 3 patients with large cell carcinomas, 1 patient with adenosquamous carcinoma, and 1 patient with pleomorphic carcinoma) had previously undergone a lung resection for primary NSCLC at our hospital. In this study, we analyzed 36 surgically resected adenocarcinoma specimens. At the time of analysis, the mean gefitinib treatment period of these 36 patients was 172 days (range, 29–542 days).

Pathological Studies. All surgical specimens were fixed with 10% formalin or absolute methanol and embedded in paraffin. The tumors were cut at approximately 5-mm intervals, and serial 4-μm sections were stained with H&E, Alcian blue-periodic acid Schiff method to visualize cytoplasmic mucin production, or Verhoeff van-Gieson method (18) to visualize elastic fibers. Lymphatic permeation and pulmonary metastases were evaluated on sections stained with H&E. Vascular invasion and pleural invasion were evaluated with the Verhoeff van-Gieson method. Three observers (Y. K., G. I., and K. T.) who were unaware of the clinical data independently reviewed all pathologic slides. The histologic diagnoses were based on the revised World Health Organization histologic classification (19). In addition, the histologic subtypes and percentage of each subtype present in the tumor were evaluated with the maximum cut surface of the tumor. The histologic patterns were divided into four distinctive subtypes: bronchioloalveolar carcinoma (BAC), acinar subtype, papillary subtype, and solid adenocarcinoma with mucin. The dominant subtype of each tumor was then documented. Tumor size was measured as the maximal diameter on the cut section of the lung. The pathologic stage was determined according to the classification of the Union Internationale Contre le Cancer (20).

Immunohistochemistry. Tissue blocks were cut into 4-μm sections and mounted on silane-coated slides (Matsunami, Tokyo, Japan). The slides were then deparaffinized in xylene, dehydrated in a graded alcohol series, and blocked for endogenous peroxidase with 3% H_2O_2 in absolute methanol. After microwave pretreatment in citrate buffer (pH 6.0) at 95°C for 20 minutes, immunostaining was done at 4°C overnight with a mouse monoclonal antihuman EGFR (Novocastra, Newcastle, United Kingdom; ref. 21) at a dilution of 1:10, a mouse monoclonal antihuman phosphorylated EGFR (provided by Kyowa-hakko, Tokyo, Japan) that recognizes Try-1173 of the activated EGFR at a dilution of 1:10. As for the use of the antihuman phosphorylated EGFP (p-EGFR), a synthetic peptide (CG-STENAEpYLRVAPQSS), the amino acid sequence of which corresponds to COOH-terminal region of human EGFR, was used as an immunogen to generate a monoclonal antibody specific for the tyrosine-phosphorylated EGFR molecule. Obtained monoclonal antibody (KM2911) was further characterized by ELISA, Western blot assay, and immunohistochemical staining to verify the specificity and sensitivity. Furthermore, we compared the immunostaining of KM2911 with that of another monoclonal antibody against tyrosine-phosphorylated EGFR (MAB3052, Chemicon International, Inc., Temecula, CA) and confirmed the same specificity and sensitivity. The tissues were then exposed to DAKO EnVision+ (DAKO, Glostrup, Denmark) at room temperature for 30 minutes. Staining was visualized by exposure to 3,3′-diaminobenzidine for 3 to 5 minutes. For c-erbB-2, mouse monoclonal antihuman c-erbB-2 (Ventana, Frankfurt, Germany) and the NX/EX automatic stainer (Ventana) were used (22) As positive controls, lung adenocarcinoma specimen, which had been surgically resected at our hospital and had been determined previously to be strongly positive, was used for the EGFR and p-EGFR experiments. Breast cancer specimen, also surgically resected at our hospital and known to be strongly positive, was used for the c-erbB-2 experiment. Negative controls for each antibody were done with nonimmune serum instead of the primary antibodies. The expression of each receptor was scored as follows: − = no discernible staining, or <10% of cells stained; 1+ = >10% of

Table 2 Univariate analysis of clinicopathological factors (N = 36)

	Responder (N = 17)	Non-responder (N = 19)	P value
Age			
≥70	3	7	0.2742
<70	14	12	
Gender			
Male	7	10	0.5251
Female	10	9	
PS			
<2	16	16	0.6052
≥2	1	3	
Smoking history			
Smokers	6	10	0.3351
Never-smokers	11	9	
Previous chemotherapy			
Yes	11	8	0.2021
No	6	11	
Recurrent site			
Lung only	10	13	0.7301
Others	7	6	
Dominant histological subtype			
Papillary	13	4	0.0021 *
Non-papillary	4	15	
BAC	1	6	0.0918
Non-BAC	16	13	
Solid	2	5	0.4080
Non-solid	15	14	
Acinar	1	4	0.3420
Non-acinar	16	15	
Tumor size			
≤3.0 cm	6	7	>0.9999
>3.0 cm	11	12	
Lymph node metastasis			
+	10	13	0.7362
−	6	6	
Lymphatic permeation			
+	15	13	0.2357
−	2	6	
Vascular invasion			
+	14	15	>0.9999
−	3	4	
Pleural invasion			
+	13	8	0.0489 *
−	4	11	
Pulmonary metastases			
+	5	6	>0.9999
−	12	13	

cytoplasmic staining, or plasma membrane staining with weak intensity; 2+ = >10% of plasma membrane staining with moderate intensity; and 3+ = >10% of plasma membrane staining with strong intensity. Staining of 2+ and 3+ were evaluated as positive. As for EGFR and p-EGFR, no universal evaluation criteria exist at present; therefore, we applied the same criteria as c-erbB-2. Although this evaluation criteria basically followed HercepTest (23), we added some modification to evaluate cytoplasmic staining.

Statistical Analysis. All statistical analyses were done with the statistical program StatView, version 5.0 (Abacus Concepts, Berkley, CA). The significance of the relationships between individual clinicopathologic factors; the expression of EGFR, p-EGFR, and c-erbB-2; and a univariate analysis with the Fisher exact probability test was used to evaluate the response to gefitinib. A multivariate regression analysis was conducted according to the Cox proportional hazard model. Kaplan-Meier method was used to calculate survival rates, and a log-rank test was used to evaluate the statistical significance of any differences. A P value of less than 0.05 was considered significant.

RESULTS

Clinical Characteristics. The patient characteristics are shown in Table 1. All clinical data were retrieved from medical records. The mean age of the patients was 70 years (range, 43–82 years). Seventeen patients were male and 19 were female. The Eastern Cooperative Oncology Group performance status was 0 for 7 patients, 1 for 25 patients, and 2 for 4 patients. Sixteen patients were current or ex-smokers. Nineteen patients had been treated previously with chemotherapeutic agents for postoperative recurrences. The Response Evaluation Criteria in Solid Tumors (24) was used to evaluate the response of the patients. Seventeen patients experienced a partial response (PR) to gefitinib, 16 patients had a stable disease (SD), and 3 patients had a progressive disease (PD); the overall response (OR) rate was 47%. No clinical differences were observed between the responders and the non-responders (Table 2). Mean duration of response was 258 days; however, 11 of 17 responded patients were continuing gefitinib at the time of analysis.

Pathologic Findings of Surgical Specimens. The details of the pathologic findings for the surgical specimens are sum-

Table 3 Pathological findings of adenocarcinoma cases (N = 36)

Patient no.	Pathological stage		Histological subtype(%)				Dominant histological subtype	Tumor size	Lymph node metastases	Ly	v	P	pm
			BAC	Acinar	Papillary	Solid							
1	$T_2N_2M_0$	IIIA	20	20	60	0	Papillary	4.5 cm	+	+	+	+	−
2	$T_4N_2M_0$	IIIB	20	30	50	0	Papillary	2.9 cm	+	+	+	+	−
3	$T_2N_2M_0$	IIIA	60	40	0	0	BAC	3.3 cm	+	+	+	+	−
4	$T_2N_1M_0$	IIB	10	0	0	90	Solid	3.5 cm	+	+	+	+	−
5	$T_4N_xM_0$	ND	0	40	0	60	Solid	1.5 cm	ND	−	+	−	−
6	$T_4N_2M_0$	IIIB	30	20	50	0	Papillary	4.3 cm	+	+	+	+	+
7	$T_3N_2M_0$	IIIA	20	30	50	0	Papillary	6.0 cm	+	−	+	+	−
8	$T_4N_0M_0$	IIIB	0	0	100	0	Papillary	4.2 cm	−	+	−	+	+
9	$T_3N_0M_0$	IIIA	30	0	70	0	Papillary	2.8 cm	−	+	+	−	−
10	$T_4N_2M_0$	IIIB	0	0	100	0	Papillary	3.2 cm	+	+	+	+	+
11	$T_4N_1M_0$	IIIB	30	10	60	0	Papillary	2.8 cm	+	+	+	+	−
12	$T_2N_0M_0$	IB	30	10	60	0	Papillary	3.2 cm	−	+	+	+	+
13	$T_1N_0M_0$	IA	0	80	10	10	Acinar	1.5 cm	−	+	+	-	−
14	$T_2N_2M_0$	IIIA	20	10	70	0	Papillary	2.0 cm	+	+	−	+	−
15	$T_4N_0M_0$	IIIB	0	0	100	0	Papillary	4.8 cm	−	+	−	−	+
16	$T_2N_0M_0$	IB	20	10	70	0	Papillary	3.2 cm	−	+	+	+	−
17	$T_2N_1M_0$	IIB	0	10	80	10	Papillary	3.5 cm	+	+	+	+	−
18	$T_4N_2M_0$	IIIB	0	30	0	70	Solid	3.2 cm	+	+	+	−	+
19	$T_2N_2M_0$	IIIA	60	0	40	0	BAC	2.5 cm	+	+	+	+	−
20	$T_4N_2M_0$	IIIB	20	0	80	0	Papillary	2.8 cm	+	+	+	−	+
21	$T_3N_1M_0$	IIIA	0	60	0	40	Acinar	2.8 cm	+	+	+	+	−
22	$T_2N_0M_0$	IB	60	0	40	0	BAC	3.5 cm	−	−	−	−	−
23	$T_2N_2M_0$	IIIA	0	10	0	90	Solid	4.5 cm	+	+	+	+	−
24	$T_1N_0M_0$	IA	80	0	20	0	BAC	2.2 cm	−	−	−	−	−
25	$T_2N_0M_0$	IB	80	10	10	0	BAC	3.2 cm	−	−	−	−	−
26	$T_1N_0M_0$	IA	80	20	0	0	BAC	1.6 cm	−	−	−	+	−
27	$T_2N_1M_0$	IIB	10	0	90	0	Papillary	3.3 cm	+	+	+	−	−
28	$T_2N_0M_0$	IB	0	100	0	0	Acinar	1.8 cm	−	+	+	+	−
29	$T_1N_2M_1$	IV	0	70	0	30	Acinar	2.2 cm	+	+	+	+	+
30	$T_2N_2M_0$	IIIA	0	20	50	30	Papillary	8.4 cm	+	+	+	−	−
31	$T_2N_2M_0$	IIIA	30	0	0	70	Solid	3.9 cm	+	−	+	−	−
32	$T_4N_2M_0$	IIIB	0	10	0	90	Solid	4.0 cm	+	+	+	+	+
33	$T_4N_2M_0$	IIIB	0	20	80	0	Papillary	8.0 cm	+	+	+	−	+
34	$T_2N_2M_0$	IIIA	0	50	20	30	Acinar	6.5 cm	+	+	+	+	−
35	$T_4N_2M_0$	IIIB	0	20	0	80	Solid	7.5 cm	+	+	+	−	+
36	$T_2N_0M_0$	IB	80	10	10	0	BAC	5.5 cm	−	−	+	−	−

Abbreviations: BAC, bronchioloalveolar carcinoma; Acinar, acinar subtype; Papillary, papillary subtype; Solid, solid adenocarcinoma with mucin; ly, lymphatic permeation; v, vascular invasion; p, pleural invasion; pm, pulmonary metastases; ND, not determined.

marized in Table 3. The pathologic stage was IA in 3 cases, IB in 6 cases, IIA in 0 cases, IIB in 3 cases, IIIA in 11 cases, IIIB in 11 cases, and IV in 1 case. The stage IV disease was caused by pulmonary metastasis to another lobe. The pathologic stage could not be determined in one case, because only a partial resection had been done. All but 3 specimens were adenocarcinomas of mixed subtype. The dominant histologic subtype was BAC in 7 cases, acinar subtype in 5 cases, papillary subtype in 17 cases, and solid adenocarcinoma with mucin in 7 cases. Both a dominant papillary subtype ($P = 0.0021$) and the presence of pleural invasion ($P = 0.0489$) were significantly associated with the response to gefitinib when examined with a univariate analysis (Table 2), but a multivariate analysis revealed that a dominant papillary subtype was the only significant factor (Table 4). In addition, the survival period of the dominant papillary subtype patients was longer than that of the non-papillary subtype patients (Fig. 1). The representative histologic features of the papillary subtype are shown in Fig. 2.

Immunohistochemistry. The immunohistochemical evaluation was done according to the scoring system described in Patients and Methods. Immunohistochemistry was not done in one patient, because a tissue block was not available. Nine patients (28%) were positive for EGFR, and 14 (40%) were positive for p-EGFR. None of the patients were positive for c-erbB-2. No substantial association was observed between the immunohistochemical expression of each receptor and the response to gefitinib. The results of immunohistochemistry are summarized in Table 5.

DISCUSSION

Adenocarcinomas were known to be significantly sensitive to the treatment with gefitinib, despite their lower expression rates of EGFR compared with squamous cell carcinomas (13). Some investigators have reported that gefitinib is particularly

Table 4 Multivariate analysis

Parameter	Odds ratio	95% CI	*P* value
Dominant histological subtype (papillary subtype)	14.902	2.497–88.916	0.0030 *
Pleural invasion (present)	0.167	0.027–1.044	0.0556

Abbreviation: CI, confidence interval.

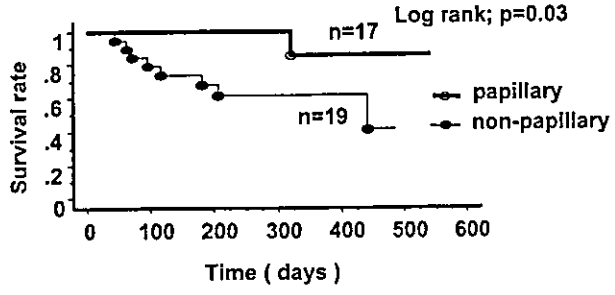

Fig. 1 Survival curves of the adenocarcinoma patients treated with gefitinib.

Fig. 2 Representative case of papillary adenocarcinoma (HE× 100). Tumor cells are growing with their own fibrovascular stroma and displaying complicated secondary and tertiary papillary branches. Abbreviation: HE, hematoxylin and eosin.

effective especially in BAC (16, 17), and clinical trials targeting BAC are now under way (25). In fact, as Hirsch *et al.* (8) reported, a high expression level of both EGFR and c-erbB-2 was seen in the BAC in a preclinical study. However, the association between the overexpression of EGFR or c-erbB-2 and the sensitivity to gefitinib is still controversial (26–28), and no data supporting an association has been obtained in clinical studies (29). Moreover, none of the previous studies examined surgical specimens, although BAC cannot be diagnosed with small biopsy specimens (19). In the present study, we investigated the clinicopathological and immunohistochemical features of surgically resected specimens from adenocarcinoma patients who were treated with gefitinib for postoperative recurrences. The surgical specimens were used to determine the dominance of the histologic subtypes according to the revised World Health Organization classification and to precisely evaluate the expressions of EGFR, p-EGFR, and c-erbB-2.

Clinical factors, including age, gender, performance status, smoking history, previous chemotherapy, and recurrence site, were not associated with the response to gefitinib in this study. The immunohistochemical expression profiles of EGFR, p-EGFR, and c-erbB-2 were also not associated with the response. However, both the dominant histologic subtype and the presence of pleural invasion differed significantly between responders and non-responders according to a univariate analysis, whereas a multivariate analysis revealed that only the dominant histologic subtype was a significant factor. In other words, a dominant papillary subtype was the feature that most favored a response to gefitinib, and the survival period of patients with this feature was significantly longer than that of patients with non-papillary subtypes.

The finding that gefitinib is more effective in papillary subtype lesion is of great interest. Drug delivery might be more effective in this histologic subtype, because cancer cells with a papillary structure usually line the fibrovascular stroma. In an *in vitro* and *in vivo* study, Hirata *et al.* (30) showed that the

Table 5 Results of immunostaining ($N = 35$)

	Score	Responders; $N = 17$ (%)	Non-responders; $N = 18$ (%)	Total (%)	P value
EGFR					
Negative	−/1+	11 (65)	15 (85)	26 (72)	0.2642
Positive	2+/3+	6 (35)	3 (15)	9 (28)	
p-EGFR					
Negative	−/1+	10 (59)	11 (61)	21 (60)	>0.9999
Positive	2+/3+	7 (41)	7 (39)	14 (40)	
c-erbB2					
Negative	−/1+	17 (100)	18 (100)	35 (100)	
Positive	2+/3+	0 (0)	0 (0)	0 (0)	

antitumor effect of gefitinib was partly mediated by the inhibition of tumor angiogenesis through direct effects on microvascular endothelial cells that express EGFR. In the papillary subtype, this direct effect on microvascular endothelial cells might be more efficient than in other subtypes.

The results of the present immunohistochemical study suggest that EGFR expression is not a useful predictor of the response to gefitinib. Recently, Paez *et al.* (31) and Lynch *et al.* (32) originally showed that EGFR mutations may predict sensitivity to gefitinib. These epoch-making studies arouse an interest about association of EGFR mutations with histologic subtypes.

In the present study, 9 patients (28%) were positive for EGFR and 14 (40%) were positive for p-EGFR. It seems somewhat strange that the positive rate of p-EGFR surpassed that of EGFR; however, we consider that it is simply because the p-EGFR antibody was more sensitive than the EGFR antibody.

The response rate of our study was high even for adenocarcinoma patients; however, patients were not selected at a point of administration of gefitinib for the most likely respond and patient's selection in the present study strictly followed the definition described in the Patients and Methods section. The relatively high proportion of female (53%) and never-smoker (56%) might lead to this result.

A micropapillary pattern (MPP) of lung adenocarcinoma, which was not included in the revised World Health Organization histologic classification, was first described by Silver and Askin (33). Lung adenocarcinomas characterized by MPP are thought to be more likely to metastasize and have a poor prognosis (34, 35). Most MPP-positive adenocarcinoma cases were included in the papillary subtype in the present study. Miler *et al.* (16) reported that a never-smoker status was a significant predictor of the response to gefitinib, whereas Wu *et al.* (36) reported that all of their patients who achieved a complete response with gefitinib had bilateral diffuse small pulmonary metastases. Both a never-smoker status and diffuse pulmonary metastases are frequently observed in MPP-positive adenocarcinoma (35). These reports, combined with the results of the present study, suggest that gefitinib might be effective against MPP-positive adenocarcinoma. In fact, MPP-positive adenocarcinomas (12 cases) were more sensitive to gefitinib than MPP-negative lesions (24 cases) in the present study ($P = 0.0328$).

In conclusion, the results of the present study indicate that a dominant papillary adenocarcinoma subtype can be an important predictor of the response to gefitinib. Even in patients with pathologic stage IA NSCLC who undergo a complete resection, the 5-year survival rate is about 70% at best (2). Therefore, adenocarcinoma with a dominant papillary subtype might be susceptible to postoperative adjuvant treatments with gefitinib. However, the precise mechanism of how this agent works is still obscure. Additional studies are needed to reveal the relation between the sensitivity to gefitinib and the histology of papillary subtype.

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ORIGINAL ARTICLE

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Phase II study of the combination of vinorelbine and cisplatin in advanced non-small-cell lung cancer

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Abstract *Purpose*: To evaluate the efficacy and safety of combination chemotherapy with cisplatin and vinorelbine for the treatment of previously untreated patients with advanced non-small-cell lung cancer (NSCLC). *Patients and methods*: Eligible patients were those with measurable NSCLC. They were treated with two or more cycles of a regimen consisting of vinorelbine 25 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 on day 1 every 3 weeks. *Results*: A total of 45 patients were enrolled. The response rate was 51.1% (23/45; 95% CI 35.8% to 66.3%). The median survival was 286 days with a 1-year survival rate of 40%. The median number of treatment cycles was 2. The major toxic effect was neutropenia of grade 3 or higher (84%). Nonhematological toxicities, including vomiting (62%), were mild (grade 2 or less). There were no treatment-related deaths. *Conclusion*: The high response rate and good tolerability proved this combination therapy to be a safe and effective treatment for advanced NSCLC.

Keywords Non-small-cell lung cancer · Vinorelbine · Cisplatin · Phase II study

Introduction

Vinorelbine ditartrate [1], a vinca alkaloid derivative, shows antitumor activity mainly by inhibiting microtubule polymerization in tumor cells just as other vinca alkaloid drugs do [2, 9]. Clinical studies of vinorelbine (VNR) have shown a good therapeutic outcome in non-small-cell lung cancer (NSCLC) and breast cancer, and a reduction in peripheral neuropathy that occurs frequently with vinca alkaloids [5, 7, 10, 12]. The combination of VNR and cisplatin (CDDP) (VP therapy) has shown a synergistic effect in vitro, while the main side effects are different between the drugs [4]. A phase I-II study has demonstrated efficacy of this combination in NSCLC [3]. VP therapy is considered a promising combination regimen for NSCLC on account of its higher response rate and longer survival compared with VNR or CDDP alone, or CDDP combined with vindesine [8, 17].

In clinical studies performed in Europe and the US, patient compliance rate was as low as 50% or less with regard to VNR when VP therapy, as VNR 25 mg/m^2 weekly and CDDP 80 mg/m^2 on day 1, was repeated every 4 weeks. This indicates the need to reconsider the dosing schedule of VNR [17]. Another dosing schedule for VP therapy (VNR 20 to 30 mg/m^2 on days 1 and 8 and CDDP 80 mg/m^2 on day 1 every 3 weeks) showed almost complete compliance and was found to be beneficial since the response rate was 28.3% to 56.7% and the survival 9.2 to 10.6 months [6, 13, 15, 17].

VP therapy is an effective regimen against advanced NSCLC. A multicenter joint phase III study is being planned in Japan to compare four regimens for advanced NSCLC: CDDP plus irinotecan used as a reference arm, CDDP plus VNR every 3 weeks, CDDP plus gemcitabine and carboplatin plus paclitaxel. A phase II study of VP therapy has not been conducted in Japan. We therefore carried out a phase II study of VNR 25 mg/m^2 on days 1 and 8 plus CDDP 80 mg/m^2 on day 1 given every 3 weeks in advanced NSCLC to evaluate the efficacy and safety of VP therapy.

Patients and methods

Patient selection

Patients eligible for the study were those admitted to our hospital between August 1999 and October 2001 who were histologically or

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cytologically diagnosed as having NSCLC and who were in clinical stage III or IV with unresectable disease, or in whom radiotherapy with curative intent was not possible, including those who had pleural effusion and dissemination, those with intrapulmonary metastasis within the ipsilateral lobe, those in whom the irradiation field exceeded one-half of one lung, those with metastasis to the contralateral hilar lymph nodes, and those with reduced lung function. None of the patients had received prior therapy. Other eligibility criteria included expected survival of 12 weeks, age ≤ 75 years, Eastern Cooperative Oncology Group performance score (PS) of 0–2, measurable lesions, adequate hematological function (WBC ≥4000/mm^3, platelet count ≥100,000/mm^3, hemoglobin ≥10 g/dl), renal function (serum creatinine ≤ 1.5 mg/dl, creatinine clearance ≥60 ml/min), and hepatic function (total serum bilirubin ≤ 1.5 mg/dl, serum GOT and serum GPT less than twice the upper limit of normal). Written informed consent was obtained from every patient with the statement that the patient was aware of the investigational nature of this treatment regimen. Pretreatment evaluation included medical history, physical examination, complete blood count, serum biochemical analyses, chest roentgenogram, electrocardiogram and urinalysis. All patients underwent radionuclide bone scan and computerized tomography of the brain, thorax, and abdomen.

Treatment

The anticancer drugs were administered via the intravenous route, VNR 25 mg/m^2 (Navelbine, Kyowa Hakko Kogyo) on days 1 and 8 and CDDP 80 mg/m^2 (Randa, Nippon Kayaku) on day 1. This combination therapy repeated every 3 weeks constituted a cycle of treatment. The minimal number of cycles to be evaluated was two. On day 8, the physician examined the patient and evaluated the development of adverse events, and if leukocytes had decreased to below 2000/mm^3, platelets had decreased to below 75,000/mm^3 or fever with infection had occurred, administration of VNR on that day was withheld at the discretion of the physician. To proceed with the second and subsequent cycles, patients were required to have a neutrophil count ≥1500/mm^3 and a platelet count ≥100,000/mm^3. Those patients receiving granulocyte colony-stimulating factor (G-CSF) were observed for 3 days after the final dose of G-CSF to ensure that their neutrophil count was 1500/mm^3 or more. Serum creatinine levels were required to be below the upper limit of normal and serum GOT/GPT levels below twice the upper limit of normal. In the presence of liver dysfunction due to apparent liver metastasis, however, serum GOT and GPT levels were required to be below three times the upper limit of normal. If fever occurred or if the PS advanced to grade 3 or worse, the subsequent cycle was postponed until the temperature fell below 38°C or until the PS returned to 2 or less. In the presence of grade 2 peripheral neuropathy dosing was temporarily postponed; with improvement to grade 1 or less treatment was cautiously resumed, but medication was discontinued if 6 weeks passed without any improvement. Peripheral neuropathy (including transient) grade 3 or higher required discontinuation of treatment. For the third and subsequent cycles, VNR or CDDP was decreased by 25% in accordance with the treatment-related adverse events observed during the preceding cycle. Steroid and HT_3-antagonist were administered to prevent nausea and vomiting.

Target population size and interim analysis

Simon's two-stage minimax design [16] was used to estimate the number of patients required for interim and final analyses at a threshold response rate (P_0) of 0.20, an expected response rate (P_1) of 0.40, $\alpha = 0.05$ and $\beta = 0.10$. If the interim analysis revealed 6 responding patients out of 24, recruitment would be continued until the target population size was achieved. The combination therapy was considered effective if 14 or more of 45 patients showed response in the final analysis.

Since an interim response rate of 48.1% (13/27) [11] was obtained, it was necessary to enroll up to 45 patients for the final analysis.

Evaluation of response and toxicity

Response and toxicity were evaluated on the basis of tumor images obtained by CT and other techniques, laboratory data and subjective/objective symptoms before, during and after administration of the study drugs and during the period from completion of treatment to the final analysis. Measurable disease parameters were determined every 4 weeks by various means such as computerized tomography. Evaluation was made in compliance with Response Evaluation Criteria in Solid Tumors (RECIST) guidelines [14] for antitumor activity and with NCI Common Toxicity Criteria version 2 for safety. The Institutional Ethical Review Committee gave approval to the study.

Results

Patient characteristics

Table 1 gives characteristics of the patients included. Their median age was 59.5 years (range 35 to 75 years). Male, PS 1 and adenocarcinoma predominated. There were 26 patients (58%) with stage IV disease and 19 (42%) with stage IIIB disease.

Treatments administered

The total number of cycles administered was 126 with a median of two per patient (ranging from one to four cycles; Table 2) and 43 patients received two cycles or more. In the two patients who received fewer than two cycles, treatment was discontinued because of CDDP-induced renal dysfunction in one and patient refusal in the other. Patients who completed two cycles or more accounted for 96% of patients (43/45). Except the two patients who received only one cycle, the every-3-week

Table 1 Patient characteristics

Eligible patients (*n*)	45
Age (years)	
Median	59.5
Range	35–75
Sex (*n*)	
Male	34
Female	11
Performance status (*n*)	
0	11
1	32
2	2
Histology (*n*)	
Adenocarcinoma	30
Squamous cell carcinoma	9
Other	6
Stage (*n*)	
IIIB	19
IV	26

Table 2 Efficacy of treatment ($n=45$)

No. of cycles	
Median	2.0
Range	1–4
Response	
Partial response	23
No change	21
Not evaluable	1
Response Rate (%)	51.1
95% CI (%)	35.8–66.3
1-year survival rate (%)	40

dosing schedule was adhered to by 88% of patients (38/43) in the second cycle, 68% (17/25) in the third and 92% (12/13) in the fourth, with a total of 83% (67/81). Only in two cycles was VNR withheld on day 8. The dose of VNR was reduced in 9% of dose administrations (22/250) and the does of CDDP was reduced in 8% (10/126). The planned dose intensities were 16.7 mg/m^2 per week for VNR and 26.7 mg/m^2 per week for CDDP while the actual dose intensities were 16.4 and 24.7 mg/m^2 per week, respectively. The median delivered dose intensity for CDDP (day 1) and VNR (days 1 and 8) of each course together was 90% or more (Table 3).

Efficacy of treatment

Of the 45 patients, 23 showed a partial response, 21 showed no change and 1 was not evaluable (Table 2). The response rate was 51.1% (23/45; 95% CI 35.8% to 66.3%; Table 2). The nonevaluable patient died of sudden hemoptysis on the 22nd day after the start of the second cycle (43rd day after the start of treatment) and could not be evaluated. Ten patients were alive at the time of this report. The time to progressive disease was 172 days and the median survival was 286 days (95% CI 248 to 404 days; Table 2). The 1-year survival rate was 40%.

Table 3 Median delivered dose intensity

	Median dose intensity (%)			
	Course 1	Course 2	Course 3	Course 4
CDDP	100	98.8	96	92.3
VNR				
Day 1	100	98.6	95.5	93.8
Day 8	97.8	98.6	95.5	93.8

Toxicities

Table 4 lists toxicities observed during the study. Hematological and blood biochemical reactions included a high incidence of leukopenia and neutropenia, i.e. leukopenia and neutropenia of grade 3 or higher occurred in 73% of patients (33/45) and 84% (38/45), respectively. Neutropenia-associated fever was limited to two patients. All neutropenic patients recovered upon treatment with G-CSF. Platelets decreased in 4% of patients (2/45). Creatinine was temporarily elevated in 15.6% (7/45).

Subjective and objective symptoms observed were of grade 2 or less and included vomiting in 77.8% of patients (35/45), hiccough in 33.3% (15/45), constipation in 40% (18/45), diarrhea in 22% (10/45), rash in 31.1% (14/45) and injection site reaction in 26.7% (12/45). All of these toxicities disappeared or improved with symptomatic treatment. There were no toxic deaths.

Discussion

As for the VP regimen for advanced NSCLC, the every-3-week dosing schedule has been tried in several medical facilities [6, 13, 15, 17]. Table 5 summarizes the clinical outcomes of every-3-week VP therapy reported in the literature and in this study. Response rates range from 28% to 57% and median survival is approximately 10 months. The results are similar among the studies.

In 96% of patients (43/45), two or more cycles of VP therapy were administered. The every-3-week dosing

Table 4 Toxicities ($n=45$)

Toxicity	Grade (Common Toxicity Criteria)				Grade 3/4 (%)
	1	2	3	4	
Leukopenia	4	3	25	8	33 (73%)
Neutropenia	2	2	13	25	38 (84%)
Anemia	12	3	1	4	5 (11%)
Thrombocytopenia	5	1	2	0	2 (4%)
Creatinine	5	2	0	0	–
Vomiting	29	6	0	0	–
Hiccough	15	0	0	0	–
Constipation	13	5	0	0	–
Diarrhea	9	1	0	0	–
Rash	10	4	0	0	–
Neuropathy	4	0	0	0	–
Injection site reaction	4	8	0	0	–
Alopecia	3	0	0	0	–

Table 5 Outcomes of studies of VP therapy (VNR days 1 and 8, CDDP day 1, every 3 weeks)

Reference	VNR (mg/m^2)	CDDP (mg/m^2)	Response	Median survival time (months)
4	25	80	28.3% (28/99)	9.2
10	25	80	56.7% (42/74)	10
11	20–25	80	46.7% (14/30)	10.6
1	30	80	36.2% (47/130)	–
Present study	25	80	51.1% (23/45)	9.6

schedule was adhered to in 85% of all cycles administered. In cycles in which noncompliance was seen, medication was postponed to the 4th to 5th week because, in most cases, the neutrophil count in the 3rd week failed to meet the criterion for going on to subsequent cycles. The planned dose intensity was almost attained since the actual dose intensity was 16.4 mg/m^2 per week for VNR and 24.7 mg/m^2 per week for CDDP, accounting for 98% and 93% of the planned values, respectively [13].

Most adverse reactions were hematological. In particular, leukopenia and neutropenia of grade 3 or worse occurred in 73% and 84% of 45 patients, respectively. Others have reported the incidence of leukopenia of grade 3 or worse to be 8% to 33% [6, 13, 17]. Although the difference in patient characteristics hinders simple comparison and analysis of these data, it can be said that leukopenia was more frequent in our study. The leukocyte count improved rapidly upon treatment with G-CSF. Nonhematological toxicities were mild and adverse reactions of grade 3 or higher were not noted.

The combination of VNR 25 mg/m^2 on days 1 and 8 and CDDP 80 mg/m^2 on day 1 was administered every 3 weeks to 45 patients with advanced NSCLC in this phase II study. The response rate was 51.1%; the main adverse effect was neutropenia. The high response rate and good tolerability indicate that this combination therapy is a safe and effective treatment for advanced NSCLC. Its usefulness will be further verified in phase III studies.

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Objective definition and measurement method of ground-glass opacity for planning limited resection in patients with clinical stage IA adenocarcinoma of the lung☆

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Abstract

Objective: The standard operation for patients with stage IA lung adenocarcinoma is considered to be a lobectomy. Recently, some researchers have reported that patients with tumors showing greater proportions of ground-glass opacity (GGO) at computed tomography (CT) could be candidates for limited resection, because of its less aggressive nature. However, the lack of a precise definition or standard measuring method of GGO prevents its general use as an index for planning limited resection. Therefore, we attempted to define GGO based on CT number and measured it more objectively. **Methods:** Between 1998 and 2001, 90 patients with clinical stage IA adenocarcinoma, who underwent standard or intentional limited resection and whose images of chest high-resolution CT were preserved in Digital Imaging and Communications in Medicine (DICOM) format, constituted the study population. The tumor shadow seen on the solid window (WL, −160 HU; WW, 2 HU) was regarded as the central solid area of the tumor seen on the lung window, and GGO was defined as the whole tumor area with the exception of the central solid area. Each area was measured using Scion Image (Scion Corp., Frederick, MD). We analyzed the relationship between the proportion of GGO and both of pathologic findings and recurrence. **Results:** Among the 90 tumors, 31 (34.4%) were calculated to have a GGO area greater than or equal to 50%. Of these, 27 (87%) tumors were bronchioloalveolar carcinoma. Lymphatic and vascular invasions, or nodal involvement were found only in patients with a smaller proportion of GGO (<50%) ($P < 0.05$). During the follow-up period (median 36 months), recurrences occurred in eight patients who were diagnosed as having tumors showing smaller proportion of GGO (<50%). **Conclusions:** Tumors with a greater proportion of GGO measured by our method are thought to have a less invasive nature. Our objective measuring method of GGO could be useful for future multicenter trials to elucidate the value of limited resection for clinical stage IA adenocarcinoma based on the proportion of GGO.

Keywords: Lung neoplasms; High-resolution computed tomography; Lung neoplasms; Adenocarcinoma; Bronchioloalveolar carcinoma; Limited operation; Ground-glass opacity

1. Introduction

The standard operation for patients with T1N0M0 stage IA non-small cell lung cancer is still lobectomy with systematic nodal dissection, because limited resection for such patients was reported to increase local recurrence and decrease the survival rate compared to lobectomy in a randomized control trial conducted by the Lung Cancer Study Group [1]. Candidates for limited resection, therefore, are thought to be rather a group of patients that have less invasive tumors and a better prognosis than the whole group of stage IA non-small cell lung cancer patients [2]. Much research has been conducted to identify the group of patients with less invasive tumors preoperatively based on the tumor size. However, the tumor size turned out to be less useful, because the incidence of lymph node metastasis in patients with tumors smaller than 2 cm in diameter were reported to be 10–20% [3,4].

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Jang et al. reported that the focal area of ground-glass opacity (GGO) on high-resolution computed tomography (HRCT) could be an early sign of localized bronchioloalveolar carcinoma (BAC) [5]. We demonstrated that in patients with clinical T1N0M0 adenocarcinoma the patients with a higher proportion of GGO area (≧50%) on HRCT by visual estimation had neither lymph node metastasis nor lymphatic invasion and were alive without recurrence [6]. From these results, it is considered that such patients may be candidates for limited resection.

However, the lack of a precise definition or standard measuring method of GGO prevents its general use as an index for planning limited resection. To resolve the problems, we characterized the GGO using CT number, and developed more objective measurement methods using Scion Image to quantitate the proportion of GGO area. Then, we tested whether or not this method was useful in predicting less invasive tumors in clinical stage IA adenocarcinoma patients.

2. Materials and methods

Between January 1998 and December 2001, 284 patients with primary lung cancer underwent surgical resection of the lung at our hospital. Of these, 103 patients were given a diagnosis of clinical stage IA lung adenocarcinoma. Among the patients, 90 underwent standard surgical resection or intentional limited resection and their lung images of HRCT were preserved in Digital Imaging and Communications in Medicine (DICOM) format. These patients constituted the study population. For four patients with multiple lung cancer, we investigated the most advanced tumor. Fifty-one patients were men, and the average age was 60.3 years (range 36–78 years). CT scanning was performed on X-Vigor or Aquilion (Toshiba Medical Systems, Tokyo, Japan). HRCT scans were performed over a range of 50 mm, covering the entire lesion. The scanning parameters were a tube voltage of 120 kV, a tube current of 250 mAs for X-Vigor and 150 mAs for Aquilion, 1 or 2 mm collimation, and a reconstruction interval of 1 or 2 mm by using a bone algorithm. The field of view was focused at about 20 cm. GGO was defined as a hazy increase in lung attenuation without obscuring the underlying vascular marking. We tried to define the GGO based on CT number (Hounsfield unit (HU)). When we fixed the window width of CT at 2 HU, the tumor shadow represented the area where the CT value was greater than that of the window level. We changed the window level from 40 to −320 HU to select the best window level at which the tumor shadow was visually almost identical to the central solid area on the lung window. As a result, we decided the best window level was −160 HU, and referred to the window setting as 'solid window' (window level −160 HU; window width 2 HU). The tumor shadow seen on the solid window was thought to represent the central solid area seen on the lung window. Therefore, the GGO area was defined as the tumor shadow on solid window subtracted from tumor shadow on lung window. The areas of the tumor shadows were measured with Scion Image (Scion Corp., Frederic, MD, USA) on one level of each tumor shadow equator on each window settings. Scion Image is an image processing and analysis program for windows computer that is based on the popular NIH Image (NIH, Bethesda, MD, USA) for Macintosh computer. These are freely available for download from their respective website. We used the 'Density slice' command to segment the target area. The details of how to use the Scion Image are also referable to the manual in the website.

Vessels or bronchi in the tumor shadow were erased if the areas were larger than 5% of the tumor shadow. The proportion of GGO was calculated as follows: [(Area on lung window − Area on solid window)/Area on lung window] × 100. A representative case is shown in Fig. 1.

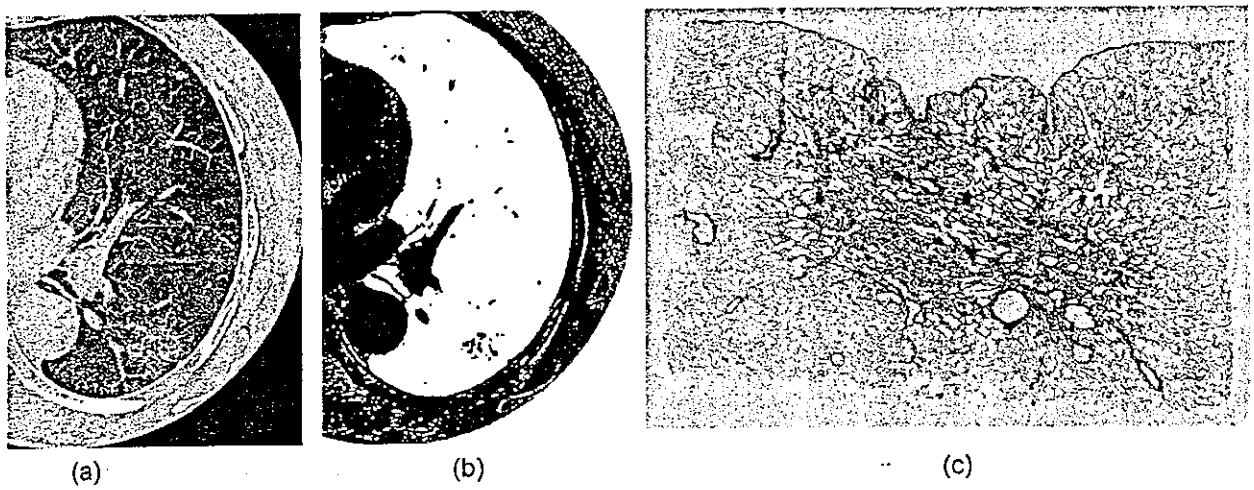

Fig. 1. Seventy-seven-year-old woman with a pulmonary nodule detected by annual screening using chest X-ray examination. Her HRCT images showed a GGO nodule dotted with small solid areas (a). The proportion of GGO was calculated at 86% (b). Pathologic examination revealed that the tumor was BAC. She was alive without any sign of recurrence at 42 months after operation (c).