under baseline in the sum of all measurable lesions, or appearance of new lesions; and stable disease (SD), residual tumor not qualified for CR, PR or PD. All evaluable lesions were measured bi-dimensionally (sum of products of longest diameter and its longest perpendicular of measurable lesions) using the same techniques as baseline, e.g. plain X-ray, CT or MRI.

At the end of 4-month treatment (or withdrawal), the best overall response was evaluated for each patient based on definitions as follows: CR, patients who qualified for CR at two sequential examination points with an interval of at least 28 days between them; PR, patients judged as PR or better at two sequential examination points with an interval of at least 28 days between them; SD, patients who were SD or better at two sequential examination points at least 28 days apart but who did not qualify as CR or PR. The first judgment of an SD case must be done at or after the first tumor assessment point (28 days after randomization); PD, the patients determined as PD at or before the first tumor assessment point (28 days after randomization); unknown, the patient does not qualify for a best response of increased disease, and all objective statuses after baseline (before randomization) and before progression are unknown.

Prior to the gefitinib treatment, tumor specimens were taken by transbronchial (TBB), skin or lymph-node biopsy with written informed consent from each patient. Ethics approval was obtained from the ethics committee of the individual institutes. Biopsy samples were frozen immediately, embedded in TissueTek OCT medium (Sakura, Tokyo, Japan), and stored at $-80°C$. All samples were examined microscopically, and samples from 28 patients (17 learning and 11 test cases) that contained enough cancer cells for analysis of expression profiles were initially selected for further analysis. For validation of the prediction system, a blinded set of samples from five newly enrolled cases (four PD and one SD) were also added to the 11 test cases. EGFR and AKT protein expression in tumor tissues, and plasma concentration of gefitinib were measured as additional biological factors in this study. Tissue sections from 19 suitable cases were used for assessment of EGFR protein expression as %positive cells with immunohistochemistry (DakoCytomation, Glostrup, Denmark). p-EGFR, AKT and p-AKT positivity were assessed on available tissue sections as absent or positive using individual monoclonal antibodies (Cell Signaling Technology, Inc., Beverly, MA, USA). Clinical and histological information about these patients is summarized in Tables 1–3.

Microdissection

In view of significant differences in the proportions of cancer cells and various types of parenchymal cells that are present from one tumor to another, microdissection is a necessary means of obtaining precise gene-expression profiles on cDNA microarrays. Therefore, we stained 8 μm thick frozen sections with hematoxylin and eosin and collected cancer cells selectively, using the μCUT laser-microbeam microdissection system (Molecular Machines & Industries AG, Glattbrugg, Switzerland) (31). In this system, tissue sections are mounted on a thin supporting polyethylene membrane that will be cut together with the target tissue; a pulsed ultraviolet (UV) narrow-beam-focus laser cuts out cancer cells along a pre-selected track that can be observed on a video screen. The material to be extracted is never directly exposed to the laser but only circumscribed by it; unlike other LMM systems, this one allows recovery of dissected cells to proceed without radiation. Moreover, the membrane protects the tissue on the slide against cross-contamination. Using this system we were able to isolate small areas of tissue rapidly, and to isolate single cells from histological sections (Fig. 1).

RNA extraction and T7-based RNA amplification

Total RNA was extracted from individual microdissected populations of cancer cells using RNAeasy mini kits and RNase-free DNase kits (QIAGEN, Hilden, Germany) according to the manufacturer's protocols. Total RNAs were subjected to T7-based RNA amplification, as described previously (32). Two rounds of amplification yielded 40–200 μg of aRNA (>100 000-fold) from each sample. As a control probe, normal human lung poly(A)$^+$RNA (BD Biosciences Clontech, Palo Alto, CA, USA and BIOCHAIN, Hayward, CA, USA) was amplified in the same way. Aliquots (2.5 μg) of aRNA from individual samples and from the control were reversely transcribed in the presence of Cy5-dCTP and Cy3-dCTP, respectively.

cDNA microarray

Our 'genome-wide' cDNA microarray system contains 27 648 cDNAs selected from the UniGene database of the National Center for Biotechnology Information (32). Fabrication of the microarray, hybridization, washing and detection of signal intensities were described previously (32). To normalize the amount of mRNA between tumors and controls, the Cy5/Cy3 ratio for each gene's expression was adjusted so that the averaged Cy5/Cy3 ratio of 52 housekeeping genes was equal to one. We assigned a cutoff value to each microarray slide using analysis of variance, and the Cy5/Cy3 ratio of the gene was calculated as follows: (1) if Cy5 (cancer sample) was lower than the cutoff level, then the Cy5/Cy3 ratio of the gene was substituted by 2.5 percentile among the Cy5/Cy3 ratios of other genes whose Cy5 and Cy3 were higher than the cutoff level; (2) if Cy3 (control sample) was lower than the cutoff level, then the Cy5/Cy3 ratio of the gene was substituted by 97.5 percentile among the Cy5/Cy3 ratios of other genes whose Cy5 and Cy3 were higher than the cutoff level; (3) if both Cy5 and Cy3 were lower than the cutoff level, then the Cy5/Cy3 ratio of the gene was left blank.

Extraction of genes for predicting responsiveness to gefitinib

To discover genes that might be associated with sensitivity to gefitinib, individual measurements of about 27 648 genes were compared between the two groups of patients, one classified as responders to gefitinib (PR) and the other as non-responders (PD). To reduce the dimensionality of the number of potent genes that could discriminate between the two classes, we extracted only genes that fulfilled two criteria: (1) signal intensities were higher than the cutoff level in at least 60% of either group, and (2) $|MED_{PR} - MED_{PD}| \geq 1$, where MED indicates

the median calculated from log-transformed relative expression ratios in each group. Then random permutation tests were applied to estimate the ability of individual genes to distinguish between the two classes (PR and PD); mean (μ) and standard deviations (σ) were calculated from the log-transformed relative expression ratios of each gene in both groups. A discrimination score (DS) for each gene was defined as follows:

$$DS = \frac{\mu_{PR} - \mu_{PD}}{\sigma_{PR} + \sigma_{PD}}$$

The samples were randomly permutated 10 000 times for each pair of groups. Since the DS dataset of each gene showed a normal distribution, we calculated a P-value for the user-defined grouping.

Calculation of drug-response scores

We calculated GRS reflecting the expression levels of candidate prediction-genes according to procedures described previously (33,34). Each gene (gi) votes for either responder (PR) or non-responder (PD) depending on whether the expression level (xi) in the sample is closer to the mean expression level of one group or the other in reference samples. The magnitude of the vote (Vi) reflects the deviation of the expression level in the sample from the average of the two classes:

$$Vi = \left| xi - \frac{\mu_{PR} + \mu_{PD}}{2} \right|$$

We summed the votes to obtain total votes for responders (V_{PR}) and non-responders (V_{PD}), and calculated GRS values as follows:

$$GRS = \frac{V_{PR} - V_{PD}}{V_{PR} + V_{PD}} \times 100$$

where the GRS value reflects the margin of victory in the direction of either responder or non-responder. GRS values range from -100 to $+100$; the higher an absolute value of GRS, the stronger the prediction.

Cross-validation of scores and evaluation of the prediction system

The prediction scores of all samples were obtained by a leave-one-out approach, in which one sample at a time was removed from the sample set; permutational P-values and mean values of the two classes were calculated for each gene using the remaining samples. The drug response of the withheld sample was predicted by calculating the prediction score. These procedures were repeated for each sample (33,34).

To evaluate the reliability of the prediction system, we calculated a 'classification score' (CS) using the GRS values of responders and non-responders in each gene set, as follows (34):

$$CS = \frac{\mu_{GRSpr} - \mu_{GRSpd}}{\sigma_{GRSpr} + \sigma_{GRSpd}}$$

A larger value of CS indicates better separation of the two groups by the prediction system.

Hierarchical clustering

We used web-available software ('Cluster' and 'TreeView') written by M. Eisen (http://genome-www5.stanford.edu/MicroArray/SMD/restech.html) to create a graphic representation of the microarray data and to create a dendrogram of hierarchical clustering. Before the clustering algorithm was applied, the fluorescence ratio for each spot was first log-transformed and then the data for each sample were median-centered to remove experimental biases.

Semi-quantitative RT–PCR analysis

Aliquots (5.0 μg) of the same aRNA hybridized to the microarray slides from individual samples and from the normal control lung were reversely transcribed using oligo(dT)$_{12-18}$ primer and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Semi-quantitative RT–PCR experiments were carried out with the following sets of synthesized primers specific to the 12 top-ranked genes used for establishing a GRS or with beta-actin (*ACTB*)-specific primers as an internal control: *FLJ22662*, 5′-GCCATAAGTGGTCCCACAGT-3′ and 5′-GTCTTCTAGTCCGTCATCTCCCT-3′; Amphiregulin (*AREG*), 5′-CCATAGCTGCCTTTATGTCTGC-3′ and 5′-CTTTTTACCTTCGTGCACCTTT-3′; coronin, actin binding protein, 1C (*CORO1C*), 5′-TAATCTGCTGAGGACCTTTTGTC-3′ and 5′-TAATTCACTGTCCTCTTCTGGGA-3′; apoptosis, caspase activation inhibitor (*AVEN*), 5′-GCTCACAGCAGTAAATGCCTA-3′ and 5′-TGCTATGCTGTAAACACTGGCTA-3′; dual specificity phosphatase 3 (*DUSP3*), 5′-GGATCCTTTATTGGTGGTAGAGC-3′ and 5′-CCAGAGTGACCCTGAAGATAAAT-3′; *DJ473B4*, 5′-ACCTGATTCTCTAGGTGCAGTTT-3′ and 5′-GTCGTTTCAACCAGGTAGTTTTG-3′; pleckstrin homology-like domain, family A, member 2 (*PHLDA2*), 5′-GGGCGCCTTAAGTTATTGGA-3′ and 5′-GGATGGTAGAAAAGCAAACTGG-3′; RNA binding motif protein 7 (*RBM7*), 5′-TGTAATGGAGATTGTACAGGTTG-3′ and 5′-AGGAACAGTACAAATGCTGTGGT-3′; *BX092512* (EST), 5′-GCACTCCTTGAAGGTACACTAAC-3′ and 5′-ATTTGTATTCACTCAGCCATGC-3′; oncostatin M receptor (*OSMR*), 5′-ACCCAACTTCAAAACTAGGACTC-3′ and 5′-ACAGCTTGATGTCCTTTCTATGC-3′; glutamate–cysteine ligase, catalytic subunit (*GCLC*), 5′-TCATGAAAGGCACTGAGTTTTG-3′ and 5′-GTTAGCTGAAGCAGCTTTATTGC-3′; collagen, type IV, alpha 3 binding protein (*COL4A3BP*), 5′-ATATGCACAATCCTGGAAGTGA-3′ and 5′-TGCCTTACTAGCATTACCACCAT-3′; *ACTB*, 5′-GAGGTGATAGCATTGCTTTCG-3′ and 5′-CAAGTCAGTGTACAGGTAAGC-3′. PCR reactions were optimized for the number of cycles to ensure product intensity within the logarithmic phase of amplification. We performed phosphorimager quantification analysis (Molecular Imager FX: Bio-Rad Laboratories, Hercules, CA, USA), and RT–PCR band intensities were quantitatively compared with normalized Cy5/Cy3 ratio of gene expression from the microarray data.

Immunohistochemical analysis

To confirm the differential expression of AREG and transforming growth factor-alpha (TGFA) proteins, both of which encode the ligand for EGFR and other ERBB members, and other three candidate markers [a disintegrin and metalloproteinase domain 9 (ADAM9), CD9 antigen (p24) and OSMR], which are also known to relate to the EGFR signaling, for predicting responders versus non-responders to gefitinib, we stained clinical tissue sections obtained by fiberscopic transbronchial biopsy (TBB) and lymph-node biopsy using ENVISION+ Kit/HRP (DakoCytomation). Briefly, after endogenous peroxidase and protein blocking reactions, anti-human AREG polyclonal antibody (Neo Markers, Fremont, CA, USA), anti-human TGFA monoclonal antibody (Calbiochem, Darmstadt, Germany), anti-human ADAM9 monoclonal antibody (R&D Systems Inc. Minneapolis, MN, USA), anti-human CD9 monoclonal antibody (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) or anti-human OSMR monoclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was added, and then HRP-labeled anti-rabbit or anti-mouse IgG as the secondary antibody. Substrate chromogen was then added and the specimens were counterstained with hematoxylin.

Frozen tissue samples from 11 patients were selected for analysis of immunohistochemistry. Positivity of immunostaining was assessed semi-quantitatively by scoring intensity as absent or positive by three independent investigators without prior knowledge of the clinical follow-up data. Cases were accepted only as positive if reviewers independently defined them thus.

ELISA

Serum was obtained from an independent set of 35 lung-adenocarcinoma patients who were treated with gefitinib based on the same protocol as this clinical study at Hiroshima University hospital in Japan (five for PR, 10 for SD and 20 for PD). The sera of all the patients were obtained with informed consent at the time of diagnosis and every 4 weeks after the beginning of treatment, and stored at −80°C. The serum TGFA levels were measured by an ELISA using a commercially available enzyme test kits (TGF-alpha ELISA kit: Oncogene Research Products, San Diego, CA, USA).

In vitro gefitinib treatment and AREG-autocrine assay

Human NSCLC (adenocarcinoma) cell lines PC-9, NCI-H358 and NCI-H522 were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). To detect expression of *AREG* in these NSCLC cells, total RNA from each line was reverse-transcribed for single-stranded cDNAs using oligo(dT)$_{12-18}$ primer and Superscript II (Invitrogen). Semi-quantitative RT–PCR was carried out as described previously (19). gefitinib [4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline: Iressa, ZD1839], an inhibitor of epidermal growth factor receptor tyrosine kinase, was provided by AstraZeneca Pharmaceuticals (Macclesfield, UK). The drug was dissolved in DMSO at a concentration of 10 mM and kept at −20°C.

We performed flow cytometry to determine the sensitivity of lung adenocarcinoma cell lines to gefitinib treatment. Cells were plated at densities of 5×10^5 cells/100 mm dish and treated with 1.0 μM of gefitinib in appropriate serum-free medium. The cells were trypsinized 72 h after the treatment, collected in PBS and fixed in 70% cold ethanol for 30 min. After treatment with 100 μg/ml RNase (Sigma-Aldrich Co., St Louis, MO, USA), the cells were stained with 50 μg/ml propidium iodide (Sigma-Aldrich) in PBS. Flow cytometry was performed on a Becton Dickinson FACScan and analyzed by ModFit software (Verity Software House, Inc., Topsham, ME, USA). The percentages of nuclei in G_0/G_1, S and G_2/M phases of the cell cycle and sub-G1 population were determined from at least 20 000 ungated cells.

To investigate whether AREG functions as an autocrine anti-apoptotic factor in lung adenocarcinoma cells treated with gefitinib, we carried out the following assay. First, gefitinib-sensitive PC-9 cells, which do not express *AREG*, were cultured in serum-free medium for at least 8 h prior to gefitinib treatment. These cells were then incubated with 0.5 or 1.0 μM of gefitinib for 72 h in media that were either serum-free or supplemented with 10% FCS, or in serum-free conditioned medium collected from 72 h cultures of *AREG*-expressing cells (NCI-H358 or NCI-H522). Each medium was replaced once with the same medium containing gefitinib at the 48 h time point. To detect the response of each cell line to gefitinib, viability was evaluated by MTT assays using Cell Counting Kits (WAKO, Osaka, Japan).

To confirm the autocrine effect of AREG on the gefitinib-resistance of NSCLC cells, we cultured PC-9 cells for 72 h in serum-free medium containing 1.0 μM of gefitinib and recombinant AREG protein (Genzyme-Techne, Minneapolis, MN, USA) in final concentrations of 1–100 ng/ml. Cell viability was evaluated by MTT assays. A possible effect of AREG itself on the viability of NSCLC cells was evaluated also, by culturing the PC-9 cells in serum- and gefitinib-free medium containing only recombinant AREG protein. MTT assays were performed as above.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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Retrospective analysis of the predictive factors associated with the response and survival benefit of gefitinib in patients with advanced non-small-cell lung cancer

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Summary *Background:* The purpose of the study was to identify the potential predictive features associated with the response and survival benefit of gefitinib administration. We have retrospectively reviewed data of all patients who received a single regimen of gefitinib in our institution from August 1998 until July 2003. *Methods:* Overall 101 patients with non-small-cell lung cancer (NSCLC) who have received a single use of gefitinib were analyzed. Potential factors associated with the response of gefitinib included smoking index, gender, histology, performance status (PS), number of pre-treatments, age and stage. Univariate analysis was performed for these strata by Fisher's exact test and multivariate analysis was then performed using the logistic regression model. *Results:* The overall response rate was 19.8%. Univariate analysis revealed that significant predictive factors were associated with the response for 'adenocarcinoma', 'female', 'good PS' (0–1) and 'non-smoker' categories. Multivariate analysis limited the predictive factors associated with the response for 'female' ($P = 0.0032$), 'good PS' ($P < 0.02$) and 'non-smoker' ($P = 0.0417$). In survival analyses, 'female' ($P < 0.005$), 'good PS' ($P < 0.0001$), and a low level of the smoking index ($P < 0.05$) indicated significantly prolonged survival. Response and survival data in elderly patients were equivalent to those in younger patients. Adverse events (AEs) were generally mild and were almost always skin reactions and diarrhea. Interstitial lung disease (ILD) occurred in 4% of the group under observation. *Conclusions:* Gefitinib provided clinical benefit for the following factors 'female', 'good PS' and 'non-smoker'. A low smoking index is reported as a novel predictive prognostic factor following a single regimen of gefitinib.
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Abbreviations: NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; IDEAL-1, Iressa dose evaluated advanced lung cancer-1; PS, performance status; NCI-CTC, National Cancer Institute-Common Toxicity Criteria; INTACT-1, Iressa NSCLC trial assessing combination treatment-1; INTACT-2, Iressa NSCLC trial assessing combination treatment-2

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1. Introduction

Patients with advanced non-small-cell lung cancer (NSCLC) have a poor prognosis with 1—5% 5-year survival rates [1]. A recent meta-analysis demonstrated that platinum-based combination chemotherapy is currently considered to be the most effective treatment for advanced NSCLC, and these have improved the median survival time (MST) by 2 months and caused a 10% increase in 1-year survival rates [2]. As platinum-based chemotherapy improves survival and quality of life in advanced NSCLC patients, most patients will receive second line chemotherapy. With recurrence or progression, docetaxel has been approved as a second line chemotherapy treatment due to demonstrated survival benefit compared with best supportive care (BSC) or vinorelbine/ifosfamide [3,4]. Currently, there is no proven effective chemotherapy for patients previously treated with platinum-based and docetaxel therapies.

The epidermal growth factor receptor (EGFR) is a promising target for anticancer therapy because many types of cancer cells express or overexpress EGFR (including NSCLC, renal cell carcinoma and breast cancer) [5,6]. EGFR overexpression has been reported as a poor prognostic factor in many types of human solid tumors including NSCLC in several studies [7—9]. Currently, monoclonal antibodies that bind to the extracellular domain of EGFR and intracellular tyrosine kinase inhibitors have been developed [10,11]. Gefitinib is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways implicated in the proliferation, angiogenesis, invasion, metastasis and survival of cancer cells [12,13]. Several phase I trials demonstrated safety and tolerability of gefitinib in pretreated patients with solid tumors, in which trials an 11% response rate was seen in 100 patients with heavily pretreated advanced NSCLC [14]. On the other hand, in Japan, a phase I trial demonstrated five responders out of a total of 31 patients who all had adenocarcinoma of the lung [12]. To confirm anti-tumour activity and the safety profile of gefitinib, an international phase II study (IDEAL-1) and United States trial (IDEAL-2) were conducted as a second or third line treatment in patients with advanced NSCLC [15,16]. Patients enrolled in these studies were randomized into two different doses, 250 and 500 mg/day. These trials demonstrated that toxicity was mild and showed an encouraging response rate with an RR of 18.4 and 11.8% of patients in the 250 mg arm, respectively, and an improvement in disease related symptoms and quality of life were observed. The IDEAL-1 study has also confirmed that there were statistically significant differences in efficacy for 'adenocarcinoma' and 'female' using multivariate analysis. Two large randomized phase III studies [17,18], which are standard chemotherapy (cisplatin/gemcitabine or carboplatin/paclitaxel) with or without gefitinib, failed to demonstrate a survival benefit for advanced NSCLC patients as a first line chemotherapy. Although the results of the phase III studies were negative, gefitinib is still considered a promising molecular targeted agent as a new generation treatment in patients with advanced NSCLC. Information on the clinical prognostic factors following a single regimen of gefitinib should be helpful in finding which patients are likely to receive benefit, and in the development of a future treatment. Although the previous phase II trial (IDEAL) showed that several predictive factors were associated with the response to gefitinib, the population was essentially biased towards the young, with good performance status (PS) and conserved, good organ functions.

In this study, to find factors associated with an objective response and survival benefit of gefitinib, we retrospectively analysed patients who received a single regimen of gefitinib at our institute.

2. Methods

All patients with stage IIIB or IV NSCLC, who received a single regimen of gefitinib from August 1998 until July 2003 at the Kinki University School of Medicine, Osaka, were retrospectively reviewed. We evaluated patients who participated in clinical trials (phase I trial, phase II trial; IDEAL-1), or phase II trial for investigating surrogate gene therapy, and in 53 patients who were administered the drug after marketing (including elderly or poor performance status patients). Patients who received gefitinib as part of a compassionate use program were excluded. All patients were checked for age, gender, histology, Eastern Cooperative Oncology Group (ECOG), PS, stage, pre-treatment regimen, number of prior regimen, and smoking status before treatment of gefitinib. Smoking status was evaluated by the Brinkmann index; number of cigarettes per day multiplied by number of years. We analyzed the response, overall survival rate and the adverse effects of gefitinib, and investigated predictive factors associated with response and prognosis. The response was assessed using physical examination, biochemical profile, chest X-ray, chest computed tomography (CT), head CT or magnetic resonance imaging (MRI) scan, abdominal echo-graphic or abdominal CT scan, bone scinti-graph, bronchoscope, and was evaluated according to the response eval-

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uation criteria in solid tumor (RECIST) [19]. The severity of all the adverse events (AEs) that related to gefitinib administration was assessed by the NCPCTC (version 2.0) grading system. The predictive factors associated with the response that were analyzed in this study were age, gender, PS, histology, stage, number of prior regimen and smoking status. Variables were tested for any possible relationship with the response to gefitinib, at first by univariate analysis, and subsequently by the application of a multivariate model. Response rates were compared between strata using Fisher's exact test. Logistic regression models were used to explore observed differences and identify baseline factors that may independently predict for response rates. The survival curves were estimated using the Kaplan—Meier method and compared using the log-rank test. *P*-values less than 0.05 were considered significant.

3. Results

3.1. Patient profiles

From August 1998 until July 2003 at our institute, a total of 105 patients, who were already cytologically or histologically diagnosed as NSCLC, were treated by a single regimen of gefitinib. Patients received gefitinib until disease progression or intolerable toxicity. Of these, 101 patients were evaluated as suitable for analysis; four patients were excluded from analysis because they received gefitinib as part of a compassionate use program. As shown in Table 1, the 101 patients included: 2 patients who received gefitinib at a once daily dose of 50 mg; single patients who each received 100, 225, 400, 525 and 700 mg, respectively; 89 patients who received 250 mg; and 5 patients who received 500 mg. In the phase I trial, we used an intermittent administration schedule with 14 days continuous dosing followed by 14 days off.

Table 1 Patient characteristics

	Number of patient ($N = 101$)
Phase I	7
50 mg	2
100 mg	1
225 mg	1
400 mg	1
525 mg	1
700 mg	1
Phase II (IDEAL-I)	11
250 mg	6
500 mg	5
Phase II (gene expression) (250 mg)	30
Post marketing (250 mg)	53

Table 2 Patient characteristics ($N = 101$)

	Number of patients
Age (year)	
Median (range)	62 (31—84)
<69	74
≥70	27
Gender	
Male	64
Female	37
Performance status	
0	15
1	62
2	17
3	7
Tumor histology	
Adenocarcinoma	81
Squamous	18
Large-cell	2
Stage	
III	18
IV	83
Previous treatment	
No treatment	5
Failed 1 previous chemotherapy regimens	53
Failed 2 previous chemotherapy regimens	34
Failed 3 previous chemotherapy regimens	9
Smoking (smoker:never-smoker)	55:46
Index[a] 0:1—999:1000	46:32:23

[a] Index: number of cigarettes per day multiplied by number of years.

Patient characteristics are shown in Table 2. The median age was 62 years (ranging from 31—84) and 74 patients (73.3%) were less than 69 years old. 63.4% of the patients were male, 76.2% had performance status (ECOG) 0—1, 80.2% had adenocarcinoma of which 83.2% had stage IV disease. Fifty-three patients had received one prior regimen, 43 had more than two prior regimens and only five had previously been untreated. 54.5% of them were smokers, and the non-smokers were almost all female. This study included patients

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Table 3 Overall objective response

	Number	%
Number of patients evaluated	101	
Complete response (CR)	1	1.0
Partial response (PR)	19	18.8
Stable disease (SD)	52	51.5
Progressive disease (PD)	25	24.8
Not evaluable	4	4.0
Response rate		
% (95% CI)	19.8 (12.0–27.6)	
Disease control rate[a]		
% (95% CI)	71.3 (62.5–80.1)	

[a] CR + PR + S.D.

who had failed several previous chemotherapy regimens, and patients with an ECOG PS score of 3.

3.2. Response to treatment

Table 3 shows an objective response observed in this study. Twenty responders were evaluated and the overall response rate was 19.8%. One patient achieved a complete response, 19 patients exhibited a partial response and 52 patients had stable disease, resulting in a disease control rate (objective responses plus stable disease) of 71.3%. When evaluated using patient characteristics, we determined the response rate detailed in Fig. 1. All patients that responded had adenocarcinoma of the lung as the histological subtype. In addition, for the factors 'female' and 'never-smoker', there were higher response rates than in 'male' and 'smoker' respectively, while RR was similar for age, stage and pre-treatment. The response rate of 'female' and 'never-smoker' were 37.8 and 32.6%, respectively. Using the Fisher's exact test, the predictive factors which were associated with a response were 'female' (37.8% versus 9.4%; $P = 0.0006$), 'adenocarcinoma' (24.7% versus 0%; $P = 0.0104$), 'good PS' (0–1) (26.0% versus 0%; $P = 0.0028$), and never-smoker (32.6% versus 9.1%; $P = 0.0025$). There were no significant differences for age, stage and pre-treatment (Table 4). A multivariate analysis was performed against the four significant predictive factors in univariate analysis (Table 5). Because the incidence of the female factor is very strongly correlated to the never-smoker factor, the statistical assay was rather unstable if the two factors were analyzed simultaneously. We then investigated two patterns of multivariate analysis. One analysis excluded smoking and the other excluded gender. If smoking status was extracted, then female and good performance status were statistically significant. If gender was extracted, then non-smoking and good performance were statistically significant. The odds of a response were over three times higher for patients with adenocarcinoma than for patients with other histologies, however, this is not considered to be statistically significant because the group in this study was of a small size and included a high percentage of adenocarcinoma.

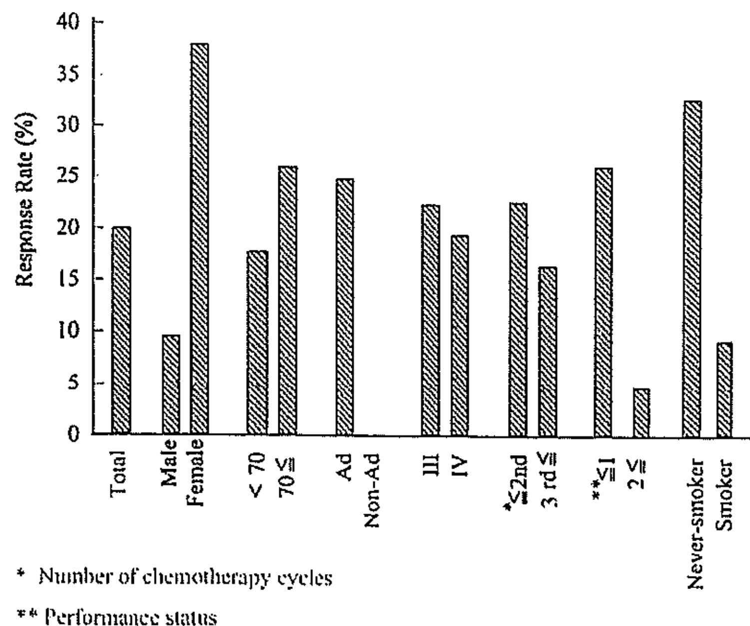

Fig. 1 Tumor response rate of the subgroups.

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Table 4 Predictive factors associated with an objective response by univariate analysis

Parameter	*N*	Responder	RR (%)	*P*-value
Smoking index				
Non-smoker	55	15	32.6	
Smoker	46	5	9.1	0.0025
Gender				
Female	37	14	37.8	
Male	64	6	9.4	0.0006
Histology				
Adenocarcinoma	81	20	24.7	
Others	20	0	0.0	0.0104
PS				
0–1	77	20	26.0	
≥2	24	0	0.0	0.0028
Pre-treatment				
≤2 regimens	58	13	22.4	
≥3 regimens	43	7	16.3	N.S.
Age (years)				
≤70	74	13	17.6	
≥71	27	7	25.9	N.S.
Stage				
IIIB	18	4	22.2	
IV	83	16	19.3	N.S.

Abbreviations: N.S., not significant.

3.3. Toxicity

Drug-related AEs of all patients are shown in (Table 6). A total of 101 patients were evaluated for toxicity. The most frequent drug-related AEs were a rash, dry skin and diarrhea. Most of these AEs were mild (Grade 1 or Grade 2) and were controllable. Of all the drug-related AEs evaluated, Grade 3 or Grade 4 AEs were seen in less than 5%, and Grade 4 drug-related AEs were only pneumonitis. Grade 3 or 4 AEs required a treatment interruption, but recovered after discontinuation of gefitinib, except with pneumonitis. Four patients developed greater than Grade 3 pneumonitis requiring hospitalization. All patients had a fever and severe hypoxemia on admission. As soon as possible, all patients were administered steroid therapy. While two patients recovered with the steroid therapy, two patients died within 40 days after the administration of gefitinib. Hematological toxicities were not observed.

3.4. Survival

The median survival time of the patients who were 'good PS' (0 or l) and 'poor PS' (2 or 3) was 353 and 97 days, respectively, and this difference was significant ($P = 0.0001$, log-rank test) (Fig. 2A). The MST of females was significantly longer than that of males (596 days versus 178 days, $P = 0.004$) (Fig. 2B). Furthermore, a low smoking index (<900) significantly prolonged survival (MST: 301 days versus 149 days, $P = 0.031$) (Fig. 2C). Age did not influence the survival benefit of the patients treated with gefitinib (Fig. 2D).

4. Discussion

Gefitinib is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways, and is one of the promising molecular targeted drugs used in the treatment of advanced NSCLC [16,17,20]. Although the large scale of the phase II study (IDEAL-1) [15] has already confirmed that there were statistically significant differences in efficacy for 'adenocarcinoma' and 'female' by multivariate analysis, the population was essentially biased towards young people with good performance status who had conserved, good organ functions. To clarify the predictive prognostic fac-

Table 5 Predictive factors associated with an objective response by multivariate analysis

Parameter	Odds ratio	95% CI	*P*-value
Extraction of smoking			
Gender (female vs. male)	0.163	0.040–0.585	0.0032
Performance status (1 vs. 2)	0.061	0.000–0.415	0.0018
Histology (Adeno[a] vs. others)	3.326	0.435–infinity	N.S.
Extraction of gender			
Non-smoking (non vs. ≥1)	0.297	0.063–0.959	0.0417
Performance status (1 vs. 2)	0.096	0.000–0.628	0.0101
Histology (Adeno vs. others)	4.385	0.588–infinity	N.S.

Abbreviations: N.S., not significant; CI, confidence interval.
[a] Adenocarcinoma.

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Table 6 Patients with drug-related adverse events (NCI-CTC)

Adverse event	Number of patients ($N = 101$)				
	Grade 1	Grade 2	Grade 3	Grade 4/5	Total
Rash	33 (32.6%)	21 (20.8%)	3 (3.0%)	0	57 (56.4%)
Dry skin	24 (23.7%)	3 (3.0%)	0	0	27 (26.7%)
Pruritis	9 (9.0%)	7 (7.0%)	0	0	16 (16.0%)
Diarrhea	19 (18.8%)	4 (4.0%)	0	0	23 (22.8%)
Nausea	6 (6.0%)	1 (1.0%)	0	0	7 (7.0%)
Vomiting	3 (3.0%)	0	0	0	3 (3.0%)
Anorexia	7 (7.0%)	0	0	0	7 (7.0%)
ALT increased	5 (5.0%)	2 (2.0%)	5 (5.0%)	0	12(13.0%)
AST increased	8 (8.0%)	2 (2.0%)	3 (3.0%)	0	13 (13.0%)
Pneumonitis	0	0	2 (2.0%)	2[a] (2.0%)	4 (4.0%)

[a] Treatment-related death (Grade 5).

tors in a practical setting, we retrospectively analysed the patients who received a single regimen of gefitinib at our institute. Multivariate analysis demonstrated that the predictive factors which were associated with a response were 'female', 'good PS' and 'never-smoker'. In survival analyses, the factors 'female', 'good PS', and a low smoking index also significantly prolonged survival.

The mechanism by which these factors produced better prognosis has not been clarified.

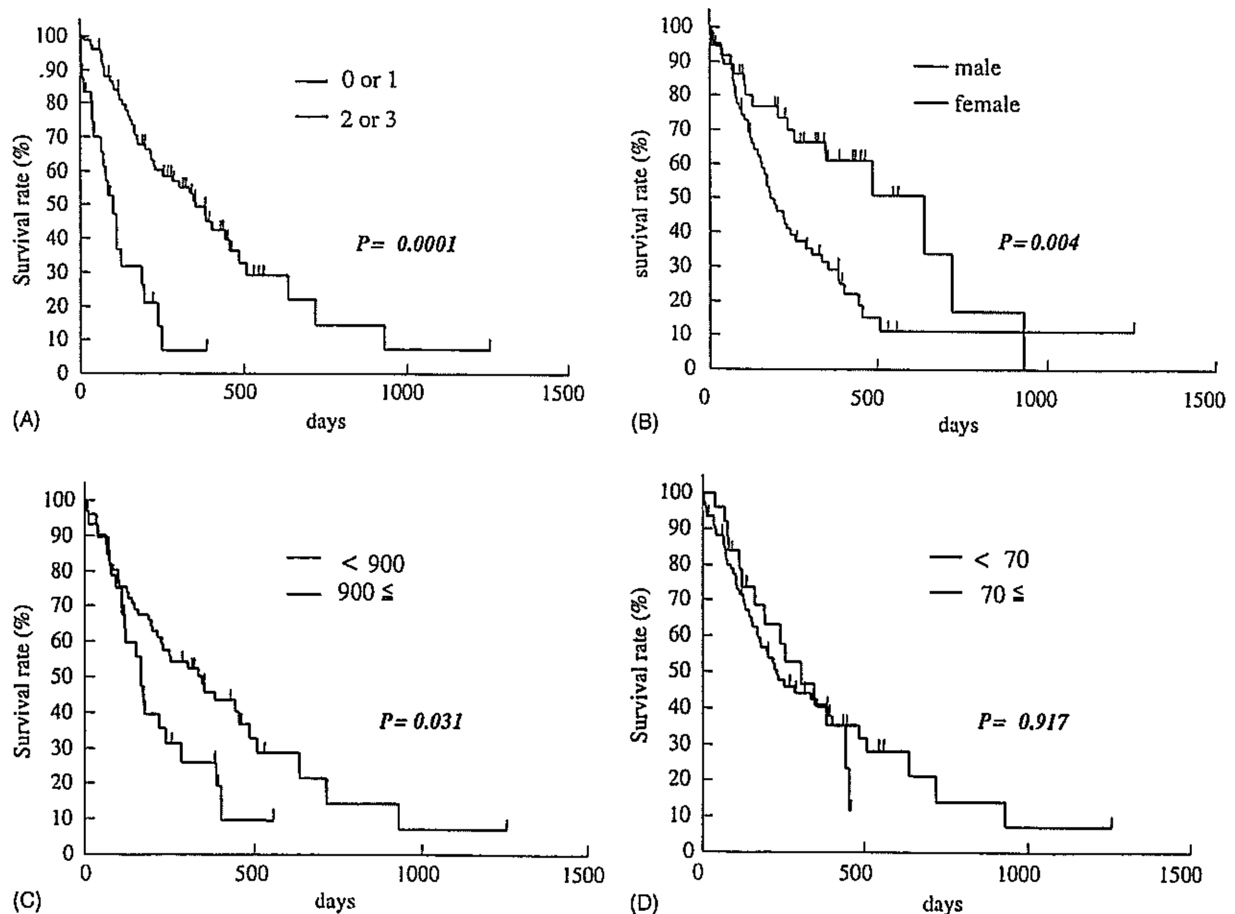

Fig. 2 A comparison of survival of: (A) PS 0, 1 vs. PS 2, 3; (B) gender: male vs. female; (C) smoking index: <900 vs. ≥ 900; and (D) age: <70 vs. ≥ 70.

Estrogen and progesterone may up-regulate EGFR in normal tissues [21], and activation of steroid hormones might impact on EGFR function in NSCLC [22]. Another explanation may be that the steroid hormone receptor might interact with EGFR and influence the response of an EGFR inhibitor.

Multivariate analysis in IDEAL-1 showed that PS was not a significant prognostic factor, however, the population of the study was restricted with regards to good PS. Although gefitinib was considered as an effector of symptom improvement in the phase II trial, the indication for patients with poor PS is controversial. Several authors described the case reports about the efficacy of gefitinib in NSCLC patients with poor PS [23,24] or with brain metastases [25]. Although 'good PS' were significant prognostic factor in this trial, gefitinib still might be a candidate drug for patients with poor PS, because of restriction of the use of other anti-cancer drug by their toxicities.

Elderly patients exhibited an equivalent response to young patients in this study. Recent data suggested, gefitinib is safe and well tolerated in elderly pretreated NSCLC patients [26]. A phase II study of gefitinib for elderly patients in NSCLC is needed.

A low smoking index was revealed as a predictive prognostic factor following a single regimen of gefitinib. Erlotinib is also administered orally and is a highly selective EGFR tyrosine kinase inhibitor [27] with a quinazolinamine-based structure similar to that of gefitinib. In the phase II study of erlotinib in NSCLC or bronchial alveolar carcinoma [28], a non-smoking history was also a prognostic factor. Chronic exposure to nicotine increases the expression level and phosphorylation status of EGFR and impairs its function [29]. Moreover, smoking produces overexpression of Her2/neu that binds to EGFR as a hetero-dimer in the tissue of normal bronchus. Expression of EGFR or Her2/neu or both in tissue samples by immunohistochemistry has not correlated in the response of gefitinib [30], however the different type of dimers formed between EGFR families might influence the response to gefitinib.

Four patients (4% of the patients) developed interstitial lung disease (ILD). Continuous smoking disrupted surfactant protein A or D [31,32], and the serum levels of the proteins were increased [33]. As 'smoking history' and 'male' are significant risk factors of ILD and also in treatment with gefitinib [34], a serum level of the surfactant protein A or D might be a predictive marker of ILD. Patients who are female and non-smokers are most likely to receive a high benefit and low risk with gefitinib treatment.

Although more basic biological research is needed to find the mechanism of action, we have found several predictive prognostic factors associated with the practical use of gefitinib. This is necessary clinical information which is important in order to set eligibility criteria for future clinical trials with gefitinib.

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Clinical

Combination phase I study of nedaplatin and gemcitabine for advanced non-small-cell lung cancer

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To establish the toxicities and maximum tolerated dose (MTD) of nedaplatin with gemcitabine, and to observe their antitumour activity, we conducted a combination phase I study in advanced non-small-cell lung cancer (NSCLC). Patients received nedaplatin (60–100 $mg\,m^{-2}$ given intravenously over 90 min) on day 1, and gemcitabine (800–1000 $mg\,m^{-2}$ given intravenously over 30 min) on days 1, 8, every 3 weeks. In total, 20 patients with locally advanced or metastatic NSCLC who received no prior chemotherapy or one previous chemotherapy regimen were enrolled. The most frequent toxicities were neutropenia and thrombocytopenia; nonhaematological toxicities were generally mild. Three out of six patients experienced dose-limiting toxicities (neutropenia, thrombocytopenia and delayed anaemia) at dose level 4, 100 $mg\,m^{-2}$ nedaplatin with 1000 $mg\,m^{-2}$ gemcitabine, which was regarded as the MTD. There were three partial responses, for an overall response rate of 16.7%. The median survival time and 1-year survival rate were 9.1 months and 34.1%, respectively. This combination is well tolerated and active for advanced NSCLC. The recommended dose is 80 $mg\,m^{-2}$ nedaplatin with 1000 $mg\,m^{-2}$ gemcitabine. This combination chemotherapy warrants a phase II study and further evaluation in prospective randomised trials with cisplatin- or carboplatin-based combinations as first-line chemotherapy for advanced NSCLC.
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Keywords: combination phase I study; maximum tolerated dose; nedaplatin; gemcitabine; non-small-cell lung cancer

Based on the results of a meta-analysis (Non-Small Cell Lung Cancer Collaborative Group, 1995), cisplatin-based chemotherapy is considered the best available therapy for patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC). Although several new agents with novel mechanisms and significant activity against NSCLC have been introduced, such as taxanes, gemcitabine and vinorelbine, any of these agents used in combination with a platinum agent provide equivalent survival improvement (Kelly *et al*, 2001; Schiller *et al*, 2002; Fossella *et al*, 2003). The prognosis of advanced NSCLC patients who receive cisplatin-based chemotherapy is still poor, and the renal and gastrointestinal toxicities caused by cisplatin often limit its clinical use. Therefore, development of different treatment strategies is necessary.

Nedaplatin is a second-generation platinum derivative that has shown equivalent antitumour activity and lower toxicity – less nausea, and lower nephrotoxicity and neurotoxicity – than cisplatin (Kameyama *et al*, 1990; Ota *et al*, 1992). A phase I study demonstrated the maximum tolerated dose (MTD) and the recommended dose (RD) for phase II studies of nedaplatin was 120 and 100 $mg\,m^{-2}$, respectively, and the dose-limiting toxicity (DLT) was thrombocytopenia (Ota *et al*, 1992). Two independent phase II studies of nedaplatin for NSCLC showed response rates of 14.7 and 20.5%, respectively, and 16.7 and 12.5% with the patients who had received chemotherapy previously (Fukuda *et al*, 1990; Furuse *et al*, 1992a). Based on these promising results, a randomised study of nedaplatin–vindesine *vs* cisplatin–vindesine was conducted for previously untreated NSCLC patients in Japan and indicated that nedaplatin-based chemotherapy yielded similar response rates and overall survival (Furuse *et al*, 1992b). Leucopenia, renal toxicities and gastrointestinal toxicities were more frequent in the cisplatin–vindesine arm, while thrombocytopenia was more frequent in the nedaplatin–vindesine arm.

Gemcitabine, an analogue of deoxycytidine, is a pyrimidine antimetabolite, that shows a reproducible response rates of >20% with a median survival time of 9 months, offering a quality of life benefit in comparison with best supportive care (Abratt *et al*, 1994; Anderson *et al*, 1994; Gatzemeier *et al*, 1996; Anderson *et al*, 2000). The main toxicity of gemcitabine is mild-to-moderate myelosuppression. The combination of gemcitabine and cisplatin showed synergistic effects in preclinical studies because gemcitabine inhibited the repair of DNA damage caused by cisplatin (Bergman *et al*, 1996), and achieved high response rates along with improvements in median survival time in clinical setting (Sandler *et al*, 2000; Schiller *et al*, 2002; Alberola *et al*, 2003).

Recently, carboplatin has attracted attention ahead of nedaplatin because it has similar activity to cisplatin with fewer nonhaematological toxicities. The available data suggest that carboplatin–paclitaxel or carboplatin–gemcitabine should be considered among standard regimen for advanced NSCLC (Kelly *et al*, 2001; Grigorescu *et al*, 2002; Rudd *et al*, 2002; Schiller *et al*, 2002).

It seems that nedaplatin has activity and toxicity profiles similar to those of carboplatin, although no randomised trial has not been done to allow direct comparison (Fukuda *et al*, 1990; Furuse *et al*,

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1992a; Ota *et al*, 1992). Moreover, Matsumoto *et al* (2001) demonstrated that the combination of nedaplatin and gemcitabine resulted in enhanced inhibition of tumour growth *in vivo* and the antitumour efficacy of the combination was superior to that of cisplatin–gemcitabine or carboplatin–gemcitabine. Based on the results of a preclinical study, we designed the present phase I study of the efficacy of the combination of nedaplatin and gemcitabine for advanced NSCLC. The purpose of this study was to establish the toxicities and MTD of this combination, to determine the RD for phase II studies, and to observe their antitumour activity.

PATIENTS AND METHODS

Patient eligibility

Patients with histologic or cytologic confirmation of locally advanced or metastatic NSCLC who received either no prior chemotherapy or one previous chemotherapy regimen were eligible. The eligibility criteria were as follows; (1) measurable lesions; (2) age $\leqslant$75 years; (3) Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–1; (4) adequate organ function (a white blood count (WBC) $\geqslant 4000\,\mu l^{-1}$, a neutrophil count $\geqslant 2000\,\mu l^{-1}$, a platelet count $\geqslant 100\,000\,\mu l^{-1}$, a haemoglobin count $\geqslant 9.5\,g\,dl^{-1}$, serum total bilirubin $\leqslant 1.5\,mg\,dl^{-1}$, serum transaminase $\leqslant 2\times$ upper normal limits, a serum creatinine $\leqslant$ upper normal limits, blood urea nitrogen (BUN) $\leqslant 25\,mg\,dl^{-1}$, $PaO_2 \geqslant 60$ mmHg or $SpO_2 \geqslant 90\%$]; and (5) normal electrocardiogram (ECG). At least 4 weeks must have passed after the completion of previous therapy and the patients had to have recovered from the toxic effects of previous therapy. The exclusion criteria consisted of pulmonary fibrosis or interstitial pneumonitis with symptoms or apparent abnormalities on chest X-ray, massive pleural effusion or ascites, acute inflammation, pregnancy, lactation, symptomatic brain metastases, active concurrent malignancies, severe drug allergies, severe heart disease, cerebrovascular disease, uncontrollable diabetes mellitus or hypertension, severe infection, active peptic ulcer, ileus, paralysis intestinal, diarrhoea and jaundice. This study was performed at Kinki University School of Medicine and was approved by the Institutional Review Board. Written informed consent was obtained from all patients. This study was conducted in accordance with Declaration of Helsinki.

Pretreatment and follow-up studies

Prior to entry, a complete history was taken and physical examination including age, height, weight, performance status, histological diagnosis, tumour stage, contents of previous treatment and presence of a complication was performed. The pretreatment laboratory investigations included a complete blood cell count, differential WBC count, platelet count, serum electrolytes, total protein, albumin, total bilirubin, transaminase, alkaline phosphatase, lactate dehydrogenase, BUN, creatinine, creatinine clearance and urinalysis. After the initiation of therapy, a complete blood cell count with a differential WBC count was performed at least twice a week. Blood chemistry profiles and chest X-ray films were obtained weekly. The lesion measurements were performed during at least every second course. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2 and tumour responses were assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (Therasse *et al*, 2000). Time to progression was measured from the date of registration to the date of first progression or death from any cause. Survival time was also measured from the date of registration to the date of death or latest follow-up, and was calculated using the Kaplan–Meier method (Kaplan and Meier, 1958).

Drug administration and dose escalation

The treatment schedule included nedaplatin, diluted with 500 ml of normal saline, given intravenously over 90 min on day 1, and gemcitabine with 100 ml of normal saline, given intravenously over 30 min after the completion of nedaplatin infusion on days 1 and 8, every 3 weeks. All patients were allowed to receive antiemetics with dexamethasone and granisetron, and post-therapy hydration with 1000 ml of normal saline. Granulocyte colony-stimulating factor (G-CSF) prophylaxis was not administered. Doses of gemcitabine on day 8 were given if the WBC count was $>2000\,\mu l^{-1}$ and/or the platelet count was $>750\,000\,\mu l^{-1}$, and/or allergic reaction, fever, elevation of transaminase and pneumonitis were less than grade 2, and/or the other nonhaematological toxicities were less than grade 3. The subsequent courses were withheld until the toxic levels returned to those specified in the eligibility criteria. The doses of both drugs were decreased by one dose level if DLTs occurred. In the case of the initial dose level, the doses of nedaplatin and gemcitabine were reduced by 20 and 200 $mg\,m^{-2}$, respectively.

Dose escalations were performed as listed in Table 1. Intrapatient dose escalation was not allowed. At least three patients were treated at each dose level, and three additional patients were entered at the same dose level if DLT was observed in one of the first three patients. The MTD was defined as the dose level at which more than two of three patients, or three of six patients experienced DLT. The definition of DLT was as follows: (1) grade 4 leukopenia, (2) grade 4 neutropenia for more than 4 days, (3) thrombocytopenia $<20\,000\,\mu l^{-1}$, (4) grade 3 febrile neutropenia, (5) grade 3 nonhaematologic toxicity except for nausea/vomiting, (6) delay of administration of gemcitabine on day 8 over a week for toxicities.

RESULTS

Between August 2001 and February 2003, 20 patients were enrolled in this study. The total and the median number of courses were 56 and 3 (range 1–6), respectively. The patients' characteristics are shown in Table 2. The majority of patients had a PS of 1. There

Table 1 Dose-escalation schema

Dose level	Nedaplatin dose ($mg\,m^{-2}$)	Gemcitabine dose ($mg\,m^{-2}$)	No. of patients (courses)
1	60	800	3 (8)
2	80	800	3 (10)
3	80	1000	8 (18)
4	100	1000	6 (20)

Table 2 Patients' characteristics

No. of patients		20
Age, years	Median	63.5
	Range	36–74
Sex	Male/female	17/3
Performance status	0/1	5/15
Histology	Adeno/squamous	13/7
Stage	IIIB/IV	4/16
Prior therapy	None	5
	Surgery	5
	Radiation	6
	Chemotherapy	14
	CDDP-based	3
	CBDCA-based	4
	Nonplatinum	4
	UFT	2
	Gefitinib	1

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were five previously untreated patients (level 3, two patients; level 4, three patients) and 15 (75%) previously treated patients. Of the previously treated patients, five had received prior surgery, five had prior radiotherapy, and 14 had prior chemotherapy. Seven had received platinum-based chemotherapy (cisplatin, three patients; carboplatin, four patients), and four a nonplatinum regimen. Responses to previous chemotherapy included partial response in five patients, stable disease in seven, progressive disease in one, and not evaluable in one. The median interval from previous treatment was 16 weeks (range 4–92.5 weeks). Out of 20 patients, 18 were assessable for toxicity and response. Two patients at level 3 were excluded from the toxicity and response evaluation because they had refused this study after registration.

Toxicities

The haematological and nonhaematological toxicities observed during the first course are shown in Tables 3 and 4, respectively. The most frequent toxicities observed in the first cycle were neutropenia and thrombocytopenia (Table 3). One-third of the patients had grade 3 thrombocytopenia, and one patient received a platelet transfusion during the first course. Three patients had grade 4 neutropenia for no longer than 4 days. The nadir for neutropenia and thrombocytopenia occurred on day 15 (median, range 5–18), and on day 15 (median, range 8–18), respectively. Nonhaematological toxicities generally were mild because none of the patients had experienced more than grade 3 in the first course (Table 4). The major toxicities following all courses are listed in Table 5. Grade 3 thrombocytopenia occurred in 16 out of 56 courses, and three patients received platelet transfusion (one patient at level 1, one at level 3 and one at level 4). However, no patient had haemorrhagic complications. The most frequent nonhaematological toxicities were elevation of transaminase activity, nausea and appetite loss, but all were mild. One previously untreated patient at level 3 experienced grade 3 pneumonitis after the fifth course, probably induced by this treatment, and the patient's condition improved after the administration of steroid. There was no treatment-related death. One of the 18 patients at level 4 underwent dose reduction after the first course due to neutropenia, and two patients at level 3 did not receive gemcitabine on day 8 because they had neutropenia, thrombocytopenia and high transaminase activity. Delays in the commencement of subsequent courses occurred in 11 courses, and the median length of the delay before starting the subsequent course was 21 days (21–35 days).

MTD and DLTs

At levels 1 and 2, none of the patients had developed a DLT. Haematological and nonhaematological toxicities were generally mild at these levels, although one patient had grade 3 thrombocytopenia at level 1. At level 3, two of six assessable patients had developed DLTs. Both could not receive their scheduled dose of gemcitabine on day 8 because they had neutropenia, thrombocytopenia and high transaminase activity. At level 4, three of six patients had developed DLTs. One patient received G-CSF for neutropenia, not lasting more than 4 days, which was considered as the DLT. Another patient required a platelet infusion because of thrombocytopenia $<20\,000\,\mu l^{-1}$. The third patient could not receive the second course due to the delayed anaemia, also considered as DLT. Therefore, dose level 4, 100 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine was regarded as the MTD. The recommended dose level for further phase II study was determined to be 80 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine (dose level 3 in this study).

Response and survival

There were three partial responses, for an overall response rate of 16.7%. As for squamous cell carcinoma, only one out of seven

Table 3 Haematological toxicity following first course of nedaplatin and gemcitabine

		WBC grade					ANC grade					plt grade					Hb grade				
Dose level	No. of patients	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	3	0	2	1	0	0	0	1	2	0	0	0	1	1	1	0	0	2	1	0	0
2	3	1	0	2	0	0	1	0	1	1	0	0	3	0	0	0	0	1	2	0	0
3	6	1	1	2	1	0	2	0	0	3	1	1	2	1	2	0	3	3	0	0	0
4	6	1	0	3	2	0	0	0	3	1	2	0	2	1	3	0	0	3	3	0	0

Table 4 Nonhaematological toxicity following first course of nedaplatin and gemcitabine

		Nausea grade					Vomiting grade					Fatigue grade					Transaminase grade				
Dose level	No. of patients	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	3	3	0	0	0	0	3	0	0	0	0	2	1	0	0	0	3	0	0	0	0
2	3	1	1	1	0	0	3	0	0	0	0	1	2	0	0	0	1	2	0	0	0
3	6	2	3	1	0	0	5	1	0	0	0	4	2	0	0	0	3	1	2	0	0
4	6	2	2	2	0	0	6	0	0	0	0	6	0	0	0	0	1	5	0	0	0
		Infection grade					**Fever grade**					**Appetite loss grade**					**Constipation grade**				
Dose level	No. of patients	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	3	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0
2	3	2	0	1	0	0	2	1	0	0	0	1	2	0	0	0	3	0	0	0	0
3	6	6	0	0	0	0	6	0	0	0	0	2	4	0	0	0	4	2	0	0	0
4	6	4	0	2	0	0	6	0	0	0	0	2	4	0	0	0	4	2	0	0	0

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Table 5 Toxicities following all courses of nedaplatin and gemcitabine (56)

	Grade			
	1	2	3	4
WBC	13	26	10	0
ANC	15	15	13	3
Hb	24	27	1	0
Plt	22	14	16	0
Nausea	17	4	0	0
Vomiting	6	0	0	0
Appetite loss	21	0	0	0
Fatigue	15	0	0	0
Constipation	6	7	0	0
Transaminase	27	5	0	0
Neuropathy	5	0	0	0
Pneumonitis	0	0	1	0
Fever	1	0	0	0
Infection	0	3	1	0

patients had a partial response. The median progression-free survival time was 5.1 months. The median survival time and 1-year survival rate were 9.1 months and 34.1%, respectively. Out of 15 patients who had received prior treatment, two (13.3%) achieved a partial response, and there was no clear relationship between responses to previous treatment and responses to this regimen. For previously treated patients, the median survival time and 1-year survival rate were 9.2 months and 40.3%, respectively. Among five previously untreated patients, one (20%) achieved a partial response and the median survival time and 1-year survival rate were 12.0 months and 50.0%, respectively.

DISCUSSION

Many recent randomised clinical trials have shown that the combinations of cisplatin with one of the new agents, such as gemcitabine, taxanes or vinorelbine, is the standard therapy for patients with locally advanced or metastatic NSCLC (Non-Small Cell Lung Cancer Collaborative Group, 1995; Kelly *et al*, 2001; Schiller *et al*, 2002; Fossella *et al*, 2003). As it is known that cisplatin strongly promotes nephrotoxicity, neurotoxicity and gastrointestinal toxicity, second-generation platinum-containing compounds including carboplatin have attracted attention. Based on several randomised trials that have shown that the combination of carboplatin with paclitaxel produces similar response rates and overall survival with a more favourable toxicity profile than the combination of cisplatin with new agents (Kelly *et al*, 2001; Scagliotti *et al*, 2002; Schiller *et al*, 2002), combined therapy of carboplatin and paclitaxel is considered to be a standard therapy. More recently, the combination of carboplatin with gemcitabine has become attractive as a therapy for advanced NSCLC. Some randomised studies have indicated that carboplatin–gemcitabine regimen offers equivalent median survival compared with cisplatin–gemcitabine or mitomycin–vinblastine–cisplatin /mitomycin–ifosfamide–cisplatin (Danson *et al*, 2003; Zatloukal *et al*, 2003), and results in significant improvements in overall survival over those for gemcitabine alone or the older cisplatin-containing regimens (Grigorescu *et al*, 2002; Rudd *et al*, 2002; Sederholm, 2002). However, neutropenia and thrombocytopenia were more common in carboplatin–gemcitabine regimens than others; thrombocytopenia was particularly common.

Like carboplatin, nedaplatin is also a second-generation platinum derivative that appears to have a similar mechanism and toxicity profile to carboplatin, although direct comparison has not been performed. Moreover, *in vivo* study suggested that nedaplatin–gemcitabine resulted in more enhanced inhibition of tumour growth than cisplatin–gemcitabine or carboplatin–gemcitabine. These results prompted us to investigate nedaplatin-based combinations and to conduct this phase I study.

With respect to toxicities, the most frequent toxicities were haematological toxicities, especially neutropenia and thrombocytopenia. Eight of 18 patients (44.4%) developed more than grade 3 neutropenia after the first courses, and after 16 out of 56 (28.6%) courses overall. On the other hand, six out of 16 patients (37.5%) developed grade 3 thrombocytopenia after the first courses, and after 16 out of 56 courses (37.5%) overall. However, patients required platelet transfusions during only three courses. In addition, one previously untreated patient developed drug-related pneumonitis, which improved with the administration of steroid, at level 3 after the fifth course.

Overall, the toxicities of the combination of nedaplatin with gemcitabine were generally mild and this combination chemotherapy is both well tolerated and active against advanced NSCLC.

The overall response rate of 16.7%, the median survival time of 9.1 months, and 1-year survival rate of 34.1% in this study were quite acceptable because most patients had been given prior chemotherapy. As evaluation of antitumour activity was not a primary objective, and our patient population was small and heterogeneous, we are unable to draw definitive conclusions about the activity of this regimen. Currently, it is still controversial whether novel platinum compounds such as carboplatin and nedaplatin could replace cisplatin for the treatment of advanced NSCLC. However, when not only antitumour activity but also palliation are the main goals of treatment, these new platinum compounds might play a useful role because of their favourable toxicity profile. Therefore, nedaplatin–gemcitabine warrants a phase II study, and further evaluation in prospective randomised trials with cisplatin- or carboplatin-based combinations as a first-line chemotherapy for advanced NSCLC in order to investigate whether nedaplatin could replace cisplatin or carboplatin.

In conclusion, the combination of nedaplatin with gemcitabine is well tolerated and active for advanced NSCLC. The MTD and recommended dose level are 100 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine and 80 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine, respectively.

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Effect of re-treatment with gefitinib ('Iressa', ZD1839) after acquisition of resistance

A 70-year-old man with adenocarcinoma of the lung developed pulmonary metastases 7 months after middle and lower lobectomy of the right lung in October 1998. He received four courses of first-line chemotherapy with docetaxel/irinotecan from June to September 1999. The best response was stable disease and, after 6 months of treatment, there was evidence of progressive disease with increase in size and number of pulmonary metastases. Therefore, we recommended enrollment in a phase I study of gefitinib ('Iressa') [1], an orally active epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor.

The patient began to take gefitinib 700 mg/day in March 2000. Remarkable tumor regression was immediately achieved in April 2000 (Figure 1). This response lasted for 18 months. However, pulmonary metastases again developed (considered to be progressive disease), and gefitinib was discontinued in October 2001. The patient received a combination of nedaplatin, a second-generation platinum complex with high antitumor activity against non-small-cell lung cancer [2], and gemcitabine in November 2001. Significant tumor regression was achieved, and a total of six courses from November to April 2002 were administered. Pulmonary metastases progressed again and pulmonary effusion developed in August 2002. Although progressed, he had few symptoms, and was considered to have a performance status of 0. We planned to use a chemotherapy regimen that had not previously been used for this patient, but instead commenced re-treatment with gefitinib at the patient's request on September 3, 2002 (gefitinib 250 mg/day had by this time been approved for use in Japan). One month later, a significant response had been achieved (Figure 1).

This is an interesting case in which acquired resistance to gefitinib could be overcome. There are some possible explanations. First, resistance to gefitinib might naturally change over time, but there is no report of this so far. Secondly, because platinum-based cytotoxic chemotherapy was administered after the first treatment with gefitinib, the proportion of sensitive or resistant cells might have been modified. Thirdly, treatment with cytotoxic chemotherapy might produce genetic changes in EGFR or other unknown associated genes that regulate resistance to gefitinib. Saltz et al. reported that a combination of the EGFR inhibitor cetuximab (C225) and irinotecan produced a 22.5% partial response in patients with irinotecan-refractory colorectal cancer with high EGFR expression [3]. In contrast to that report, cytotoxic agents have the possibility of modifying resistance to cytostatic agents. Recently, two large phase III studies to compare concurrent use of conventional platinum-based chemotherapy (carboplatin/paclitaxel or cisplatin/gemcitabine) and gefitinib with conventional chemotherapy alone were reported [4, 5]. No differences in overall survival were found. These results suggested that gefitinib and chemotherapy may be targeting the same cells with the possibility of overlapping activity. If cytotoxic agents altered sensitivity to gefitinib by genetic modification, chemotherapy followed by gefitinib might be superior to concurrent use. Gefitinib is a very promising agent, but little knowledge is available concerning the types of cases for which gefitinib should be administered, or how gefitinib should be combined with conventional cytotoxic agents. Further investigations are needed to answer these questions.

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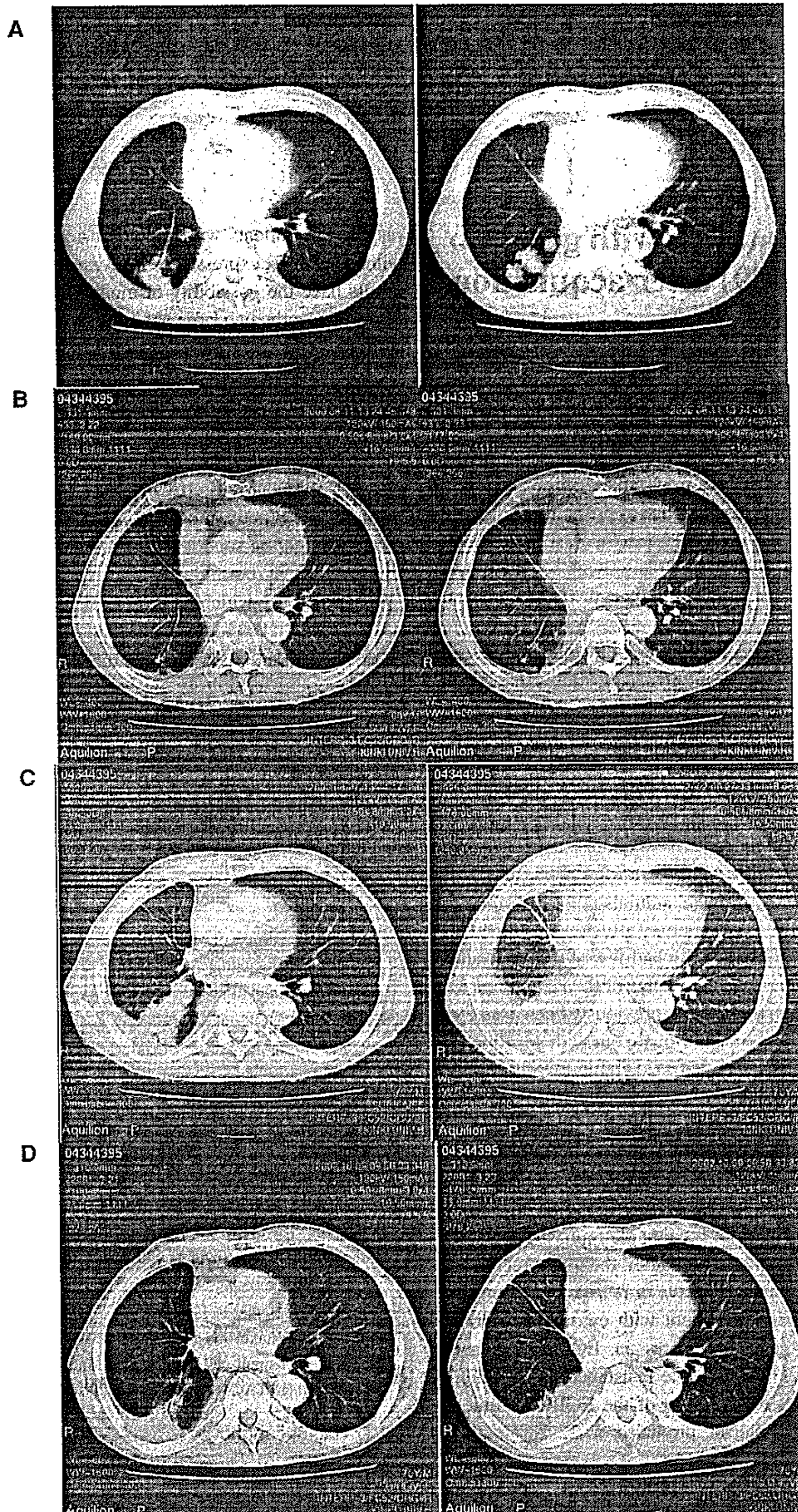

Figure 1. A 70-year-old man with adenocarcinoma of the lung. CT scan before treatment of gefitinib (**A**), after initiation of treatment (**B**), before re-treatment (**C**) and after initiation of re-treatment (**D**).

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Clinical

Randomised phase II study of docetaxel/cisplatin *vs* docetaxel/irinotecan in advanced non-small-cell lung cancer: a West Japan Thoracic Oncology Group Study (WJTOG9803)

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Docetaxel plus cisplatin and docetaxel plus irinotecan are active and well-tolerated chemotherapy regimens for advanced non-small-cell lung cancer (NSCLC). A randomised phase II study compared their efficacy and toxicity in 108 patients with stage IIIb/IV NSCLC, who were randomised to receive docetaxel 60 mg m^{-2} and cisplatin 80 mg m^{-2} on day 1 (DC; $n = 51$), or docetaxel 60 mg m^{-2} on day 8 and irinotecan 60 mg m^{-2} on day 1 and 8 (DI; $n = 57$) every 3 weeks. Response rates were 37% for DC and 32% for DI patients. Median survival times and 1- and 2-year survival rates were 50 weeks (95% confidence interval: 34–78 weeks), 47 and 25% for DC, and 46 weeks (95% confidence interval: 37–54 weeks), 40 and 18% for DI, respectively. The progression-free survival time was 20 weeks (95% confidence interval: 14–25 weeks) with DC and 18 (95% confidence interval: 12–22 weeks) with DI. Significantly more DI than DC patients had grade 4 leucopenia and neutropenia ($P < 0.01$); more DC patients had grade $\geqslant 2$ thrombocytopenia ($P < 0.01$). Nausea and vomiting was more pronounced with DC ($P < 0.01$); diarrhoea was more common with DI ($P = 0.01$). Three treatment-related deaths occurred in DC patients. In conclusion, although the DI and DC regimens had different toxicity profiles, there was no significant difference in survival.
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Unfortunately, non-small-cell lung cancer (NSCLC) is a member of the group of neoplastic diseases that is relatively chemoresistant. Recent meta-analyses show that cisplatin-based chemotherapy improves survival (Non-Small Cell Lung Cancer Collaborative Group, 1995), and it is considered a standard treatment for NSCLC, Most cisplatin-based regimens have substantial toxicities that require close monitoring and supportive care. Thus, there is a need to develop active and less toxic chemotherapy regimens that include new active compounds with novel mechanisms of action.

In the 1990s, several new, active therapies with single-agent response rates of 15–30% became available for NSCLC, including irinotecan, docetaxel, paclitaxel, vinorelbine, and gemcitabine. Because irinotecan and docetaxel were approved for NSCLC earlier than the other drugs in Japan, development of regimens containing irinotecan or docetaxel is more advanced. Docetaxel 60 mg m^{-2} showed good antitumour activity against advanced NSCLC (Kunitoh *et al*, 1996), and the combination of docetaxel plus cisplatin (DC) is one of the most effective regimens for advanced NSCLC (Rodriguez *et al*, 2001; Schiller *et al*, 2002). Studies in Japan included a phase II study in which DC yielded a response rate of 42% (Okamoto *et al*, 2002), and a phase III study in which DC was associated with better survival than the vindesine and cisplatin (VC) combination (Kubota *et al*, 2002).

Irinotecan demonstrated activity similar to that of VC in stage IIIb/IV NSCLC (Negoro *et al*, 2003), and significant longer overall survival time than VC in stage IV NSCLC (Fukuoka *et al*, 2000). We reported a phase I study of docetaxel plus irinotecan (DI) in patients with advanced NSCLC, in which a promising response rate of 48% and the median survival time of 48 weeks were achieved with acceptable toxicities (Masuda *et al*, 2000). Thus, DI appeared to be a promising non-cisplatin-containing regimen.

Based on the above findings, we conducted a randomised trial of DC *vs* DI in patients with advanced NSCLC to compare the respective response rates, survival data, and toxicity profiles of the two regimens. This was a multicentred phase II study.

PATIENTS AND METHODS

Patients

Patients enrolled in this trial had histologically or cytologically confirmed stage IIIb or IV NSCLC. Patients with stage IIIb disease who were not candidates for thoracic radiation and patients with stage IV disease were eligible if they had not received previous therapy, had measurable disease, and had a life expectancy of at least 3 months. Additional entry criteria were age $\geqslant 20$ years, performance status of 0 or 1 on the Eastern Cooperative Oncology Group (ECOG) scale, adequate bone marrow function (leucocyte

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