tate aminotransferase and alanine aminotransferase less than twice the upper limit of the normal range, serum creatinine ≤1.5 mg/dL, and creatinine clearance more than 50 mL/minute. None of the patients had received prior chemotherapy for the primary lesion. Written informed consent for chemotherapy and a genetic analysis of tumor tissue was obtained in every case.

Chemotherapy

All patients with nonprogressive cancer were treated with 2 or more courses of chemotherapy. Response criteria were evaluated according to the World Health Organization criteria.[4] Toxicities were evaluated according to the NCI-CTC version 2 criteria.[5]

Tumor Samples

Transbronchial biopsy specimens of tumors were obtained before chemotherapy. Half the specimens were fixed in formalin for pathologic diagnosis and the other half were immediately frozen for storage at −80°C until genetic analysis.

Extraction and Purification of RNA and Preparation of Probes

The total RNA of each sample was isolated and treated with DNase I to avoid contamination of genomic DNA by silica membrane affinity chromatography using Macherey–Nagel's total RNA isolation kit (MACHEREY–NAGEL GmbH & Co. KG, Germany). One hundred nanograms of the total RNA for each sample was reverse transcribed into cDNA and amplified by SMART polymerase chain reaction (PCR) technology[6] using the Super SMART PCR cDNA Synthesis kit (BD Biosciences Clontech, CA) according to the manufacturer's instructions. Each cDNA sample was subjected to microarray expression profiling using the BD Atlas Human Cancer 1.2 Array (Clontech) based on the manufacturer's protocol described previously.[2,3]

cDNA Microarray

Each labeled probe was then hybridized into a separate Atlas Array. The signal intensity for each spot, which corresponds to each gene examined, was determined using a STORM image analyzer (Amersham Bioscience, Picataway, NJ). The hybridization pattern and signal intensity were analyzed to determine changes in gene expression levels using AtlasImage 2.01 software (CLONTECH, Laboratory, Inc., Japan).

Statistical Methods

t-tests were used to identify differences in mean expression levels among benefits and toxicities of chemotherapy. We compared the differences of gene expression between grade 3 or grade 4 (worst grade) and others for hematologic toxicities, and between grade 0 and others for nonhematologic toxicities. To determine whether gene expression profiles were associated with variety in cases of survival, Kaplan–Meier survival plots and log-rank tests were used. The influence of each gene expression on each outcome of chemotherapy was examined in stepwise multivariate regression analysis. $P < 0.01$ was considered significant.

RESULTS

Between September 2000 and December 2001, 47 patients were registered in the study (Table 1). Thirty-six patients were men and 11 were women, with a median age of 66 years (range, 35–81 years). Eighteen patients had small cell lung cancer (SCLC), and the rest had nonsmall cell lung cancer (NSCLC). Of the patients with SCLC, 2 had limited disease and the other 16 had extensive disease. Of the patients with NSCLC, 12 had locally advanced disease and 17 had metastatic disease. No patients had received prior chemotherapy. All the patients, except for 3 who had been prescribed paclitaxel and irinotecan, were given full-dose platinum-based chemotherapy. Sixteen of the 18 patients with SCLC (89%) and 12 of the 29 patients with NSCLC (41%) responded to chemotherapy.

The expression levels of 1176 genes in the tumor specimens were analyzed using cDNA microarray screening. Four housekeeping genes that were expressed in all 47 tumor

TABLE 1. Patient Characteristics

	No. of Patients
Total	47
Gender	
Male	36
Female	11
Smoker	38
PS(ECOG)	
0	5
1	30
2	9
3	3
Pathology	
SCLC	
Stage	
LD	2
ED	16
NSCLC	
Stage	
IIB/IIIA	4
IIIB	8
IV	17

PS, performance status; ECOG, Eastern Cooperative Oncology Group; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; LD, limited disease; ED, extensive disease.

samples were used as controls for gene expression: ubiquitin, liver glyceraldehyde 3-phosphate dehydrogenase, 23-kDa highly basic protein, 60S ribosomal protein L13A, and 40S ribosomal protein S9.

When we analyzed the relationship between gene expression and chemotherapy-related hematologic toxicity, 2 and 22 genes were identified as showing significantly higher expression in patients with grade 4 neutropenia and grade 3 anemia in comparison with grade 0 to grade 3 neutropenia and grade 0 to grade 2 anemia respectively. We also identified 17, 19, 4, and 1 genes that showed significantly higher expression in patients who experienced diarrhea, infection, increased serum creatinine, and pneumonitis respectively than in patients who did not (grade 0). Stepwise multivariate regression analysis revealed that 1, 3, 3, 1, and 1 genes were independent factors, each of which was correlated with toxicities such as neutropenia, anemia, diarrhea, infection, and increased serum creatinine respectively (Table 2, $P < 0.01$). We were unable to identify any genes that were correlated with thrombocytopenia, emesis, increased total bilirubin, and increased GPT.

As previously presented, stepwise multivariate regression analysis revealed that 3 genes—allograft inflammatory factor 1, HLA-DR antigen-associated invariant subunit, and MHC class HLA-DR-β precursor—were factors independently associated with chemoresistance ($P < 0.0001$, Table 3). When we analyzed the relationship between gene expression level and survival, G1/S-specific cyclin, type II cGMP-dependent protein kinase, and hepatocyte growth factor-like protein were significantly correlated (log-rank test, $P < 0.01$, Table 3). Thus, not only chemotherapeutic benefits but also some toxicities were predicted by cDNA microarray using tumor specimens obtained before chemotherapy.

DISCUSSION

We examined the expression of cancer-related genes in samples of lung cancer obtained before chemotherapy using cDNA microarray screening, and analyzed the relationship between gene expression levels and clinical outcome after chemotherapy. We previously reported 3 genes with expression levels that were each correlated with the tumor response to chemotherapy[2] or patient survival.[3] One surprising finding was that chemoresistant genes related to host immunity were different from survival-related genes. This is because patient survival is influenced by not only the effect of chemotherapy on the tumor but also by tumor growth and metastasis.

The current study revealed some specific genes with expression levels that were correlated with chemotherapy-related toxicity. Cytohesin-1 was identified as a genetic factor that predicted neutropenia resulting from chemotherapy. This is a guanine nucleotide exchange factor that regulates members of the ADP–ribosylation factor family of small GTPases. An analysis of granulocytic maturation of HL-60 cells has revealed a marked increase in the level of cytohesin-1 expression during dibutyryl–cyclic AMP-induced granulocyte differentiation.[7] These data suggest that cytohesin-1 may be useful as a potential marker of granulocytic differentiation.

Three genes—MAD3, DNAX activation protein 12, and interleukin-1β precursor—were identified as predictors of anemia induced by chemotherapy. MAD3 is one of the

TABLE 2. Genes Closely Associated With Chemotherapeutic Toxicities

Factor	Description	Gene Expression (mean ± SD)		Coefficient	SE	*P*
Neutrophil		grade 0–3 (n = 35)	grade 4 (n = 12)			0.0056
	Cytohesin-1	1.8 ± 3.5	6.6 ± 7.9	0.033	0.011	
Hemoglobin		grade 0–2 (n = 43)	grade 3 (n = 4)			<0.0001
	Major histocompatibility complex enhancer-binding protein MAD3	7.0 ± 9.4	43.5 ± 83.7	−0.009	0.004	
	DNAX activation protein 12	10.2 ± 13.1	53.8 ± 63.7	0.005	0.002	
	Interleukin-1 beta precursor	13.1 ± 13.5	529.3 ± 1034.6	0.001	0.0003	
Infection		grade 0 (n = 43)	grade 1–3 (n = 4)			0.0003
	Hemoglobin alpha subunit	7.7 ± 8.8	44.3 ± 61.0	0.007	0.002	
Creatinine		grade 0 (n = 41)	grade 1–2 (n = 6)			0.0021
	Matrix metalloproteinase 10	12.3 ± 19.1	62.2 ± 64.3	0.005	0.001	
Diarrhea		grade 0 (n = 35)	grade 1–3 (n = 12)			0.0002
	ICH-2 protease	16.1 ± 17.5	42.4 ± 36.8	0.008	0.073	
	Interferon-inducible RNA-dependent protein kinase	4.3 ± 6.8	12.9 ± 14.0	−0.028	0.013	
	Collagen 16 alpha 1 subunit precursor	2.8 ± 4.5	15.9 ± 20.0	0.031	0.01	

TABLE 3. Genes Closely Associated With Chemotherapeutic Benefits

Factor	Description	Coefficient	SE	P
Survival	G1/S-specific cyclin D2			0.0055
	Type II cGMP-dependent protein kinase			0.0016
	Hepatocyte growth factor-like protein			0.0075
Tumor effect on chemotherapy	Allograft inflammatory factor 1			<0.0001
	HLA-DR antigen-associated invariant subunit	−0.014	0.002	
	MHC class II HLA-DR-beta precursor	−0.001	0.0003	
		−0.01	0.002	

metaphase checkpoint proteins involved in cell division, and interleukin-1 is one of the monokines that can elicit many of the defective host responses to infection. DNAX activation protein 12 is a membrane adaptor molecule that contains an immunoreceptor tyrosine-based activation motif, which activates calcium signaling in immune cells. However, the mechanisms by which these 3 genes influence the incidence of chemotherapy-related anemia remain unclear.

ICH-2, found to be a predictor of diarrhea, is a novel human gene encoding a member of the interleukin-1β converting enzyme cysteine protease family. ICH-2 mRNA is widely expressed in human tissue and appears to play a primary role in apoptosis.[8] Another predictor of diarrhea, protein kinase regulated by RNA, plays an important role in many cellular processes, including virus multiplication and cell growth, differentiation, and apoptosis.[9] It is also still unclear how these genes, including collagen 16, participate in susceptibility to chemotherapy-related diarrhea.

Although this study revealed a number of genes related to the beneficial and toxic effects of chemotherapy, their mechanisms of action remain to be explained. This may be because we used mononuclear cells from peripheral blood of healthy volunteers as a control for gene expression. A major objective of this study was to clarify predictors of not only beneficial but also toxic effects of cancer chemotherapy. The genetic characteristics of various tissues are believed to differ from one another. Therefore cancer cells need to be examined to clarify the factors related to tumor susceptibility to chemotherapy, and blood cells need to be examined for susceptibility to hematologic toxicities. Malignant tumor tissues are heterogeneous and contain a number of cell types, and specimens of lung cancer obtained by transbronchial biopsy are not considered to reflect the general characteristics of tumor tissue. The fact that genetic information on tumor cells can predict not only tumor susceptibility to chemotherapy but also toxicity suggests that certain genetic characteristics may be common to all somatic cells, irrespective of whether they are malignant or normal. If this hypothesis is correct, then nonmalignant normal cells may also be used for analysis of informative genetic factors that can predict the antitumor effects and toxicities of chemotherapy.

We need to undertake prospective evaluations to determine whether the genes revealed in this study are truly important and potentially useful for predicting the beneficial or toxic effects of chemotherapy. Accumulation of such data could eventually allow chemotherapy to become "personalized" using anticancer drugs that would be effective and nontoxic in individual patients.

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Phase I study of cisplatin, vinorelbine, and concurrent thoracic radiotherapy for unresectable stage III non-small cell lung cancer

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To determine the recommended phase II dose of vinorelbine in combination with cisplatin and thoracic radiotherapy (TRT) in patients with unresectable stage III non-small cell lung cancer (NSCLC), 18 patients received cisplatin (80 mg/m^2) on day 1 and vinorelbine (20 mg/m^2 in level 1, and 25 mg/m^2 in level 2) on days 1 and 8 every 4 weeks for 4 cycles. TRT consisted of a single dose of 2 Gy once daily for 3 weeks followed by a rest of 4 days, and then the same TRT for 3 weeks to a total dose of 60 Gy. Fifteen (83%) patients received 60 Gy of TRT and 14 (78%) patients received 4 cycles of chemotherapy. Ten (77%) of 13 patients at level 1 and all 5 patients at level 2 developed grade 3–4 neutropenia. Four (31%) patients at level 1 and 3 (60%) patients at level 2 developed grade 3–4 infection. None developed ≥grade 3 esophagitis or lung toxicity. Dose-limiting toxicity was noted in 33% of the patients in level 1 and in 60% of the patients in level 2. The overall response rate (95% confidence interval) was 83% (59–96%) with 15 partial responses. The median survival time was 30.4 months, and the 1-year, 2-year, and 3-year survival rates were 72%, 61%, and 50%, respectively. In conclusion, the recommended dose is the level 1 dose, and this regimen is feasible and promising in patients with stage III NSCLC. (Cancer Sci 2004; 95: 691–695)

Stage III locally advanced non-small cell lung cancer (NSCLC) accounts for about 25% of all lung cancer cases.[1)] Successful treatment of this disease rests on the control of both clinically apparent intrathoracic disease and occult systemic micrometastases, and therefore a combination of systemic chemotherapy and thoracic radiotherapy is indicated in many patients with good performance status and no pleural effusion.[2)] Concurrent chemoradiotherapy is superior to the sequential approach, as shown by recent phase III trials in unresectable stage III NSCLC, in which the median survival time was 15.0 to 17.0 months in the concurrent arm and 13.3 to 14.6 months in the sequential arm, although acute esophagitis was more severe in the concurrent arm.[3–5)] Chemotherapy regimens combined with simultaneous thoracic radiotherapy have consisted of cisplatin plus etoposide and cisplatin plus vinca alkaloids,[3,4)] and a combination of cisplatin plus vindesine, with or without mitomycin, has been widely used in Japan.[5–8)]

Vinorelbine, a new semisynthetic vinca alkaloid with a substitution in the catharanthine ring, interacts with tubulin and microtubule-associated proteins in a manner different from the older vinca alkaloids, and it more selectively depolymerizes microtubules in mitotic spindles.[9)] Several randomized trials have shown vinorelbine to be more active against advanced or metastatic NSCLC than vindesine as a single agent or in combination with cisplatin.[10–13)] Thus, incorporation of vinorelbine into concurrent chemoradiotherapy instead of vindesine is an important strategy for the treatment of locally advanced NSCLC. The objective of this study was to determine the maximum tolerated dose (MTD) and recommended dose of vinorelbine for phase II studies in combination with cisplatin, with or without mitomycin, and thoracic radiotherapy for patients with unresectable stage III NSCLC. We planned to start with the cisplatin and vinorelbine combination and then add mitomycin.

Patients and Methods

Patient selection. The eligibility criteria were: histologically or cytologically proven NSCLC; unresectable stage IIIA or IIIB disease; no previous treatment; measurable disease; tumor within an estimated irradiation field no larger than half the hemithorax; age between 20 years and 74 years; Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1[14)]; adequate bone marrow function (12.0×10^9/liter ≥white blood cell [WBC] count ≥4.0×10^9/liter, neutrophil count ≥2.0×10^9/liter, hemoglobin ≥10.0 g/dl, and platelet count ≥100×10^9/liter), liver function (total bilirubin ≤1.5 mg/dl and transaminase ≤twice the upper limit of the normal value), and renal function (serum creatinine ≤1.5 mg/dl and creatinine clearance ≥60 ml/min); and a PaO_2 of 70 Torr or more. Patients were excluded if they had malignant pleural or pericardial effusion, active double cancer, a concomitant serious illness, such as uncontrolled angina pectoris, myocardial infarction in the previous 3 months, heart failure, uncontrolled diabetes mellitus, uncontrolled hypertension, interstitial pneumonia or lung fibrosis identified by a chest X-ray, chronic obstructive lung disease, infection or other diseases contraindicating chemotherapy or radiotherapy, pregnancy, or breast-feeding. All patients gave their written informed consent.

Pretreatment evaluation. The pretreatment assessment included a complete blood cell count and differential count, routine chemistry determinations, creatinine clearance, blood gas analysis, electrocardiogram, lung function testing, chest X-rays, chest computed tomographic (CT) scan, brain CT scan or magnetic resonance imaging, abdominal CT scan or ultrasonography, and radionuclide bone scan.

Treatment schedule. The dose levels and doses of each anticancer agent are shown in Table 1. Cisplatin and vinorelbine were administered at dose levels 1 and 2. It was planned to give cisplatin, vinorelbine, and mitomycin at dose levels 3–5, but because the MTD was determined to be dose level 2, dose levels 3–5 were not used. Cisplatin was administered on day 1 by intravenous infusion over 60 min together with 2500 to 3000 ml of fluid for hydration. Vinorelbine diluted in 40 ml of normal saline was administered by bolus intravenous injection on days 1 and 8. All patients received prophylactic antiemetic ther-

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apy consisting of a 5HT3-antagonist and a steroid. This chemotherapy regimen was repeated every 4 weeks for 4 cycles.

Thoracic radiotherapy with photon beams from a liniac or microtron accelerator with energy between 6 and 10 MV at a single dose of 2 Gy once daily given 15 times over 3 weeks was begun on day 2 of the first cycle of cisplatin and vinorelbine chemotherapy, and followed by a short rest period of 4 days. The same radiotherapy was begun on day 1 of the second cycle of chemotherapy to a total dose of 60 Gy. The clinical target volume (CTV) was based on conventional chest X-ray and CT scans, and included the primary lesion (CTV1), involved lymph nodes whose short diameter was 1 cm or larger (CTV2), and the ipsilateral pulmonary hilum and bilateral mediastinum area (CTV3). Anterior and posterior parallel opposed fields encompassed the initial planned target volume (PTV), consisting of CTV1–3 with the superior and inferior field margins extended to 1 to 2 cm and the lateral field margins extended to 0.5 cm for respiratory variation and fixation error. The boost PTV included only CTV1–2 based on the second CT scans with the same margins. The spinal cord dose was limited to 40 Gy by using oblique parallel opposed fields.

Toxicity assessment and treatment modification. Complete blood cell counts and differential counts, routine chemistry determinations, and a chest X-ray were performed once a week during the course of treatment. Acute toxicity was graded according to the NCI Common Toxicity Criteria version 2.0 issued in 1998, and late toxicity associated with thoracic radiotherapy was graded according to the RTOG Late Radiation Morbidity Scoring Schema.[15] Vinorelbine administration on day 8 was omitted if any of the following toxicities was noted: WBC count $<3.0\times10^9$/liter, neutrophil count $<1.5\times10^9$/liter, platelet count $<100\times10^9$/liter, elevated hepatic transaminase level or total serum bilirubin ≥grade 2, fever ≥38°C, or performance status ≥2. Subsequent cycles of chemotherapy were delayed if any of the following toxicities was noted on day 1: WBC count $<3.0\times10^9$/liter, neutrophil count $<1.5\times10^9$/liter, platelet count $<100\times10^9$/liter, serum creatinine level ≥1.6 mg/dl, elevated hepatic transaminase level or total serum bilirubin ≥grade 2, fever ≥38°C, or performance status ≥2. The doses of cisplatin and vinorelbine were reduced by 25% in all subsequent cycles if any of the following toxicities was noted: WBC count $<1.0\times10^9$/liter, platelet count $<20\times10^9$/liter, or grade 3 or severer non-hematological toxicity, except for nausea and vomiting. The dose of cisplatin was reduced by 25% in all subsequent cycles if the serum creatinine level was elevated to 2.0 mg/dl or higher. Thoracic radiotherapy was suspended if any of the following toxicities was noted: WBC count $<1.0\times10^9$/liter, platelet count $<20\times10^9$/liter, esophagitis ≥grade 3, fever ≥38°C, performance status ≥3, or PaO_2 <70 Torr. Thoracic radiotherapy was terminated if this toxicity persisted for more than 2 weeks. Granulocyte colony-stimulating factor support was used if the neutrophil count was $<0.5\times10^9$/liter for more than 4 days, the WBC count was $<1.0\times10^9$/liter, or febrile neutropenia ≥grade 3 was noted.

Dose-limiting toxicity, MTD, and recommended dose for phase II studies. The dose-limiting toxicity (DLT) was defined as a neutrophil count $<0.5\times10^9$/liter lasting 4 days or longer, febrile neutropenia ≥grade 3, platelet count $<20\times10^9$/liter, grade 3 or more severe non-hematological toxicity other than nausea and vomiting, and patient's refusal to receive subsequent treatment. Doses were escalated according to the frequency of DLT evaluated during the first and second cycles of chemotherapy and thoracic radiation. Six patients were initially enrolled at each dose level. If one or none of them experienced DLT, the next cohort of patients was treated at the next higher dose level. If 2 of the 6 patients experienced DLT, then 6 additional patients were enrolled at the same dose level to make a total of 12 patients. If 4 or fewer patients experienced DLT, the next cohort of patients was treated at the next higher dose level. If 5 or more of the 12 patients experienced DLT, that level was considered to be the MTD. If 3 of the initial 6 patients experienced DLT, that level was considered to be the MTD. The recommended dose for phase II trials was defined as the dose preceding the MTD.

Response evaluation. Objective tumor response was evaluated according to the WHO criteria issued in 1979.[16] A complete response (CR) was defined as the disappearance of all known disease for at least 4 weeks with no new lesions appearing. A partial response (PR) was defined as an at least 50% decrease in total tumor size for at least 4 weeks without the appearance of new lesions. No change (NC) was defined as the absence of a partial or complete response with no progressive or new lesions observed for at least 4 weeks. Progressive disease was defined as a 25% or greater increase in the size of any measurable lesion or the appearance of new lesions.

Study design, data management, and statistical considerations. This study was designed as a phase I study at two institutions, the National Cancer Center Hospital and Kanagawa Cancer Center. The protocol and consent form were approved by the Institutional Review Board of each institution. Registration was conducted at the Registration Center. Data management, periodic monitoring, and the final analysis were performed by the Study Coordinator. A patient accrual period of 24 months and a follow-up period of 18 months were planned. Overall survival time and progression-free survival time were estimated by the Kaplan-Meier method.[17] Survival time was measured from the date of registration to the date of death due to any cause. Progression-free survival time was measured from the date of registration to the date of disease progression or death. Patients who were lost to follow-up without event were censored at the date of their last known follow-up.

Table 1. Dose level and the dose of each anticancer agent

Dose level	Cisplatin (mg/m²)	Vinorelbine (mg/m²)	Mitomycin (mg/m²)
−1	80	15	—
1	80	20	—
2	80	25	—
3	80	15	8
4	80	20	8
5	80	25	8

Results

Registration and characteristics of the patients. From October 1999 to August 2000, 13 patients were registered at dose level 1 and 5 patients at dose level 2. The detailed demographic characteristics of the patients are listed in Table 2. All patients had unresectable IIIA-N2 or IIIB disease. One of the 6 patients enrolled at dose level 1 developed bacterial meningitis during the second cycle of chemotherapy, and that case is described in detail elsewhere.[18] We did not include it in the assessment of DLT, because the bacterial meningitis was not specifically related to treatment. We registered another patient at the same dose level, and 2 cases of DLT were noted among the initial 6 patients evaluable for DLT. We added another 6 patients, and DLT was noted in 4 of the 12 patients registered at the dose level 1. Of the 5 patients registered at level 2, 3 patients developed DLT. This dose level was determined to be the MTD, and patient accrual to this study was terminated.

Treatment delivery. Treatment delivery was generally well maintained, and it did not differ between the two dose levels (Table 3). Full dose (60 Gy) thoracic radiotherapy was completed in 77% and 100% of the patients at dose levels 1 and 2,

Table 2. Patients' characteristics

		Median (range)	*N* (%)
Number of patients			18
Gender	male		16 (89)
	female		2 (11)
Age	median (range)	59 (48–69)	
PS	0		4 (22)
	1		14 (78)
Body weight loss	<5%		12 (67)
	5–9%		4 (22)
	≥10%		2 (11)
T-factor	1		1 (6)
	2		6 (33)
	3		7 (39)
	4		4 (22)
N-factor	2		11 (61)
	3		7 (39)
Clinical stage	IIIA		9 (50)
	IIIB		9 (50)
Histology	adenocarcinoma		14 (78)
	squamous cell carcinoma		3 (17)
	adenosquamous carcinoma		1 (6)

Table 3. Treatment delivery

	Dose level 1 (*N*=13)	Dose level 2 (*N*=5)
	N (%)	*N* (%)
Initial irradiation field (cm^2) median (range)	171 (128–529)	182 (128–248)
Total dose of radiotherapy (Gy)		
60	10 (77)	5 (100)
50–59	1 (8)	0
<50	2 (15)	0
Delay of radiotherapy (days)[1)]		
<5	6 (60)	3 (60)
5≤	4 (40)	2 (40)
Number of chemotherapy cycles		
4	10 (77)	4 (80)
3	0	1 (20)
2	2 (15)	0
1	1 (8)	0
Omission of vinorelbine administration on day 8		
0	9 (69)	2 (40)
1	4 (31)	2 (40)
3	0	1 (20)

1) Evaluated in patients who received 60 Gy radiotherapy (*N*=15).

respectively. Delays in radiotherapy evaluated in patients who completed the full course of radiotherapy amounted to less than 5 days in 60% of the patients at both levels. Full cycles (4 cycles) of chemotherapy were administered to 77% and 80% of the patients at dose levels 1 and 2, respectively, but vinorelbine administration on day 8 was more frequently omitted at dose level 2 (Table 3).

Toxicity, MTD, and the recommended dose for phase II trials. Acute severe toxicity was mainly hematological (Table 4). Grade 3–4 leukopenia and neutropenia were noted in 77% and 100% of the patients at dose levels 1 and 2, respectively. Grade 3 anemia was observed in 23% and 20% of the patients at dose levels 1 and 2, respectively, but no blood transfusions were required. Thrombocytopenia was mild. Grade 4 transaminase elevation was observed in 1 patient during the first cycle of chemotherapy, but no subjective manifestations associated with liver dysfunction were noted. Chemotherapy was discontinued and the transaminases quickly decreased to within their normal ranges. Transient asymptomatic grade 3 hyponatremia was noted in 1 patient. Grade 3–4 infection was noted in 7 patients. Bacterial meningitis unassociated with neutropenia developed on day 6 of the second cycle of chemotherapy in 1 patient.[18)] The other grade 3–4 infections were all associated with neutropenia. Esophagitis was mild in this study, and no grade 3–4 esophagitis was noted. No deaths occurred during or within 30 days of therapy.

DLT was noted in 4 of the 12 (33%) evaluable patients at dose level 1, and in 3 of the 5 (60%) at dose level 2. Six of these 7 DLTs were grade 3–4 infection associated with neutropenia, and the other 1 was grade 4 transaminase elevation. Thus, we determined that dose level 2 was the MTD, and dose level 1 was recommended as the dose for phase II trials.

Table 4. Acute toxicity

Toxicity	Dose level 1 (*N*=13), Grade					Dose level 2 (*N*=5), Grade				
	1	2	3	4	3–4 (%)	1	2	3	4	3–4 (%)
Hematological										
Leukopenia	0	2	9	1	(77)	0	0	4	1	(100)
Neutropenia	1	1	7	3	(77)	0	0	1	4	(100)
Anemia	4	6	3	0	(23)	2	2	1	0	(20)
Thrombocytopenia	1	2	0	0	(0)	1	0	0	0	(0)
Non-hematological										
AST	2	0	0	1	(8)	1	0	0	0	(0)
ALT	7	0	0	1	(8)	0	1	0	0	(0)
Total bilirubin	2	1	0	0	(0)	2	0	0	0	(0)
Creatinine	2	2	0	0	(0)	1	0	0	0	(0)
Hyponatremia	6	0	1	0	(8)	1	0	0	0	(0)
Infection	1	3	2	2	(31)	0	0	3	0	(60)
Nausea	4	1	0	0	(0)	3	0	0	0	(0)
Diarrhea	0	1	0	0	(0)	0	0	0	0	(0)
Stomatitis	2	0	0	0	(0)	0	2	0	0	(0)
Esophagitis	6	1	0	0	(0)	4	0	0	0	(0)
Sensory neuropathy	2	0	0	0	(0)	0	0	0	0	(0)

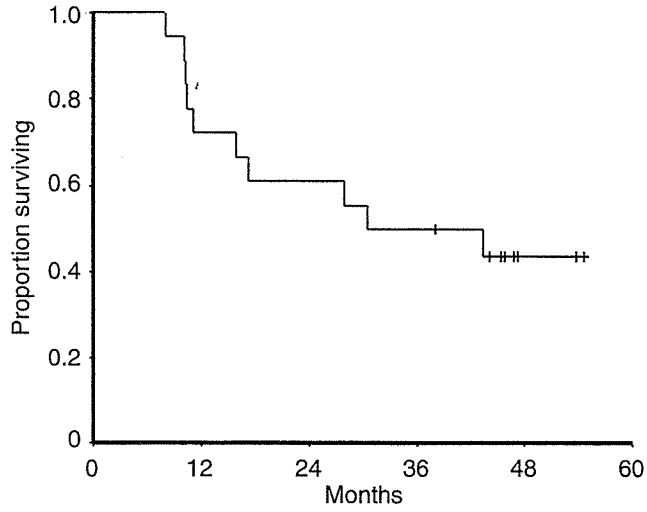

Fig. 1. Overall survival in 18 patients. The median (range) follow-up period of censored cases has been 35.4 (32.0–43.4) months, and the median overall survival time has not yet been reached.

Late lung toxicity associated with thoracic radiotherapy was grade 3 in 1 (6%) patient, grade 2 in 4 (22%) patients, and grade 1 in 8 (44%) patients. No late esophageal toxicity was noted.

Objective responses, relapse pattern, and survival. All patients were included in the analyses of tumor response and survival. No CR, 15 PRs, and 1 NC were noted, and the overall response rate (95% confidence interval) was 83% (59–96%). Relapse was noted in 12 (67%) of 18 patients. Initial relapse sites were locoregional alone in 5 (28%) patients, locoregional and distant in 3 (17%) patients, and distant alone in 4 (22%) patients. Brain metastasis was detected in 5 patients, and the brain was the most frequent site of distant metastasis. The median progression-free survival time was 15.6 months, and the median overall survival time was 30.4 months. The 1-year, 2-year, and 3-year survival rates were 72%, 61%, and 50%, respectively (Fig. 1).

Discussion

The combination of cisplatin, vindesine, and mitomycin with concurrent thoracic radiotherapy has been shown to yield an encouraging survival outcome, a median survival time of 17–19 months, and a 5-year survival rate of 16% in patients with unresectable stage III NSCLC.[5,7,8] A Japanese randomized trial revealed that replacement of vindesine by vinorelbine in combination with cisplatin and mitomycin yielded a promising response rate (57% versus 38%, $P=0.025$) and median survival time (15 months versus 11 months, $P<0.01$) in patients with stage IIIB or IV NSCLC.[13] Thus, the combination of cisplatin, vinorelbine, and mitomycin is a chemotherapy regimen with potential for combination with concurrent thoracic radiotherapy. The present study, however, showed that a DLT developed in 60% of patients who received cisplatin and vinorelbine 25 mg/m^2 days 1 and 8 (level 2), and since the DLTs were associated with myelosuppression, which is the major critical toxicity of mitomycin, we concluded that it would be impossible to incorporate mitomycin into this regimen.

The recommended doses of vinorelbine of 20 mg/m^2 on days 1 and 8 and cisplatin of 80 mg/m^2 on day 1 repeated every 4 weeks in this study are comparable to the doses used in the CALGB (vinorelbine 15 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 on day 1 repeated every 3 weeks),[19,20] and the Czech Lung Cancer Cooperative Group (vinorelbine 12.5 mg/m^2 on days 1, 8, and 15 and cisplatin 80 mg/m^2 on day 1, repeated every 4 weeks),[21] but lower than in a Mexican study (vinorelbine at 25 mg/m^2 on days 1 and 8 and cisplatin 100 mg/m^2 on day 1, repeated every 3 weeks).[22] These recommended doses are also lower than expected when compared with the recommended vinorelbine dose combined with cisplatin for metastatic NSCLC (vinorelbine 30 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 on day 1, repeated every 3 weeks),[23] and when compared with the results of vindesine, cisplatin, and mitomycin combined with thoracic radiotherapy, where the full doses can be administered concurrently.[8] Thus, vinorelbine can be safely administered with cisplatin and concurrent thoracic radiotherapy at a maximum dose of two-thirds the optimal dose without radiotherapy.

The results for response and survival in this study, however, were very encouraging. This may have been attributable to patient selection bias, but the percentage of patients who had stage IIIB disease in this study was similar to the percentage in the CALGB randomized phase II study.[20] In addition, 33% of the patients in this study had ≥5% body weight loss, whereas only 7% of the patients did in that study.[20] The median survival time was 30.4 months and exceeded the results of concurrent

chemoradiotherapy with old drug combinations that yielded a median survival time of 15–19 months.[3–8] Thus, it could be argued that the combination of cisplatin and vinorelbine is more active for locally advanced NSCLC than the older drug combinations, although there have not been any randomized trials comparing this regimen with old drug combinations in combination with thoracic radiotherapy in patients with stage III NSCLC. Our results also seem better than those of other trials using concurrent cisplatin, vinorelbine, and thoracic radiotherapy, in which the median survival time was 13 to 18 months.[20,22] Those trials used induction chemotherapy followed by chemoradiotherapy. Since the response rate to induction chemotherapy is no more than 40%, induction chemotherapy may be disadvantageous. This issue is being evaluated in an on-going CALGB phase III trial.

Severe esophagitis and pneumonitis have been DLTs in many trials of concurrent chemoradiotherapy, but neither was observed in this study. Nevertheless, since the occurrence of these non-hematological toxicities associated with thoracic radiotherapy is sporadic, the sample size in this study may have been too small to detect them. Thus, careful observation for these toxicities is needed in further phase II and phase III trials to definitely establish the safety profile of this regimen.

In conclusion, cisplatin and vinorelbine chemotherapy combined with concurrent full-dose thoracic radiotherapy is feasible, and the recommended dose of vinorelbine for phase II trials is 20 mg/m^2 on days 1 and 8 repeated every 4 weeks. This regimen was highly active in patients with stage III NSCLC.

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Genome-wide cDNA microarray screening to correlate gene expression profile with survival in patients with advanced lung cancer

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Abstract. We conducted a study using cDNA microarray analysis to determine whether expression levels of genes in tumors were correlated with survival after chemotherapy. Between September 2000 and December 2001, 47 patients were registered in the study. Eighteen patients had small cell lung cancer (SCLC), and the others had non-small cell lung cancer (NSCLC). All patients except three received platinum-based chemotherapy. Transbroncheal biopsy specimens of tumors were obtained before chemotherapy. The expression levels of 1176 genes in tumor specimens were analyzed using the Atlas™ Human Cancer 1.2 Array. The expression levels of three genes, G1/S-specific cyclin, type II cGMP-dependent protein kinase and hepatocyte growth factor-like protein, were significantly correlated with survival ($p<0.01$). Ten of the 47 patients who showed an elevated expression level of one or more of the three genes had a significantly increased chance of survival ($p=0.0056$). In conclusion, some survival-related genes were detected in the tumor tissue of lung cancer patients using cDNA microarray analysis. A prospective study is required to confirm whether expression levels of these genes can be used for prognosis.

Introduction

Lung cancer is a leading cause of cancer death and most patients with this disease are candidates for chemotherapy. To improve the prognosis of lung cancer patients, attempts have been made to develop treatment of lung cancer and thereby decrease the mortality from this disease. To develop new therapeutic strategies for lung cancer we require a better understanding of the cell biology of this disease. Although a number of clinicopathological characteristics may affect the prognosis of lung cancer, these characteristics have not yet been defined. Several molecular markers have been evaluated in association with established histological and clinical prognostic parameters of non-small cell lung cancer (NSCLC) (1-5), although the intrinsic nature of gene dysregulation that leads small tumors to metastasize remains unclear. It is suspected that tumor invasion and metastasis involve complex alterations of gene expression that may be selective for specific cancer types.

We identified that survivin and cyclin D1 are indicators of poor prognosis in small adenocarcinoma of the lung (6,7). Moreover, other factors have also been reported to be prognostic factors in resected NSCLC, including cyclin E (1), FHIT (2), VEGF (3), cadherin (4) and RAR-ß (5). These factors have different functions in tumors, such as tumor suppression, angiogenesis, apoptosis, adhesion and cell differentiation. Clarification of the many genetic abnormalities that influence tumor progression in NSCLC is clearly required when considering new therapeutic strategies for resectable NSCLC.

The cDNA microarray method is now widely used to analyze the expression of thousands of genes simultaneously in cancer tissues, and its development has facilitated the analysis of genome-wide expression profiles that can generate a large body of information concerning genetic networks related to pathological conditions. Large-scale gene expression microarray studies of lung cancer have shown that expression patterns of various genes is associated with pathological characteristics (8,9). In other studies, different sets of genes were identified which may act as predictive markers for chemosensitivity to drugs in human cancer cell lines or tumor tissues using cDNA microarray (10-12).

In the present study, we used cDNA microarray screening to examine the expression levels of specific genes in tumor tissue obtained by transbroncheal biopsy, in order to determine any correlations with survival after chemotherapy.

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Key words: microarray, lung cancer

Patients and methods

Patients. This study was approved by the Institutional Review Boards of Kanagawa Cancer Center. The patients with histologically proven lung cancer treated with chemotherapy were entered into the present study. All were eligible for treatment. They had an expected survival of at least six weeks; measurable lesions; Eastern Cooperative Oncology Group performance status (PS) score ≤3; white blood count ≥4000/μl; hemoglobin ≥10 g/dl; platelet count ≥100000/μl; total serum bilirubin <2 mg/dl; aspartate aminotransferase and alanine aminotransferase less than twice the upper limit of the normal range; serum creatinine ≤1.5 mg/dl; and creatinine clearance >50 ml/min. None of the patients had received prior chemotherapy for the primary lesion. Written informed consent for chemotherapy and a genetic analysis of tumor tissue was obtained in every case. All patients with non-progressive cancer were treated with two or more courses of chemotherapy.

Tumor samples. Transbroncheal biopsy specimens of tumors were obtained before chemotherapy. One half of the specimens were fixed in formalin for pathological diagnosis and the other half were immediately frozen for storage at -80°C until genetic analysis.

Extraction and purification of RNA and preparation of probes. The total RNA of each sample was isolated and treated with DNaseI to avoid contamination of genomic DNA by silica membrane affinity chromatography using Macherey-Nagel's total RNA isolation kit (Macherey-Nagel GmbH and Co., KG, Germany). Total RNA (100 nanograms) for each sample was reverse transcribed into cDNA and amplified by SMART polymerase chain reaction (PCR) technology (18) using the Super SMART™ PCR cDNA Synthesis kit (BD Biosciences Clontech, CA, USA) according to the manufacturer's instructions. To represent the expression profile of the initial total RNA material, the optimal conditions for PCR cycling were determined for each sample by testing the amplified cDNA with gel electrophoresis. All samples were amplified for 19 to 23 cycles. Each cDNA sample was subjected to microarray expression profiling using the BD Atlas™ Human Cancer 1.2 Array (Clontech) based on the manufacturer's protocol. The following is a brief overview of the procedures used. A radioactively labeled probe mixture for hybridization with array membranes was synthesized from each cDNA sample using the CDS Primer Mix specific for the Atlas™ Human Cancer 1.2 Array and [α-^{32}P]-dATP.

cDNA microarray. Each labeled probe was hybridized into a separate Atlas Array. After appropriate washing, array membranes were exposed to a phosphor screen and the signal intensity for each spot, which corresponds to each gene examined, was determined using a STORM image analyzer (Amersham Bioscience, Picataway, NJ). The hybridization pattern and signal intensity were analyzed to determine changes in gene expression levels using AtlasImage™ 2.01 software (Clontech, Laboratory, Inc., Japan).

Table I. Patient characteristics.

	No. of patients
Total	47
Gender	
Male	36
Female	11
Smoker	38
PS (ECOG)	
0	5
1	30
2	9
3	3
Pathology	
SCLC	
Stage	
LD	2
ED	16
NSCLC	
Stage	
IIB/IIIA	4
IIIB	8
IV	17

PS, performance status; ECOG, Eastern Cooperative Oncology Group; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; LD, limited disease; ED, extensive disease.

Statistical methods. To determine whether gene-expression profiles were associated with variety in cases of survival, Kaplan-Meier survival plots and log-rank tests were used. $p<0.01$ was considered statistically significant.

Results

Between September 2000 and December 2001, 47 patients were registered in the study. Patient characteristics are summarized in Table I. Thirty-six patients were male and eleven were female, with a median age of 66 years (range 35-81 years). Thirty-eight patients were smokers. The PS was 0 for five patients; 1 for 30 patients; 2 for nine and 3 for three patients. Eighteen patients had small cell lung cancer (SCLC), and the remaining had NSCLC. Of the patients with SCLC, two had limited disease and the other 16 had extensive SCLC. Of the patients with NSCLC, four had stage IIB/IIIA, eight had stage IIIB, and 17 had stage IV. None of the patients had received prior chemotherapy.

All patients except three who had been subscribed paclitaxel and irinotecan were given platinum-based chemotherapy. Three patients with SCLC and seven patients with NSCLC received thoracic radiotherapy concurrently or sequentially with chemotherapy (Table II). Sixteen of the 18 patients with SCLC (89%) and 12 of the 29 patients with NSCLC (41%) responded to chemotherapy, respectively. Eight out of the total 47 patients were alive at analysis.

Table II. Therapeutic regimens.

	No. of patients
SCLC	
Cisplatin + etoposide	6
Cisplatin + etoposide + TRT	2
Cisplatin + irinotecan	4
Cisplatin + irinotecan + etoposide	2
Carboplatin + etoposide	3
Cisplatin + TRT	1
NSCLC	
Cisplatin + gemcitabine	7
Cisplatin + vinorelbine	3
Cisplatin + vinorelbine + TRT	2
Cisplatin + vindesine + TRT	3
Cisplatin + irinotecan	1
Cisplatin +TRT	2
Carboplatin + etoposide	1
Carboplatin + paclitaxel	1
Nedaplatin + irinotecan	6
Paclitaxel + irinotecan	3

SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; TRT, thoracic radiation therapy.

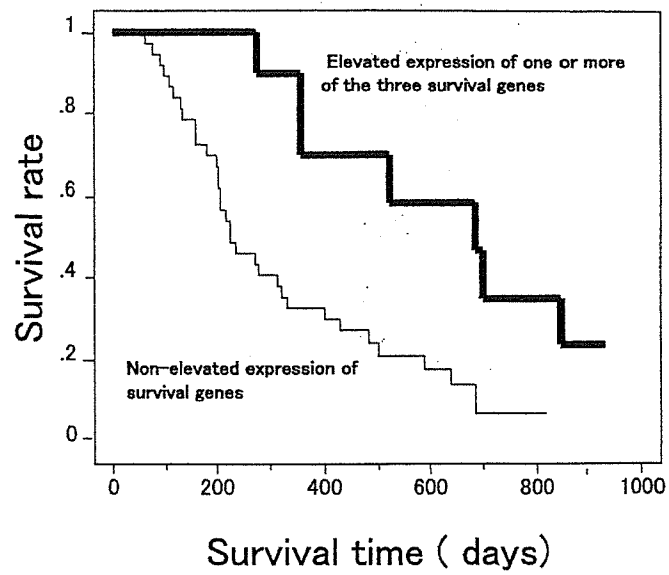

Figure 1. Survival curves constructed using the Kaplan-Meier method. Ten of 47 patients who showed an elevated expression of one or more of the three survival genes compared to the mean expression level of control genes had a significantly better chance of survival (log-rank, p=0.0056).

The expression levels of 1176 genes in the tumor specimens were analyzed using cDNA microarray screening. Four housekeeping genes which were expressed in all 47 tumor samples in the present study were used as controls for gene expression: ubiquitin, liver glyceraldehydes 3-phosphate dehydrogenase, 23-kDa highly basic protein, 60S ribosomal protein L13A and 40S ribosomal protein S9.

When we analyzed the relationship between gene expression level and survival, three genes, G1/S-specific cyclin, type II cGMP-dependent protein kinase and hepatocyte growth factor-like protein, were significantly correlated (Table III, log-rank test, $p<0.01$). Ten of 47 patients who showed an elevated expression of one or more of the three survival genes compared to the mean expression level of control genes had a significantly better chance of survival (Fig. 1, log-rank; p=0.0056).

Discussion

We examined cancer-related gene expressions in lung cancer samples obtained before chemotherapy using cDNA microarray screening, and analyzed the relationship between gene expression levels and survival after chemotherapy. We identified three genes whose expression could be used to predict the survival outcomes of patients in the present study. These genes were involved in cell cycling, adhesion and invasion. The families of G1-cyclins such as cyclins D and E, and their dependent kinases, control the transition through the restriction point of the middle and late G1 cells during cell cycles. A previous examination of gastric cancers revealed that positivity of cyclin D2 and negativity for p27 in the tumor tissue were independent of prognostic factors (13).

For cancer to metastasize, tumor cells present in the circulation must first adhere to the endothelium. An

Table III. Genes closely associated with patient survival.

Description	Symbol	p-value
G1/S-specific cyclin D2 (CCND2) + KIAK0002	M90813 + D13639	0.0055
Type II cGMP-dependent protein kinase	X94612	0.0016
Hepatocyte growth factor-like protein (HGF activator-like protein); hyaluronan-binding protein (PHBP)	D49742, S83182	0.0075

investigation of the mechanism of adhesion and trans-endothelial migration of cancer cells showed that stimulation of cancer cells by CD44 cross-linking or fragmented hyaluronan markedly induces the expression of lymphocyte function-associated antigen (LFA)-1; that stimulation of CD44 also induces expression of the hepatocyte growth factor (HGF) receptor c-Met on cancer cells; and that HGF further amplifies the LFA-1-mediated adhesion of cells (14). Another study demonstrated that HGF/SF-Met binding up-regulated the expression of CD44v6 in murine melanoma cells (15). These data support the hypothesis that HGF influences the outcome of patient survival.

Tumor hypoxia is associated with a poor prognosis for patients with various cancers, often resulting in an increased metastasis. A study demonstrated that culturing tumor cells under hypoxic conditions results in lower cyclic GMP levels. The study revealed that an important mechanism by which hypoxia increases tumor cell invasiveness requires inhibition of the nitric oxide signaling pathway involving protein kinase G activation (16). Moreover, in another study, a potent inhibitor of cyclic GMP-dependent protein kinase displayed cytostatic activity against *Toxoplasma gondii in vitro* (17). These data may support the hypothesis that the three survival genes identified in this study do influence the outcome of patient survival.

In this report we have discussed the mechanisms related to tumor cell survival with regard to three genes implicated in patient survival outcomes. We need to undertake prospective evaluations to determine whether the selected genes in this study are truly important and potentially useful for predicting patient survival. It is also necessary to determine whether administration of drugs will result in changes to the expression levels of the survival genes we identified, and if any such changes are related to survival. If the expression level of a gene changes with treatment, that gene will be the new target of cancer chemotherapy. In this study we measured the expression levels of genes in patients treated with platinum-based chemotherapy. Recently, patients with NSCLC have been treated with non-platinum chemotherapy. It is thus also necessary that the expression levels of our survival genes can be used to predict clinical outcome with non-platinum chemo-therapy. Accumulation of these data could eventually lead to the prescription of 'personalized chemotherapy' with effective anticancer drugs.

Acknowledgements

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SHORT COMMUNICATION

Phase II study of OK-432 intrapleural administration followed by systemic cisplatin and gemcitabine for non-small cell lung cancer with pleuritis carcinomatosa*

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We conducted a phase II study of OK-432 intrapleural administration followed by systemic chemotherapy using cisplatin with gemcitabine to determine their combined effects on non-small cell lung cancer (NSCLC) with pleuritis carcinomatosa. Between December 1999 and October 2001, 15 patients were registered in the study. Fourteen patients had an Eastern Cooperative Oncology Group performance status (PS) of 1, and one patient had a PS of 2. Ten patients had adenocarcinoma, one had squamous cell carcinoma, and four had malignant mesothelioma. Patients underwent thoracocentesis and received an OK-432 intrapleural injection. They were then treated every three weeks with chemotherapy consisting of 80 mg/m2 cisplatin on day 1 and 1000 mg/m2 gemcitabine on days 1 and 8. Thirteen patients received two or more courses of chemotherapy. Grade 3 or 4 neutropenia, anemia and thrombocytopenia occurred in five, two and three patients, respectively. Non-hematological toxicities were mild, except for one patient who experienced a grade 3 elevation of transaminase and two patients who experienced grade 3 nausea. Of the 15 patients, one achieved partial response (PR), 13 a stable disease (SD) rating, and one a progressive disease (PD) rating, and the overall response rate was 6.7%. The median survival time was 13.5 months and the one-year survival rate was 60.0%. In conclusion, OK-432 intrapleural administration followed by cisplatin and gemcitabine systemic chemotherapy did not reduce patients' tumors but did prolong their survival time. A large-scale phase II study of the efficacy of this combination therapy is required.

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INTRODUCTION

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death in Japan. To improve the prognosis of lung cancer patients, attempts have been made to develop tests that will facilitate the early diagnosis and treatment of lung cancer and thereby decrease the mortality from this disease. Pleuritis carcinomatosa is one type of advanced stage NSCLC, and shows poor prognosis due to micrometastatic lesions and respiratory failure by massive pleural effusion. Standard therapy for NSCLC with pleuritis carcinomatosa consists of drainage of pleural effusion followed by intrapleural administration of sclerosing agents. Until recently, there has been controversy regarding which agent was most effective for treatment of sclerosing pleural lesions. A randomized phase II study has been conducted to compare three regimens for intrapleural treatment in patients with pleuritis

carcinomatosa of NSCLC (1). The study suggested that intrapleural OK-432 administration was more effective for the management of malignant effusion compared to intrapleural administration of bleomycin or cisplatin plus etoposide.

Systemic chemotherapy is usually performed after sclerosing modality treatment for patients. In the past decade, a number of new anti-cancer agents have been approved for the treatment of advanced NSCLC, including vinorelbine, gemcitabine, docetaxel and paclitaxel. Regimens based on the combination of these drugs with platinum compounds have presented interesting new possibilities for treatment of patients with NSCLC. Randomized studies comparing these platinum-based combinations with single-agent treatment have demonstrated a small but significant survival benefit with the combination treatments (2, 3). The treatment for NSCLC with pleuritis carcinomatosa, usually performed in accordance with the chemotherapy regime for metastatic NSCLC, is controversial. A phase II study of cisplatin and gemcitabine combination chemotherapy, one of the standard therapies for metastatic NSCLC, has been conducted to determine its effects on malignant mesothelioma (4). The study reported 10 responders out of the 21 patients treated, and a median survival time of 41 weeks, suggesting an efficacy of cisplatin and gemcitabine for treating malignant pleural lesions of NSCLC.

With reference to these data, we conducted a phase II study to determine the efficacy of intrapleural administration of OK-432 followed by cisplatin and gemcitabine systemic chemotherapy for the treatment of NSCLC with pleuritis carcinomatosa. For this study, we used a gemcitabine and cisplatin regimen with a 21-day schedule. In previous phase II studies, based on a 28-day cycle, gemcitabine was given at a dose of 1000 mg/m^2 on days 1, 8 and 15 (5, 6). However, the number of omissions and reductions of the day-15 gemcitabine dose was quite high. As a previous study has shown that cisplatin and gemcitabine treatment on a 21-day cycle has a high-dose intensity with high activity (7), we chose a 21-day cycle of this combination chemotherapy for the present study. This study allowed the entry of patients with malignant mesothelioma.

PATIENTS AND METHODS

Patients

Patients with histologically or cytologically diagnosed NSCLC with pleuritis carcinomatosa or malignant mesothelioma were registered for intrapleural therapy using OK-432 followed by cisplatin and gemcitabine systemic chemotherapy. The eligibility criteria were: expected survival time ≥6 weeks, age ≦ 75 years, Eastern Cooperative Oncology Group performance status (PS) score ≤1, leukocyte count ≥4,000/μl, hemoglobin count ≥10 g/dl, platelet count ≥100,000/μl, total serum bilirubin ≤1.5 mg/dl, aspartate aminotransferase and alanine aminotransferase ≤90 IU/L, serum creatinine ≤1.5 mg/dL, and creatinine clearance ≥60 ml/min. Patients who had already received radiotherapy to their metastatic sites were not eligible for the present study. Written informed consent was obtained from every patient.

Treatment

Patients underwent thoracocentesis, and a 19-Fr chest drainage tube was kept in place until the drained volume of pleural effusion was less than 100 ml/day. Then, a 5–10 Klinische Einheit unit of OK-432 diluted by 100 ml saline was administered into the pleural cavity. The chest tube was clamped for 1–3 hours and then released for drainage. When the drained effusion volume was less than 100 ml/day, the chest tube was removed. Following intrapleural therapy, patients were treated every three weeks with two or more courses of systemic chemotherapy consisting of 80 mg/m^2 cisplatin on day 1 and 1000 mg/m^2 gemcitabine on days 1 and 8. Subsequent courses of chemotherapy were started when the leukocyte count was ≧ 4000 /μL, with a platelet count ≧ 100,000 /μL. The dose of gemcitabine was reduced to 800 mg/m^2 for the subsequent course if the patient experienced grade 4 thrombocytopenia, or grade 4 neutropenia lasting four days. Physical examination, complete blood cell counts, biochemical tests, and chest roentgenograms were obtained weekly. Tumor responses were evaluated according to the Response Evaluation Criteria for Solid Tumors (8). Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC Version 2.0).

Fifteen patients were treated in the first stage. We decided to stop the study if less than three of the 15 patients responded at this stage. If four or more patients responded, a total of 26 patients would be required. This regimen was defined as active if the number of responders was ≧ 10 and inactive if the number of responders was ≦ 9 (Simon minimax two stage; α <0.05 and β< 0.10) (9, 10). This plan allowed early termination of the study as soon as possible should it become evident that the true rate of response was less than 25% or greater than 45%. Overall survival time was estimated using the method devised by Kaplan and Meier. The Review Board of the Kanagawa Cancer Center reviewed and approved the protocol prior to commencement of the trial.

Table 1. *Patient characteristic.*

			No. of patients
Total			15
Age, years	Median		62
	Range		29 - 74
Gender	Male		10
	Female		5
Performance status		1	14
(ECOG)		2	1
Histology	Adenocarcinoma		10
	Squamous cell carcinoma		1
	Mesothelioma		4
No. ofmetastatic sites		0	10
		1	5

RESULTS

Between December 1999 and October 2001, 15 patients were registered in the study. Patient characteristics are summarized in Table 1. Ten patients were male and five were female, with a median age of 62 years (ranging from 29 to 74). Fourteen patients had a PS of 1 and one patient had a PS of 2. Ten patients had adenocarcinoma, one had squamous cell carcinoma, and four had malignant mesothelioma. No patients had received prior treatment, including any radiotherapy for metastatic lesions. All fifteen patients were assessed for their response and for toxicities. Thirteen patients received two or more courses of chemotherapy. Two patients were not given a second course of chemotherapy, one because of PD, and another because of no improvement from a depressed PS 3.

Patients' hematologic and non-hematologic toxicities are summarized in Table 2. Grade 3 or 4 neutropenia, anemia and thrombocytopenia occurred in five (33%,), two (13%) and three (20%) patients, respectively. Non-hematological toxicities were mild, except in one patient, who experienced a grade 3 elevation of transaminase, and in two patients who experienced grade 3 nausea.

The outcome of chemotherapy in 15 patients with measurable lesions is shown in Table 3. One patient achieved a PR, 13 an SD, and one a PD, and the overall response rate was 6.7%. As only one patient responded, no further patients were registered for the first stage. The overall survival curve is shown in Figure 1. The median potential follow-up time was 18.5 months (range, 10.1–34.4), and the median time to progression (MTP) was 3.7 months (range, 1.9–11.2). Four patients were still alive and the other 11 patients died during the follow-up period. The median survival time (MST) was 13.5 months and the one-year survival rate was 60.0%.

Table 2. *Toxicities*

	No. of patients with Toxicity NCI-CTC ver.2 grade				
Toxicity	**0**	**1**	**2**	**3**	**4**
Hemoblobin	0	8	5	2	0
Leukocytes	2	2	8	3	0
Neutrophils	2	2	6	3	2
Platelets	4	5	3	3	0
Bilirubin	14	0	1	0	0
AST	8	5	1	1	0
ALT	7	5	2	1	0
Creatinine	14	1	0	0	0
Nausea	4	5	4	2	0
Vomiting	9	0	5	1	0
Phlebitis	14	0	1	0	0
Headache	14	1	0	0	0
Weight loss	12	3	0	0	0
Stomototis	14	1	0	0	0

Table 3. *Chemotherapeutic response.*

Responce	Number of Patients
PR	1
NC	13
PD	1

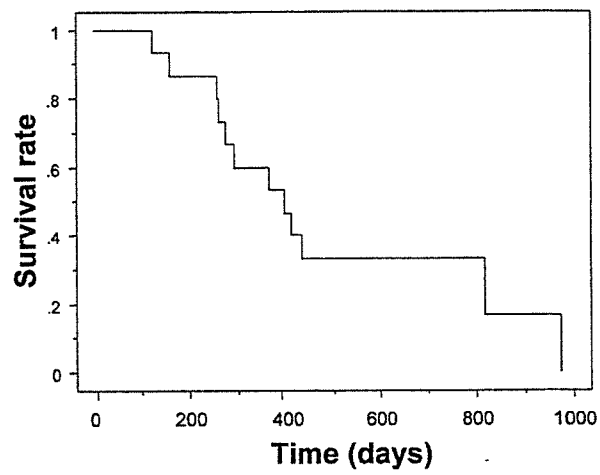

Figure 1. Kaplan-Meier estimation of overall survival of 15 patients with NSCLC with pleuritis carcinomatosa treated with cisplatin plus gemcitabine.

DISCUSSION

An effective treatment for NSCLC with pleuritis carcinomatosa has not been established. Sufferers usually experience massive pleural effusion and require pleurodesis before systemic chemotherapy. A randomized study conducted by the JCOG demonstrated the efficiency of intrapleural injection of OK-432 compared to bleomycin or cisplatin plus etoposide (1). Thirty-four patients in the study who received intrapleural treatment of OK-432 showed 28 weeks of median pleural progression-free survival and 48 weeks of MST. As these survival data were promising compared to those obtained with other treatments, we selected OK-432 administration for treating sclerosing pleural lesions in our study. Use of systemic chemotherapy after pleurodesis is also controversial, and the patients with pleuritis carcinomatosa are usually treated in accordance with the chemotherapy regimen for metastatic NSCLC. A large-scale, phase III study demonstrated an equal efficiency of cisplatin plus gemcitabine compared to cisplatin plus docetaxel, cisplatin plus paclitaxel, or carboplatin plus paclitaxel (11). We selected cisplatin plus gemcitabine for the treatment of pleuritis carcinomatosa after pleurodesis because the regimen was effective for malignant mesothelioma (4). Both pleuritis carcinomatosa and mesothelioma are pleural lesions, and the effective treatment for malignant mesothelioma is considered to also be effective for pleuritis carcinomatosa. It is also expected that cisplatin and gemcitabine shift to the thoracic cavity.

Unfortunately, only one patient responded to the combination of cisplatin and gemcitabine, so we terminated our study in the first stage. However, it should be noted that nine of the fifteen patients (60%) who entered our study survived over one year. While a combination of cisplatin and gemcitabine is one of the standard chemotherapies for advanced NSCLC, previous researchers have reported an MST of less than one year (5, 6, 11). Whether a measurable response is a good substitute for an increased survival time in the treatment of advanced cancer is still a matter of controversy (12). The survival time data in our study could not be confirmed as an outcome of treatment for pleuritis carcinomatosa because of the small number of patients analyzed, however it may suggest this combined therapy has potential for treatment of pleuritis carcinomatosa. Cisplatin and gemcitabine treatment induced a response rate similar to that of other standard chemotherapies in a randomized study against advanced NSCLC (11). The data showed that cisplatin and gemcitabine had a cytotoxic but not a cytostatic effect. The MTP was 3.7 months in our study, which is similar to other active regimens (11) and is considered to be long in spite of the poor response rate. The MTP is a measure of the quality of response, taking into account both objective response and stable disease qualifications. The reason why a good survival time was obtained in our study could not be explained; a tumor-stabilizing effect was certainly achieved with the treatment.

The JCOG study demonstrated that intrapleural sclerosing modality treatment using OK-432 is promising compared to intrapleural injection of anti-cancer agents such as bleomycin or cisplatin plus etoposide (1). OK-432 is not a cytotoxic agent and is used to achieve asclerosing effect for pleuritis carcinomatosa in Japan. The non-shrinking agent is more effective than cytotoxic agents for prolonging the survival of patients with lung cancer and pleuritis carcinomatosa, suggesting that stabilization of pleural lesions is most important for treatment of pleuritis carcinomatosa. OK-432 intrapleural administration followed by cisplatin and gemcitabine systemic chemotherapy did not reduce the tumor size in this study, but only one patient experienced tumor progression during the treatment. Chemotherapy regimens with a poor response rate usually have a 20–30% progression response and, therefore, the treatment used in this study may have the potential to stabilize pleural lesions and prolong survival.

We terminated this study in the first stage because of the poor response rate. In order to confirm the efficacy of OK-432 intrapleural administration followed by systemic chemotherapy with cisplatin and gemcitabine against pleuritis carcinomatosa, a large trial with survival time as the primary end-point is required.

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Dose Escalation Study of Paclitaxel in Combination with Fixed-Dose Irinotecan in Patients with Advanced Non-Small Cell Lung Cancer (JCOG 9807)

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Key Words
Paclitaxel · Irinotecan · Non-small cell lung cancer · G-CSF

Abstract
Background: Both irinotecan (CPT) and paclitaxel (Pac) are effective against non-small cell lung cancer (NSCLC), and besides, preclinical studies have demonstrated an additive or synergistic interaction between camptothecin and taxane. ***Methods:*** We conducted a phase I/II study of combination chemotherapy consisting of Pac and CPT to determine qualitative and quantitative toxicities and efficacy of the combination against advanced NSCLC. We fixed the dose of CPT at 60 mg/m^2 and escalated the Pac dose in 10 or 20 mg/m^2 increments from a starting dose of 80 mg/m^2, and repeated the cycle every 2 weeks. Prophylactic G-CSF was also administered. ***Results:*** Between February 1999 and April 2001, 24 patients were registered in the study. None of the patients had a history of prior chemotherapy, but surgical resection had been performed in 3 of them. None of the patients experienced dose-limiting toxicity (DLT) up to and including level 6. At dose level 7 of Pac, 180 mg/m^2, 2 patients experienced DLT, that is grades 2 and 3 dyspnea due to pneumonitis. Another patient experienced grade 1 dyspnea due to pneumonitis. Neutropenia, diarrhea, and other toxicities were mild; however, we concluded that dose level 7 of Pac was the maximum-tolerated dose. An objective response was observed in 58.3%. The median survival time was 370 days, and the 1-year survival rate was 54.2%. ***Conclusion:*** Pneumonitis was the DLT in this study, and Pac 160 mg/m^2 and CPT 60 mg/m^2 every 2 weeks are recommended for the phase II study. This combination shows appreciable activity against NSCLC.

Introduction

Current chemotherapy regimens for metastatic non-small cell lung cancer (NSCLC) are not particularly effective, and the disease cannot be cured even with the most effective cisplatin-based combination chemotherapy. New agents and new combination chemotherapies have been investigated for metastatic NSCLC, and in the past decade, a number of new anticancer agents, including vinorelbine, gemcitabine, docetaxel, and paclitaxel (Pac), have been approved for the treatment of advanced NSCLC. Regimens based on combinations of these drugs with platinum compounds have presented interesting new possibilities for treating patients with NSCLC, and randomized studies comparing platinum-based combinations with single-agent treatment have demonstrated a

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small but significant survival benefit for combined treatment [1, 2]. A trial of nonplatinum combination chemotherapy has recently been instituted [3].

The camptothecin derivative irinotecan (CPT) is a topoisomerase I inhibitor and effective against NSCLC [4]. Pac preferentially binds to microtubles and impairs microtubular disassembly, and it is also effective against NSCLC [5]. The combination of a camptothecin and a taxane is attractive, because both have been shown to have a broad spectrum of clinical activity that is dependent on the schedule of administration, and the mechanisms of action and the nonhematologic toxicity profiles of the camptothecins and taxanes are different. Preclinical studies that have evaluated combinations of a camptothecin with a taxane have yielded promising results, and several studies have demonstrated an additive or synergistic interaction between camptothecin and taxane [6–10].

Accordingly, we expected the combination of Pac and CPT to display high activity against NSCLC. Pac was initially infused every 3–4 weeks; however, weekly infusions were shown to produce a higher dose intensity and to have promising activity [11]. CPT has been used in various schedules, including weekly, every 2 weeks, and every 3 weeks. Weekly or 2-week cycles are considerably better than 3-week cycles to increase the dose intensity of both drugs. An important criterion for the feasibility of the combination of Pac and CPT is a reduced incidence of neutropenia, which is dose-limiting toxicity (DLT) of both drugs, and prophylactic granulocyte colony-stimulating factor (G-CSF) has been used to prevent neutropenia. We previously reported that prophylactic administration of G-CSF when monocytopenia is first detected can lessen neutropenia caused by chemotherapy for lung cancer without increasing the total G-CSF dose beyond the standard dose [12]. Administration of G-CSF was started on day 6–8 in this study. A 2-week cycle is reasonable for combinations of Pac and CPT when G-CSF was prophylactically used after the detection of monocytopenia.

We designed a dose escalation study to determine the maximum dose of Pac that could be given with CPT when both drugs were delivered according to a 2-weekly schedule. We fixed the dose of CPT at 60 mg/m^2, which is the dose used in combination with cisplatin [13], and escalated the Pac dose form 80 mg/m^2 in 10 or 20 mg/^{2} increments. The aims of this study were: (1) to determine the qualitative and quantitative toxicities of the combination chemotherapy; (2) to determine the recommended dose of Pac with CPT, and (3) to determine the efficacy of combination chemotherapy against advanced NSCLC by evaluating the objective response rate and survival rate.

Table 1. Patient characteristics

	Patients
Total	24
Age, years	
Median	59
Range	44–69
Gender	
Male	18
Female	6
PS (ECOG)	
0	4
1	20
Clinical stage	
IIIA/B	5
IV	16
Postoperative recurrence	3
Histology	
Adenocarcinoma	16
Squamous cell carcinoma	7
Large cell carcinoma	1
Prior treatment (surgery)	3

Patients and Methods

Patients

Patients with histologically or cytologically proven unresectable NSCLC were registered for Pac and CPT combination chemotherapy. Eligibility criteria were an expected survival of at least 6 weeks, age <70 years, Eastern Cooperative Oncology Group performance score (PS) ≤1, leukocyte count ≥4,000/μl, hemoglobin ≥10 g/dl, platelet count ≥100,000/μl, total serum bilirubin ≤1.5 mg/dl, aspartate aminotransferase and alanine aminotransferase ≤90 IU/l, serum creatinine ≤1.5 mg/dl, and creatinine clearance ≥50 ml/min. Patients who had experienced postoperative recurrence were eligible for this study, but a period of rest of 4 or more weeks was required after surgery. Patients who had received chemotherapy or radiotherapy were excluded from this study. Written informed consent was obtained from every patient.

Chemotherapy

All patients without disease progression were treated every 2 weeks for a total of 4 courses of chemotherapy. CPT was dissolved in 250 ml of 5% glucose and administered as a 90-min infusion at a fixed dose of 60 mg/m^2 on day 1. Pac was dissolved in 500 ml of 5% glucose and administered as a 3-hour infusion at a starting dose of 80 mg/m^2 on day 1 and escalated in 10 or 20 mg/m^2 increments per dose level (table 1). Premedication consisted of a 20-mg dexamethasone infusion (14 and 7 h before Pac), and a 50-mg ranitidine and 50-mg oral dose of diphenhydramine (30 min before Pac). After completion of the 90-min CPT infusion, a 30-min ranitidine infusion was administered, and a 3-hour Pac infusion was then administered. G-CSF, 50 or 2 μg/kg/day, was administered subcutaneously when the leukocyte, neutrophil, or monocyte count fell below 2,000, 1,000 or 150/μl, respectively. G-CSF was stopped if the leukocyte or neutrophil count rose above 10,000 or 5,000/μl, respectively. Patients were

given 5-HT_3 antagonist intravenously before administration of CPT on day 1. Subsequent courses of chemotherapy were started when the patients satisfied the organ function criteria: leukocyte count ≥3,000/μl, neutrophil ≥1,500/μl, platelet count ≥75,000/μl, less than grade 1 nonhematologic toxicities, except alopecia. Grade 3 nausea and vomiting did not preclude subsequent courses of chemotherapy. If the DLT was reached the dose of Pac and CPT in the subsequent course was reduced by 10 mg/m^2. Chemotherapy was repeated for a maximum of 6 courses unless the disease progressed, but it was stopped if the tumor response was judged to be no change (NC) after 4 courses. Physical examination, a complete blood cell count, biochemical tests, and chest radiographs were obtained weekly. Tumor response was evaluated according to World Heath Organization criteria [14]. Complete response was defined as the complete disappearance of all evidence of tumor for at least 4 weeks. PR was defined as at least a 50% reduction in the sum of the product of the two greatest perpendicular diameters of all indicator lesions or a reduction of more than 50% in evaluable disease for at least 4 weeks with no appearance of new lesions or progression of any existing lesions. PD was defined as at least a 25% increase in the tumor area or the appearance of new lesions. All other outcomes were classified as NC. Toxicities were evaluated according to the JCOG criteria [15].

DLT was defined as toxicity during the first 4 courses consisting of grade 4 neutropenia lasting 4 days or more, or grade 4 neutropenia with a fever of 38°C or higher, grade 4 thrombocytopenia, ≥grade 2 depression of PaO_2, grade 2 dyspnea, grade 3 or 4 other nonhematologic toxicity, except alopecia, nausea, and vomiting, or failure to complete the 4 courses within 9 weeks because of toxicity. Patient refusal was also defined as a DLT.

At least 3 patients assessable for toxicity were treated at each dose level. If none of the first 3 patients experienced a DLT, then escalation to the next dose level proceeded. If 1 patient developed a DLT, the cohort was expanded to 6 patients. We planned a 20 mg/m^2 increase by dose level after level 3 when none of the patients at levels 1 and 2 experienced DLT. The maximum tolerated dose (MTD) was defined as the dose level at which at least 2 of 3 patients or 3 of 6 patients experienced a DLT. The recommended dose of Pac for the phase II study was defined as the most dose-intensive level below the MTD. If the MTD was not defined by the Pac dose level at 210 mg/m^2, which is the recommended dose for Pac alone in Japan, the most dose-intensive level including 210 mg/m^2 of Pac would be recommended as the dose for phase II study. The Committee of JCOG and the Institutional Review Board of the Kanagawa Cancer Center reviewed and approved the protocol prior to commencement.

Results

Patient Characteristics

Between February 1999 and April 2001, 24 patients were registered in the study. The patients' characteristics are summarized in table 1. Eighteen patients were male, and 6 were female; their median age was 59 years (range 44–69 years). Four patients had a PS of 0, and the other 20 patients had a PS of 1. Sixteen patients had adenocarcinoma, 7 had squamous cell carcinoma, and 1 had large cell carcinoma. None of the patients had a prior history of chemotherapy, but surgical resection had been performed in 3 of them.

Table 2. Planned doses and administered doses of Pac and CPT

Dose level	Pac mg/m^2	CPT mg/m^2	Number of patients	Number of cycles
1	80	60	3	12
2	90	60	3	16
3	100	60	3	12
4	120	60	3	9
5	140	60	3	14
6	160	60	3	13
7	180	60	6	26

Dose Escalation and Determination of MTD

The numbers of patients and cycles at each dose level are listed in table 2. All patients were assessable for toxicity. Three patients each were registered at dose levels 1 and 2. None of the patients experienced DLT during 4 courses. At dose levels 3 and 4, 2 and 1 of the 3 patients registered developed PD during the third cycle and the first cycle, respectively, but none of them experienced DLT. None of the 3 patients registered at level 5 experienced DLT. One of the 3 patients registered at dose level 6 developed PD during the first cycle, but none of them experienced DLT. At dose level 7, 1 of the first 3 patients experienced DLT, grade 2 dyspnea, because of pneumonitis during the third cycle, and another 3 patients were registered. One of the additional 3 patients experienced a DLT, grade 3 dyspnea, because of pneumonitis in the fourth cycle, and another additional patient experienced grade 1 dyspnea because of pneumonitis in the fifth cycle. Two patients experienced DLTs at level 7, but these were both cases of pneumonitis; since another patient experienced pneumonitis, which is a very serious toxicity, we concluded that the dose level 7 of Pac, 180 mg/m^2, was the MTD. As a result, Pac 160 mg/m^2 and CPT 60 mg/m^2 every 2 weeks were recommended for the phase II study.

Treatment Administration and Toxicities during All Cycles of Treatment

The 24 patients received a total of 102 cycles of treatment, and the mean number of cycles was 4 (range 1–6). There were no treatment-related deaths. Five patients were excluded from treatment, 4 because of disease progression and 1 because of an adverse event, that is pneumonitis. Tables 3 and 4 list the overall incidence of hematologic and nonhematologic adverse events among all