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Concurrent Chemoradiotherapy with Cisplatin and Vinorelbine for Stage III Non-small Cell Lung Cancer

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Introduction: Concurrent chemoradiotherapy with full doses of cisplatin-based chemotherapy is standard treatment for inoperable stage III non-small cell lung cancer (NSCLC). Although many platinum-based two drug combinations with third-generation agents are difficult to combine fully with thoracic radiotherapy (TRT), a phase I study reported a full dose of cisplatin (CDDP) plus 80% dose of vinorelbine (VNR) was successfully combined with concurrent TRT.

Methods: Between October 2000 and October 2004, 73 patients with inoperable stage III NSCLC treated with CDDP, VNR, and concurrent TRT were retrospectively analyzed. Patients were treated with CDDP 80 mg/m^2 on day 1 and VNR 20 mg/m^2 on days 1 and 8 every 4 weeks. Radiotherapy was administered concurrently in cycle 1. The total radiation dose was 60 Gy in 30 fractions. Common Terminology Criteria for Adverse Events version 3.0 were used to assess treatment-related adverse events.

Results: Median age was 63 years (40–78). Twenty-nine patients had adenocarcinoma, 63 were male, 47 ECOG PS 1, and 47 stage IIIB. Median chemotherapy cycle was 2.0. Objective response rate was 93% and median survival time was 21 months. Three-year overall survival rate was 33%. Infield control rate was 71%. The most common grade 3 or 4 adverse event was leukocytopenia (67%). Only 3 patients (4%) experienced grade 3 esophagitis. One patient died of radiation pneumonitis 87 days after completion of chemoradiotherapy.

Conclusions: Concurrent chemoradiotherapy with CDDP and VNR was highly active and well-tolerated. This regimen could be used as a control arm in future trial for stage III NSCLC.

Key Words: Concurrent chemoradiotherapy, Non-small cell lung cancer, Cisplatin, Vinorelbine.

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Lung cancer is the leading cause of cancer-related deaths throughout the world, including Japan.[1] Stage III inoperable non-small cell lung cancer (NSCLC) constitutes approximately 30% of all newly diagnosed cases of NSCLC.[2] Historically, patients with stage III NSCLC were treated with thoracic radiotherapy (TRT) alone. Nevertheless, the survival of patients treated with TRT alone was poor, with a 5-year survival rate of approximately 5%.[3] As the treatment option of chemoradiotherapy (CRT) has developed, the survival of patients with stage III NSCLC has improved, with 3-year survival of approximately 15–20% and median survival time (MST) of 15–20 months.[4,5] Several randomized trials have demonstrated that concurrent CRT using full dose of cisplatin-based chemotherapy improves long-term survival compared with sequential CRT.[6–9] Although two-drug combinations with cisplatin (CDDP) and third-generation agents including vinorelbine (VNR), docetaxel, paclitaxel, gemcitabine, and irinotecan are standard chemotherapy regimens for stage IV NSCLC[10–12], it is difficult to deliver full doses of these regimens and concurrent TRT because of excessive toxicity.

Recently a phase I trial of CDDP, VNR, and concurrent RT was reported.[13] The recommended doses were CDDP 80 mg/m^2 on day 1 and VNR 20 mg/m^2 on days 1 and 8. Although this was a phase I study, an encouraging survival rate of 50% at 3 years was reported. On the basis of this result, we have treated inoperable stage III NSCLC patients with CDDP, VNR, and concurrent RT in clinical practice at the National Cancer Center Hospital East, Japan. Herein is our review of the efficacy and tolerability of CRT with CDDP and VNR.

MATERIALS AND METHODS

The objective of this retrospective analysis was to evaluate the efficacy and tolerability of concurrent CRT using CDDP and VNR.

Patient Selection

We reviewed consecutive 106 inoperable stage III NSCLC patients who were treated with CDDP, VNR, and concurrent TRT at the National Cancer Center Hospital East, Japan, between October 2000 and October 2004. Clinically apparent or histologically/cytologically proven N2/N3 disease or T4 otherwise pulmonary metastasis in the same lobe was considered "inoperable." Chest CT, abdominal CT/ultrasonography, bone scintigram or FDG-PET, and brain MRI/CT were performed in all patients. In general, lymph nodes that were larger than 1.0 cm in minor axis were considered as metastatic. Lymph nodes that were involved in multiple stations were considered 'clinically apparent N2/3.' To con-

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firm N2 disease, which was detected in chest CT and considered 'not apparent,' FDG-PET and/or mediastinoscopy was performed. FDG-PET (or PET/CT) was performed in 18 patients. Mediastinoscopy was performed in ten patients. In addition, there were 5 histologically/cytologically confirmed N3 (supraclavicular lymph nodes) diseases. Thirty-three patients were excluded because they participated in a clinical trial that evaluated CDDP plus VNR followed by docetaxel,[14] therefore 73 patients were evaluated in the present analysis. Data of survival, recurrence, and treatments after failure were obtained from medical records. All patients were evaluated at weekly case conference in which radiation oncologists and medical oncologists who had special expertise in thoracic oncology made treatment decisions. Inclusion criteria for CRT in our institution were generally as follows; white blood cell count $>3.0 \times 10^9$/liter, platelet count $>10.0 \times 10^9$/liter, serum creatinine <1.5 mg/dl, total bilirubin <1.5 mg/dl, and transaminase less that twice the upper limit of the normal value. Exclusion criteria were pulmonary fibrosis identified by a chest x-ray, malignant pleural or pericardial effusion, and a concomitant serious illness, such as uncontrolled angina pectoris, myocardial infarction in the previous 3 months, heart failure, uncontrolled diabetes mellitus, *severe respiratory failure* and uncontrolled hypertension. All patients gave informed consent before CRT.

Chemotherapy

Chemotherapy consisted of CDDP (80 mg/m^2 on day 1) and VNR (20 mg/m^2 days on 1 and 8). Treatment cycles were repeated every 4 weeks with a maximum of 3 cycles administered. Cisplatin and VNR were administered by intravenous infusion. All patients received prophylactic antiemetic therapy consisting of 5-HT3 antagonist, metoclopramide, and dexamethasone. If a patient experienced excessive adverse events, dose reduction of both drugs was implemented during the subsequent treatment cycle. When leukocyte or platelet counts were inappropriate, or if infection developed at day 8, VNR was withheld.

Radiotherapy

TRT was administered concurrently in cycle 1. A CT-scan based treatment planning was used in all patients. The clinical target volume (CTV) for the primary tumor was defined as the gross tumor volume plus 0.5–0.8 cm margin taking account of subclinical extension. The CTV for metastatic lymph nodes were the same as the gross tumor volume for metastatic lymph nodes. Metastatic lymph nodes were defined as the lymph nodes that were larger than 1.0 cm in minor axis. Regional lymph nodes (mainly #3, #4, #7), excluding the contralateral hilar and supraclavicular lymph nodes, were included in the CTV for elective nodal irradiation. The planning target volume for the primary tumor, the metastatic lymph nodes, and regional lymph nodes was determined as CTVs plus setup margin (0.5 cm) and internal margins according to the respiratory motion on fluoroscopy (circumferential 0.5 cm, cranial 0.5 cm, and caudal 1.0–1.5 cm). Lung heterogeneity corrections were not used, and the doses were prescribed to the center of planning target volume. Principally, the initial radiation field was planned not to exceed 50% of ipsilateral lung volume on chest radiograph, or since August 2003, V20 of the normal lung (the percent volume of normal lung receiving 20 Gy or more) was planned not to exceed 35%. The total radiotherapy dose was 60 Gy in 30 fractions (5 fractions per week) delivered over 6 weeks. Radiation therapy was delivered with megavoltage equipment (6 mV) using parallel opposed fields up to 40 Gy in 20 fractions including primary tumor, the metastatic lymph nodes, and the regional lymph nodes. A booster dose of 20 Gy in 10 fractions was given to the primary tumor and the metastatic lymph nodes according to the CT obtained after initial 40 Gy radiation, using opposed oblique fields to avoid excessive dose to the spinal cord.

Evaluation of Efficacy and Adverse Events

Overall survival was defined as time from start of chemoradiotherapy to death of any cause. Progression-free survival was defined as time from start of chemoradiotherapy to the first documented disease progression or death. Disease progression was subdivided into infield relapse or not. Chest CT was used to asses if the relapse was within the initial radiation field. Response Evaluation Criteria in Solid Tumor criteria were used to assess the best tumor response. Chest CT was reviewed independently by a radiologist. The response rate was calculated as the total percentage of patients with a complete or partial response. In principle, the chest CT was taken 2 and 4 months after starting chemoradiotherapy and as needed to evaluate the response and toxicity. Treatment-related adverse events were evaluated using the Common Terminology Criteria for Adverse Events Version 3.0. Late toxicities were scored according to the European Organization for Research and Treatment of Cancer/Radiation Therapy Oncology Group late radiation morbidity scoring scheme.

Statistical Analyses

Multivariate analyses were performed using Cox regression models. Expected prognostic factors included age (<70 years versus >70), gender (male versus female), Eastern Cooperative Oncology Group (ECOG) performance status (0 versus 1), clinical stage (IIIA versus IIIB), smoking history (<30 pack-year versus >30), histology (adenocarcinoma versus others), tumor size (<5 cm versus >5 cm), stage (IIIA versus IIIB), and weight loss (<5% versus >5%). Kaplan–Meier methods were used to graphically describe the distribution of survival. All statistical analyses were performed using SPSS II for Windows version 11.0.1J.

RESULTS

Patients' characteristics are shown in Table 1. Median number of chemotherapy cycles were 2.0 (mean 2.4, ranges 1–3). Dose reduction of chemotherapy was implemented in 11 patients mainly due to grade 4 leukocytopenia. Two patients did not receive full dose of radiotherapy. In one patient, radiotherapy was discontinued at the dose of 40 Gy because the tumor was located nearby the spinal cord, and in the other patient because of declined PS.

All 73 patients were assessable for survival, time to progression, response rate, and adverse events. No patient achieved complete response. Partial response, stable disease,

TABLE 1. Patient Characteristics

	Patients (n = 73)	
	No.	%
Age		
Median (range) (yr)	63 (40–78)	
<70 yr	48	66
≥70 yr	25	34
Gender		
Female	10	14
Male	63	86
Histological diagnosis		
Adenocarcinoma	29	40
Squamous cell carcinoma	28	38
Others	16	22
Tumor size		
Median (range) (cm)	5.4 (1.5–12.0)	
<5 cm	33	45
≥5 cm	40	55
ECOG performance status		
0	26	36
1	47	64
Smoking history		
Never smoker	5	7
<30 pack-yr	11	15
≥30 pack-yr	57	78
Stage		
IIIA	26	36
T3N1	3	4
N2	23	32
IIIB	47	64
T4[a]	40	55
N3	12	16
Body weight loss (recent 6 mo)		
<5%	58	79
≥5%	15	21

[a] Six were T4N0, 3 were T4N1, and 5 were T4N3.

TABLE 2. Overall Objective Response

	Number	%
Number of patients evaluated	73	
Complete response (CR)	0	0
Partial response (PR)	68	93.2
Stable disease (SD)	5	7.8
Progressive disease (PD)	0	0
Response rate (95% CI)		93.2 (87.2–99.1)%

CI, confidence interval.

and progressive disease were observed in 68, 5, and 0 patient, respectively (Table 2). The response rate was 93.2% (95% confidence interval; 87.2–99.1%). Median progression free survival time was 12 months and median overall survival time was 21 months with median follow-up of 35 months (ranges 23.7–61.2). Two- and 3-year survival rate was 44 and 33%, respectively. The Kaplan–Meier plots of overall survival are shown in Figure 1; Figure 2 shows progression-free survival. Multivariate analysis showed that no variables significantly affected the overall survival (Table 3).

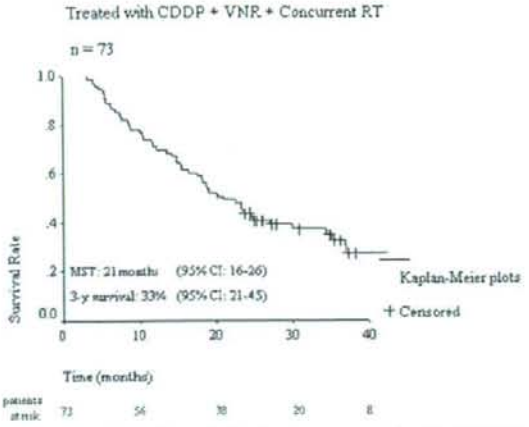

FIGURE 1. Overall survival of patients treated with CDDP + VNR + concurrent RT. CDDP, cisplatin; VNR, vinorelbine; RT, radiotherapy; MST, median survival time; 3-year survival, survival rate at 3 years.

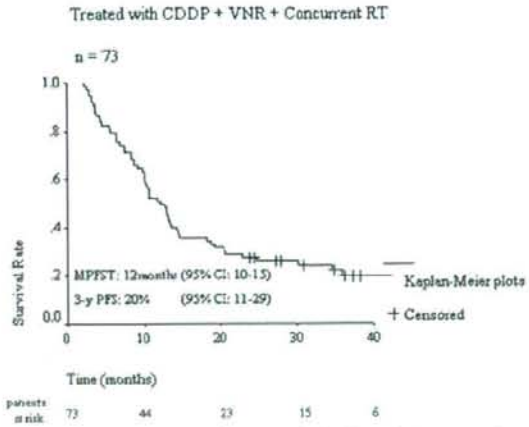

FIGURE 2. Progression-free survival of patients treated with CDDP + VNR + concurrent RT. CDDP, cisplatin; VNR, vinorelbine; RT, radiotherapy; MPFST, median progression-free survival time; 3-year survival, progression-free survival rate at 3 years.

There were 46 disease relapses and 50 deaths. Infield relapses were observed in 21 patients (11 without and 10 with relapse outside of the radiation fields); therefore infield control rate was 71%. Distant metastases were the first sites of the failure in 35 patients; brain (n = 16), bone (n = 10), adrenal gland (n = 5), liver (n = 3), and lung (n = 16). Seventeen patients received docetaxel and 12 received gefitinib as second line treatment. None responded to docetaxel and two patients (16%) responded to gefitinib (and 1 achieved partial response).

TABLE 3. Prognostic Factors Treated with CDDP + VNR + Concurrent TRT (n = 73)

Parameter	Hazard Ratio	95% CI	p
Age (<70 yr vs. ≥70)	1.787	0.941–3.394	0.076
Gender (male vs. female)	1.364	0.490–3.799	0.553
PS (0 vs. 1)	0.818	0.435–1.537	0.533
Clinical Stage (IIIA vs. IIIB)	1.109	0.588–2.093	0.749
Smoking (<30 pack-yr vs. ≥30)	0.698	0.321–1.519	0.365
Tumor size (< 5 cm vs. ≥5)	0.862	0.473–1.569	0.626
Histology (Ad vs. others)	1.565	0.766–3.198	0.219
Body weight loss (<5% vs. ≥5)	1.567	0.786–3.125	0.202

CI, confidence interval; Ad, adenocarcinoma.

The incidence of treatment-related adverse events is listed in Table 4. The most common grade 3 or 4 adverse event was leukocytopenia (67%). Grade 3 or 4 neutropenia was observed in 38 patients (52%). Grade 3 or 4 thrombocytopenia was not observed; grade 3 or 4 anemia occurred in 17 patients (23%). Only 3 patients (4%) experienced grade 3 esophagitis related to radiotherapy. Five patients (7%) developed grade 3 or 4 pneumonitis and one of them died of respiratory failure 87 days after completion of chemoradiotherapy. The autopsy revealed diffuse alveolar damage compatible with radiation pneumonitis and fibrosis. None of the 5 patients with grade 3 or 4 pneumonitis received second line chemotherapy. Another patient of them developed grade 3 pulmonary fibrosis, but no other severe late radiation morbidity was observed.

DISCUSSION

Chemoradiotherapy is standard treatment for patients with inoperable stage III NSCLC. Several trials indicate that concurrent CRT improves long-term survival compared with sequential CRT.[6–9] Nevertheless, the optimal regimen and dose of chemotherapy has not been determined yet. The efficacy of chemoradiotherapy with CDDP and vinca alkaloids or etoposide has been reported, and CDDP plus vindesine with or without mitomycin has been one of the standard chemotherapy regimens.[6,15–17]

TABLE 4. Grade 3 or 4 Treatment-Related Adverse Events (NCI-CTC vs. 3.0, n = 73)

Adverse Event	Grade 3 (%)	Grade 4 (%)
Leukocytes	32	36
Neutrophiles/granulocytes	25	27
Hemoglobin	22	1
Platelets	1	0
Febrile neutropenia	14	0
Infection with grade 3 or 4 neutropenia	1	0
Infection without neutropenia	10	0
Pneumonitis/pulmonary infiltrates	5	1[a]
Radiation esophagitis	4	0
Radiation dermatitis	0	0
Anorexia	16	0
Nausea	8	0
Vomiting	5	0
Diarrhea	1	0
Creatinine	0	0
Supraventricular arrhythmia (atrial fibrillation)	1	0

[a] One patient died from radiation pneumonitis 87 d after completion of chemoradiotherapy.

VNR is a newer semi-synthetic vinca alkaloid and more active than vindesine against metastatic NSCLC.[18] Zatloukal et al.[8] reported the efficacy of CRT with CDDP and VNR in a randomized phase II trial, which randomized concurrent CRT or sequential. Concurrent arm was favored in overall survival (MST was 16.6 months in the concurrent arm and 12.9 months in the sequential arm). Vokes et al.[19] also reported the efficacy of CRT with CDDP and VNR in randomized phase II trial, which randomized 3 CDDP-based combination chemotherapies with third-generation agents. In this series, MST of all patients were 17 months and 3 year survival of VNR arm was 23%. With these results, concurrent CRT with CDDP and VNR could be considered one of the new standard regimens for stage III NSCLC, although the employed VNR doses in each phase II study were 12.5 mg/m^2 and 15 mg/m^2. Standard doses of CDDP plus VNR for metastatic NSCLC are 80 mg/m^2 of CDDP and 25 mg/m^2 of VNR. The doses of 20 mg/m^2, employed in the present study, are close to the standard. Moreover, 20 mg/m^2 of VNR alone has reported to be active in advanced NSCLC, with response rate of 21.7%.[20]

Results of the present study were encouraging, demonstrating MST of 21 months and a 3-year survival rate of 33%. Our study confirmed clinical usefulness of combination chemotherapy with CDDP, VNR, and simultaneous TRT.

The most common treatment-related adverse events were hematological (grade 3 or 4 leukocytopenia in 67%, neutropenia in 52%, and anemia in 23%), and these were well tolerated. There were 5 patients (7%) who developed grade 3 or more pneumonitis and only one patient (2%) died of radiation pneumonitis. The incidence and mortality of radiation pneumonitis was comparable with other reports.[6,8,9,19,21–24] Recently we have evaluated dose volume histogram and plan V20 not to exceed 35% in CRT, which may contribute to reducing severe radiation pneumonitis.

Low incidence of severe radiation-related esophagitis in our study deserves special mention. In the present study grade 3 esophagitis was developed in only 3 patients (4%), which is lower than other studies of concurrent chemoradiotherapy where radiation-related esophagitis was reported to be in the range of 12–46%,[21–23] with the exception of one study using CDDP, vindesine (VDS), and mitomycin.[6] In this report, the incidence of grade 3 or more radiation-related esophagitis was only 3%. The cause of this difference is still unknown; however, low incidence of esophagitis may correlate with the use of vinca alkaloids and Japanese studies. Further examination is warranted. We believe that highly conformal therapy could reduce the rate of esophagitis. Overall, chemoradiotherapy with CDDP and VNR were well tolerated.

Although the collection of toxicity data retrospectively is of concern, most patients were treated as inpatient through-

out the treatment course, and toxicity data were recorded on medical records in detail. It should be confirmed by a prospective study.

Taxanes are also investigated widely in patient with unresectable stage III NSCLC. Weekly administration with carboplatin (CBDCA) plus paclitaxel (PTX) and concurrent RT was reported in multiinstitutional phase II study. Reported MST was promising, with 20.5 months.[25] Nevertheless, recently reported phase III trial compared induction chemotherapy plus CRT with CRT alone, which employed weekly CBDCA and PTX, showed disappointing results, with MST of 14 months and 12 months, respectively.[26] The authors concluded that the routine use of weekly CBDCA and PTX with simultaneous TRT should be re-examined. Chemotherapy with docetaxel (DOC) plus CDDP and concurrent TRT was also reported in a phase I/II study.[21] The result was promising, with MST of 23 months, and phase III trial comparing DOC and CDDP to CDDP, VDS, and mitomycin is currently underway.

Local recurrence was observed in 21 patients (29%), and the brain was also a major site of treatment failure (16 patients, 22%). These results are comparable to the literature.[21] On the basis of these observations, other radiation approaches such as hyperfractionated radiotherapy or high-dose thoracic radiation to improve local control should be considered.[27–31] Moreover, whether prophylactic cranial irradiation reduces the incidence of brain metastases should be confirmed.

Advanced age did not correlate with worse prognosis and it is compatible with literature.[32] Gender, tumor size, body weight loss, smoking status did not significantly correlate with shorter overall survival, and it may be due to the small sample size of our study.

We excluded 33 patients who participated in the trial evaluated consolidation docetaxel after concurrent CRT with CDDP and VNR.[14] Sekine and colleagues reported that majority of patients could not continue with consolidation docetaxel after concurrent CRT with CDDP and VNR because of pulmonary toxicity. Although consolidation therapy using docetaxel seems to be highly effective in SWOG phase II study,[33] randomized phase III trial failed to demonstrate that addition of consolidation docetaxel improves survival.[34]

Two patients did not receive full dose of radiotherapy. Nevertheless, these two patients were treated initially with curative intent. Therefore we included these two patients in this analysis. Moreover, exclusion of these two patients did not alter the results (data not shown).

In conclusion, chemoradiotherapy with CDDP and VNR was promising and well tolerated. This regimen could be used as a control arm in future trial for stage III NSCLC.

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Clinical Outcome of Chemoradiation Therapy in Patients with Limited-Disease Small Cell Lung Cancer with Ipsilateral Pleural Effusion

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Background: The indications for definitive thoracic radiotherapy (TRT) in limited-disease small cell lung cancer (LD-SCLC) and ipsilateral pleural effusion have not been thoroughly investigated. We retrospectively investigated the clinical outcome of LD-SCLC patients with ipsilateral pleural effusion.

Methods: The medical records of SCLC patients who received treatment at the National Cancer Center Hospital East between July 1992 and December 2006 were reviewed. Sixty-three of the 373 LD-SCLC patients (17%) had ipsilateral pleural effusion. Of these, 62 patients received chemotherapy as an initial treatment, and were included in this study. Since about 1998, definitive TRT was routinely performed if the patient's pleural effusion disappeared after induction chemotherapy. The 62 patients were divided into three subgroups: group A included patients who received chemotherapy and TRT ($n = 26$), group B included patients who did not receive TRT in spite of the disappearance of pleural effusion after first-line chemotherapy ($n = 8$), and group C included patients who did not receive TRT and whose pleural effusion persisted after first-line chemotherapy ($n = 28$).

Results: The response rate for first-line chemotherapy was 74%. Ipsilateral pleural effusion disappeared after first-line chemotherapy in 34 patients (55%). The median overall survival time was 11.8 months, and the 2 and 3-year survival rates were 21 and 10%, respectively. In groups A, B, and C, the median survival times were 19.2, 10.5, and 9.2 months, respectively, and the 2-year survival rates were 38, 25, and 7%, respectively.

Conclusion: Long-term survival was achieved by LD-SCLC patients with ipsilateral pleural effusion who successfully underwent chemoradiotherapy.

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Lung cancer is the leading cause of cancer-related deaths worldwide. In Japan, over 56,000 people died of lung cancer in 2003. Small cell lung cancer (SCLC) accounts for about 15% of all forms of lung cancer. SCLC has a more aggressive biologic behavior than non-small cell lung cancer. At the time of presentation, two-thirds of patients exhibit disseminated disease. SCLC is sensitive to chemotherapy, with a response rate of 70 to 80%. A clinical two-stage system proposed by the Veterans Administration Lung Study Group distinguishes limited-disease (LD) and extensive-disease (ED) in SCLC.[1] LD is defined as being limited to one hemithorax, including mediastinal, contralateral hilar, and ipsilateral supraclavicular lymph nodes, whereas ED represents tumor spread beyond these regions. The current standard care for LD-SCLC is a combination of chemotherapy and thoracic radiotherapy (TRT). On the other hand, ED-SCLC is treated with chemotherapy alone. The original definition of LD was a tumor volume that could be encompassed by a reasonable radiotherapy plan. According to the International Association for the Study of Lung Cancer (IASLC)'s consensus report, on the other hand, the classification of LD-SCLC includes bilateral hilar and/or supraclavicular nodal involvement and ipsilateral pleural effusion.[2] However, the indication for definitive TRT in patients with LD-SCLC and ipsilateral pleural effusion have not been thoroughly investigated. Recently, the IASLC proposed the seventh edition of the tumor, node, metastasis (TNM) classification for lung cancer. In the proposals, the presence of a pleural effusion is considered as M1 disease.[3–6]

Definitive TRT is contraindicated in lung cancer patients with malignant pleural effusion. We have sometimes treated SCLC cases in which the ipsilateral pleural effusion disappeared after induction chemotherapy. Should definitive TRT be indicated in SCLC patients if the ipsilateral pleural effusion disappears after induction chemotherapy? Since about 1998, we have routinely performed definitive TRT if the patient's pleural effusion disappeared after induction chemotherapy. In this retrospective study, we investigated the clinical course and outcome of LD-SCLC patients with ipsilateral pleural effusion and exam-

ined the overall survival in patients who received chemotherapy and TRT, comparing with that of ED-SCLC or LD-SCLC patients without ipsilateral pleural effusion. We also applied the proposed seventh edition of the TNM stage to our cohort.

PATIENTS AND METHODS

We retrospectively reviewed the medical records of lung cancer patients who received treatment at the National Cancer Center Hospital East between July 1992 and December 2006. During this period 699 patients were newly diagnosed as having SCLC. Three-hundred and seventy-three patients were diagnosed as having LD-SCLC, and 326 were diagnosed as having ED-SCLC using conventional staging procedures, including a medical history and physical examination, chest radiography, computed tomography (CT) scan of the chest, CT scan or ultrasound of the abdomen, bone scan, and CT scan or magnetic resonance imaging of the brain. In this study, LD-SCLC was defined as disease limited to one hemithorax, including mediastinal, contralateral hilar, and supraclavicular lymph nodes, ipsilateral pleural effusion, and pericardial effusion; ED-SCLC was defined as tumor spread beyond these manifestations.[2] Sixty-three of the 373 LD-SCLC patients (17, 95% confidence interval (CI): 13–21%) had ipsilateral pleural effusion. Thirty-seven SCLC patients underwent surgical resection as an initial treatment, and 13 patients received only TRT and/or best supportive care. Remaining 649 patients received chemotherapy as an initial treatment. Of these, 62 LD-SCLC patients had ipsilateral pleural effusion, and were included in this study. The patient characteristics are shown in Table 1. The breadth of the pleural effusion was measured using a CT scan of the chest (Figure 1). Cytologic examination of the pleural effusion prior to treatment was performed in 26 patients. Eleven patients had cytologically positive effusion. Ten patients also had pericardial effusion. Three patients had solid pleural tumor and pleural effusion detected on CT scan. Twenty-six patients had atelectasis. Of these, 14 patients received cytologic examination of the pleural effusion, and four patients had cytologically positive effusion.

We collected clinical data on the patients from their medical records; this data included the chemotherapy regimen that was received, the response to first-line chemotherapy, whether pleural effusion disappeared after first-line chemotherapy, and whether the patient underwent definitive TRT. The World Health Organization's response criteria were used.[7]

Overall survival was defined as the interval between the start of treatment and death or the final follow-up visit. Median overall survival was estimated using the Kaplan-Meier analysis method.[8] Survival data was compared among groups using a log-rank test. The breadth of pleural effusion was compared using the Mann-Whitney U test. All reported p values are two-sided.

RESULTS

The induction chemotherapy regimens were shown in Table 2. Most common regimen was cisplatin or carboplatin plus etoposide. In LD patients with ipsilateral pleural effusion, there were three complete responses, 43 partial responses, seven no changes, and six progressive diseases. Response was not evaluated in three patients because of early death. The response rate was 74% (95% CI: 62–84%). Ipsilateral pleural effusion disappeared after first-line chemotherapy in 34 patients (55, 95% CI: 42–68%).

TABLE 1. Patient Characteristics

	LD-SCLC without Ipsilateral Pleural Effusion	LD-SCLC with Ipsilateral Pleural Effusion	ED-SCLC
No. of patients	270	62	317
Sex			
Male	226	50	262
Female	44	12	55
Age, yr			
Median	66	67	66
Range	38–87	46–79	28–85
Performance status			
0	71	2	20
1	178	45	203
2	14	10	59
3	6	5	28
4	1	0	7
Breadth of pleural effusion on CT scan, cm			
Median		2.3	
Range		0.5–9.4	
Cytology of pleural effusion			
Positive		11	
Negative		15	
Not examined		36	

Patients who received chemotherapy as an initial treatment were included.
LD, limited-disease; SCLC, small cell lung cancer; ED, extensive-disease; CT, computed tomography.

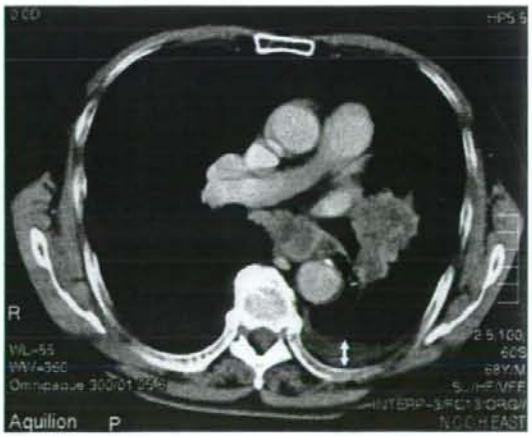

FIGURE 1. Ipsilateral pleural effusion. The arrow indicates the breadth of pleural effusion.

TABLE 2. Induction Chemotherapy Regimens and Response

	LD-SCLC without Ipsilateral Pleural Effusion	LD-SCLC with Ipsilateral Pleural Effusion	ED-SCLC
Chemotherapy regimens			
Platinum + ETP	252	54	154
Cisplatin and irinotecan containing regimens	10	2	92*
CODE	7	5	52
CAV/PE	1	1	11
Other	0	0	8
Response			
CR	64	3	28
PR	189	43	213
NC	8	7	37
PD	5	6	18
NE	4	3	21
Response rate (%) (95% CI)	94 (90–96)	74 (62–84)	76 (71–81)

*Nine patients received chemotherapy of cisplatin and topotecan.

LD, limited-disease; SCLC, small cell lung cancer; ED, extensive-disease; ETP, etoposide; CODE, weekly cisplatin, vincristine, doxorubicin, plus etoposide; CAV/PE, cyclophosphamide, doxorubicin, plus etoposide alternating with cisplatin plus etoposide; CR, complete response; PR, partial response; NC, no change; PD, progressive disease; NE, not evaluable; CI, confidence interval.

Since about 1998, definitive TRT to the primary lesion and mediastinum was routinely performed in patients whose pleural effusion disappeared after chemotherapy. We divided the 62 patients in this study into three subgroups: group A included patients who received chemotherapy and TRT (n = 26), group B included patients who did not receive TRT in spite of the disappearance of pleural effusion after first-line chemotherapy (n = 8), and group C included patients who did not receive TRT and whose pleural effusion persisted after first-line chemotherapy (n = 28).

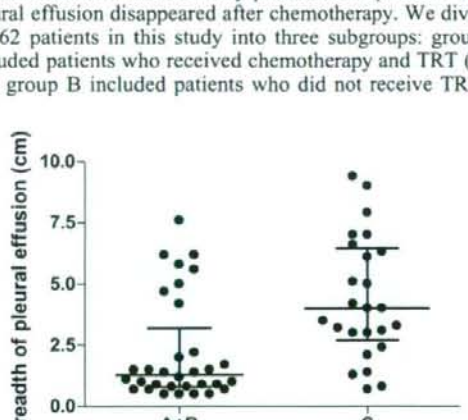

FIGURE 2. Breadth of pleural effusion in subgroup A + B, and C. Group A included patients who underwent chemotherapy and thoracic radiotherapy (TRT) (n = 26), group B included patients who did not undergo TRT in spite of the disappearance of pleural effusion after first-line chemotherapy (n = 8), and group C included patients who did not undergo TRT and whose pleural effusion persisted after first-line chemotherapy (n = 28). The line represents the median with the interquartile range.

The median (range) breadth of pleural effusion was 11.2 cm (0.5–7.6 cm) in group A, 1.8 cm (0.5–5 cm) in group B, and 4 cm (0.7–9 cm) in group C. Combining group A and B, the median breadth of pleural effusion was 1.3 cm, which was significantly lower than that of group C (p = 0.0007) (Figure 2).

In group A, all but two patients received platinum-based chemotherapy. One patient received weekly cisplatin, vincristine, doxorubicin, plus etoposide (PE) therapy, and the other patient received cyclophosphamide, doxorubicin, PE alternating with cisplatin PE therapy. Three of the 26 patients in group A underwent TRT (twice daily, 45 Gy in total) concurrently with the first course of chemotherapy. The breadths of pleural effusion in those three patients were 0.7, 0.8, and 1.0 cm. Two, seven, and one patient underwent TRT (once daily, 50 Gy in total) concurrently with the second, third, and fourth courses of chemotherapy, respectively. Thirteen patients underwent TRT (once daily, 50 Gy in total) sequentially after chemotherapy. Six patients received prophylactic cranial irradiation (PCI) of 25 Gy.

Figure 3*A* showed the survival of the all 699 SCLC patients by the proposed seventh edition of TNM stage. Figure 3*B* showed the survival of the 649 SCLC patients who received chemotherapy as an initial treatment. The survival of LD patients with ipsilateral pleural effusion was intermediate between those of LD patients without effusion and ED patients ($p < 0.0001$). The median survival time in LD patients with ipsilateral pleural effusion was 11.8 months (95% CI: 9.2–16.6), and the 1, 2, 3 and 5-year survival rates were 48, 21, 10 and 8%, respectively. Four patients have survived for over 5 years. One patient had a cytologically negative pleural effusion, and cytologic examinations were not performed for the remaining three patients. Breadth of pleural effusion of these four patients ranged from 1.0 to 1.5 cm. Two of these four patients have not shown any progression for more than 5 years. One patient who received only chemotherapy as an initial treatment developed a local recurrence 3 years after the first-line treatment. This patient received concurrent chemoradiotherapy and achieved a complete response. Unfortunately, he developed brain metastasis 9 years after the first-line chemotherapy and received whole brain radiotherapy. The other patient developed cervicular and inguinal node metastases 8 months after the initiation of first-line chemotherapy and concurrent TRT with three courses of chemotherapy. This patient received second, third, and fourth-line chemotherapy, radiotherapy to the cervicular and inguinal node metastases, and surgical resection of the recurrent inguinal node metastasis. He has not shown any signs of progression for 3 years and 3 months after the final surgical resection of the metastatic inguinal node. All three patients who had solid pleural tumor died within 31 months.

Survival analyses for the subgroups in LD patients with ipsilateral pleural effusion are shown in Figures 4, 5 and Table 3. In group A, the median survival time was 19.2 months (95% CI: 16.7–27.9) and the 1 and 2-year survival rates were 81 and

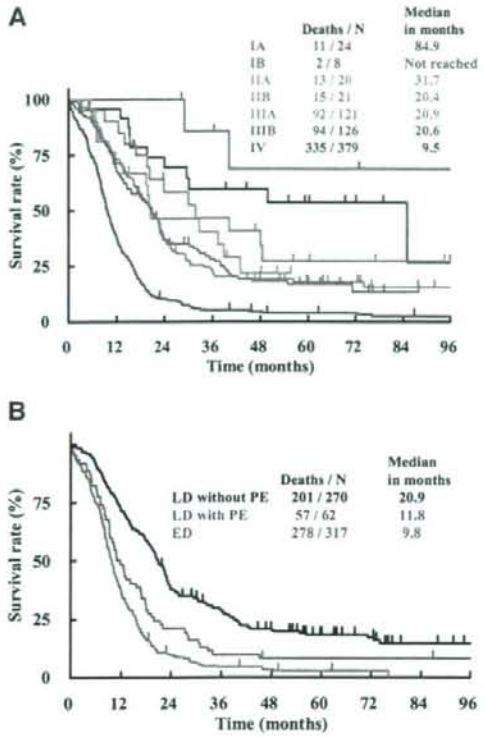

FIGURE 3. *A*, Overall survival in the all 699 patients with small cell lung cancer by the proposed seventh edition of the tumor, node, metastasis stage. *B*, Overall survival in the 649 patients who received chemotherapy as an initial treatment. LD, limited-disease; SCLC, small cell lung cancer; ED, extensive-disease.

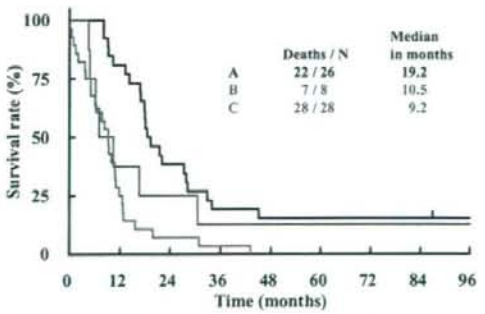

FIGURE 4. Overall survival in subgroups A, B, and C.

38%, respectively. The median survival time of patients with cytologically positive and negative pleural effusion were 9.3 months (95% CI: 3.8–14.2) and 12.7 months (95% CI: 5.1–17.9), respectively. The median survival time of those patients

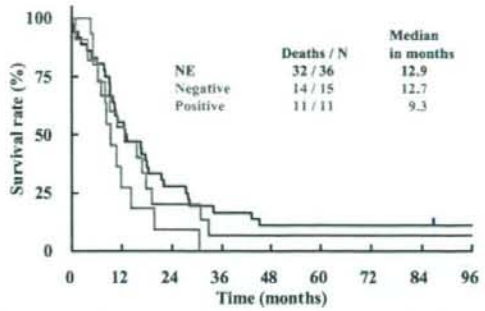

FIGURE 5. Overall survival according to the results of cytologic examination for ipsilateral pleural effusion. NE, not examined.

whose pleural effusions were not examined cytologically was 12.9 months (95% CI: 9.2–18.4). This difference was not statistically significant ($p = 0.1959$).

Disease progression was confirmed in 21 of the 26 patients in group A. The sites of first disease progression included the brain ($n = 10$), regional lymph nodes ($n = 5$), primary lesion ($n = 3$), distal lymph nodes ($n = 2$), liver ($n = 1$), adrenal gland ($n = 1$), and bone ($n = 1$). Twelve (57%) were distant, seven (33%) were local-regional, and two (10%) were both local-regional and distant. Brain metastasis was the only site of recurrence in nine patients. These nine patients had not received PCI. At the time of disease progression, ipsilateral pleural effusion recurred in 10 of the 18 patients.

DISCUSSION

LD-SCLC with ipsilateral pleural effusion accounted for 9% of all the patients with SCLC (63 of 669 patients) and 17% of all the patients with LD-SCLC (63 of 373 patients). Twenty-six (41%) of the LD-SCLC patients with ipsilateral pleural effusion received chemotherapy and definitive TRT. The median survival time of these patients was 19.2 months (95% CI: 16.7–27.9), and the 1 and 2-year survival rates were 81 and 38%, respectively. This overall survival time was comparable to that of LD patients without ipsilateral pleural effusion.

Among the LD-SCLC patients with ipsilateral pleural effusion, the median survival time was 11.8 months (95% CI: 9.2–16.6), and the 1 and 2-year survival rates were 48 and 21%, respectively. This survival was intermediate between those of LD patients without ipsilateral pleural effusion and ED patients. An analysis of 2,580 patients treated in the Southwest Oncology Group trials demonstrated that the survival of patients with LD-SCLC and ipsilateral pleural effusion was not significantly different from that of patients with ED-SCLC and a single metastatic lesion. The median survival times were 13.0 and 12.0 months ($p = 0.85$), respectively.[9] Thus, our data was compatible with that of the Southwest Oncology Group trials. Another analysis of 5,758 patients with SCLC from the IASLC database also demonstrated consistent results.[10]

According to the proposed seventh edition of the TNM classification for lung cancer, LD patients with ipsilateral

TABLE 3. Survival Data

Subgroup	No. of Patients	Median Survival Time (mo) (95%CI)	1-yr Survival Rate (%)	2-yr Survival Rate (%)	3-yr Survival Rate (%)
ED	317	9.8 (8.8–10.6)	37	10	4
LD without ipsilateral pleural effusion	270	20.9 (19.1–22.7)	72	38	29
LD with ipsilateral pleural effusion	62	11.8 (9.2–16.6)	48	21	10
Receiving TRT	26	19.2 (16.7–27.9)	81	38	19
Not receiving TRT	36	9.1 (6.0–10.8)	28	11	6
Not receiving TRT in spite of disappearance of pleural effusion	8	10.5 (4.5–30.6)	38	25	13
Not receiving TRT and persistent pleural effusion after chemotherapy	28	9.2 (5.1–10.8)	25	7	4
Cytologically positive pleural effusion	11	9.3 (3.8–14.2)	27	9	0
Cytologically negative pleural effusion	15	12.7 (5.1–17.9)	53	20	7
Without cytological examination	36	12.9 (9.2–18.4)	56	28	17

CI, confidence interval; ED, extensive-disease; SCLC, small cell lung cancer; LD, limited-disease; TRT, thoracic radiotherapy.

pleural effusion will be classified as stage IV.[3–6] However, prognosis of LD patients with ipsilateral effusion is better than that of ED patients with distant metastasis. If surgical cases such as clinical stage I cases were excluded, the simple staging system, LD or ED, seemed to be sufficient to select treatment strategy.

In our study, four LD patients with ipsilateral pleural effusion have survived for more than 5 years. Three patients received chemotherapy and TRT as an initial treatment. The remaining one patient received only chemotherapy as an initial treatment but received chemotherapy and TRT after a local recurrence. TRT probably contributed to local control and long-term survival in those LD-SCLC patients with ipsilateral pleural effusion. A previous systematic review demonstrated that an early timing of TRT contributed to a significant improvement in long-term survival, compared with a late timing.[11] In patients whose ipsilateral pleural effusion disappears after chemotherapy, definitive TRT should be considered as early as possible.

Disease progression was confirmed in 21 out 26 patients (81%) who received chemotherapy and definitive TRT. The most common site of first failure was the brain. Nine of the 10 patients had not received PCI. In these nine patients, brain metastasis was the only site of recurrence. In LD-SCLC patients with ipsilateral pleural effusion who undergo chemotherapy and definitive TRT, PCI may further improve treatment outcome.

Cytologic examinations of the pleural effusion before treatment were only performed in 26 patients (42%). These cytologic results did not significantly affect overall survival. However, all nine patients with cytologically positive pleural effusion died within 31 months. A similar observation was reported in a cohort of IASLC database.[10]

Chemotherapy regimens were heterogeneous between LD and ED patients. More patients with ED received cisplatin and irinotecan containing regimens. However, response rates were similar between LD with ipsilateral pleural effusion and ED patients (74 and 76%).

In conclusion, long-term survival was achieved by LD-SCLC patients who underwent definitive TRT after their ipsilateral pleural effusion disappeared after induction chemotherapy. A prospective randomized trial is warranted to compare chemotherapy alone with chemoradiotherapy in LD-SCLC patients with ipsilateral pleural effusion. This work was supported in part by a Grant from the Ministry of Health, Labor, and Welfare for the 3rd term Comprehensive Strategy for Cancer Control and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor, and Welfare, Japan.

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Immunohistochemical expression of BCRP and ERCC1 in biopsy specimen predicts survival in advanced non-small-cell lung cancer treated with cisplatin-based chemotherapy☆

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ABSTRACT

Purpose: The aim of this study was to determine the prognostic value of expression of ATP binding cassette (ABC) transporter proteins and DNA repair gene proteins by immunohistochemically staining tumor biopsy specimens from patients with advanced non-small-cell lung cancer (NSCLC) being treated with platinum-based chemotherapy.
Experimental design: Expression of ABC transporter proteins, including BCRP (breast cancer resistance protein) and MRP2 (multidrug resistance proteins 2), and the DNA-repair-related proteins, ERCC1 (excision repair cross-complementation group 1) and BRCA1 (breast cancer type 1 susceptibility protein) was assessed immunohistochemically in 156 tumor samples from untreated stage IV NSCLC patients. All of the patients had received platinum-based chemotherapy. Response to chemotherapy, progression-free survival (PFS), and overall survival were compared in relation to expression of each of the proteins and to clinicopathological factors.
Results: High ERCC1 expression was associated with short survival (237 days vs. 453 days, log-rank $P = 0.03$), but not with response to chemotherapy or PFS. And high BCRP expression was associated with short survival (214 days vs. 412 days, log-rank $P = 0.02$) but not with response to chemotherapy or PFS. Multivariate analysis confirmed that negativity for the expression of BCRP tends to be an independent variable related to overall survival ($P = 0.06$).
Conclusions: This study examined ERCC1 and BCRP expression in biopsy specimens as candidates for predictors of the survival of patients with advanced NSCLC treated with platinum-based chemotherapy.

1. Introduction

More than half of all patients with non-small-cell lung cancer (NSCLC) have advanced stage IIIB or IV disease when first diagnosed, and patients with advanced NSCLC are candidates for systemic chemotherapy. Despite the increasing number of active chemotherapeutic agents available, patients with advanced NSCLC still have a median survival time of only 1 year. Intrinsic or acquired tumor-mediated drug resistance is a major clinical problem that can result in lack of tumor response to chemotherapy. Clinical investigators have recognized that several genetic abnormalities underlying NSCLC contribute to the development of the chemotherapeutic patterns that influence chemotherapeutic sensitivity to certain cytotoxic drugs. If the resistance to drugs could be explained by a simple, widely applicable method, such as immunohistochemical analysis of tumor biopsy specimens, the most effective drug candidates for the treatment could be more accurately identified.

The mechanisms of chemoresistance are likely to involve multiple gene products, and understanding of the potential modes of chemotherapeutic resistance to platinum-based chemotherapy has recently been achieved through studies that have correlated cytotoxicity with DNA repair or drug efflux [1,2]. Breast cancer

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resistance protein (BCRP) and multidrug resistance protein 2 (MPR2), a member of the superfamily of ATP binding cassette (ABC) transporter proteins, are involved in membrane transport during drug metabolism, and elevated expression of BCRP and MRP2 in vitro causes resistance to anticancer drugs [3–8]. Expression of BCRP and MRP2 has been found to be characterized by a reduced intracellular drug level. Moreover, there is convincing evidence that BCRP expression in biopsy specimens from patients with advanced NSCLC predicts response to chemotherapy or outcome [9].

The cytotoxic effect of anticancer platinum drugs is principally attributable to the formation of platinum-DNA adducts. Repair of these lesions in genomic DNA is mediated by both NER and Interstrand Cross-Link Repair (ICL-R) pathways, both in whose ERCC1 is a critical element [10–15]. Further, high ERCC1 expression is associated with resistance of human cancers to platinum-containing therapy [16–20].

Mutations in Breast cancer type 1 susceptibility protein (BRCA1), another DNA repair protein, can induce resistance to cisplatin-mediated apoptosis. BRCA1 is also involved in the repair of DNA damage induced by platinum drugs [21,22].

Attempts to overcome resistance have mainly involved the use of combination therapy with different classes of drugs in this study. We focused on the two different classes of proteins involved in resistance: ABC transporter proteins and DNA damage repair proteins. We quantified expression of ABC transporter (BCRP, MRP2) proteins and DNA repair genes (ERCC1, BRCA1) proteins by immunohistochemical staining of tumor biopsy specimens collected before chemotherapy. We also evaluated the value of these proteins for predicting tumor response and survival in NSCLC patients treated with platinum-based combination therapy.

2. Materials and methods

2.1. Subjects

A total 200 of stage IV NSCLC patients received platinum-based combination chemotherapy at the National Cancer Center Hospital East between February 1996 and December 2004 because they had a PS of 0 or 1 on the Eastern Cooperative Oncology Group scale. Adequate tumor biopsy specimens collected before chemotherapy were available for 156 of these patients, and they were analyzed in this study. All tumor specimens analyzed were collected before chemotherapy. The histological classification was based on a WHO report. Clinical staging was based on an initial evaluation that consisted of a clinical assessment, chest X-ray, computed tomography of the chest and abdomen, computed tomography or magnetic resonance imaging of the brain, and bone scintigraphy. The current International Staging System was used to stage clinical disease. The clinicopathological characteristics of all of the patients are listed in Table 1. Their median age at diagnosis was 62 years (range, 39–79 years), and 44 of the 156 stage IV patients were women. All of the patients were treated with platinum-based combination chemotherapeutic regimens, which are considered standard regimens for patients with advanced NSCLC. After obtaining informed consent in accordance with institutional guidelines, all of the patients underwent tumor biopsy and chemotherapy.

2.2. Chemotherapy

All of the patients received at least 2 courses of platinum-based chemotherapy and received courses until the appearance of progressive disease. The platinum regimens were vinorelbine 25 mg/m^2 on days 1 and 8 plus cisplatin 80 mg/m^2 on day 1 of a 21-day cycle (68 patients), docetaxel 60 mg/m^2 on day 1 plus cisplatin 80 mg/m^2 on day 1 of a 21-day cycle (20 patients), irinotecan 60 mg/m^2 on days 1, 8, and 15 plus cisplatin 80 mg/m^2 on day 1 of a 28-day cycle (16 patients), gemcitabine 1000 mg/m^2 on days 1 and 8 plus cisplatin 80 mg/m^2 on day 1 of a 21-day cycle (15 patients), and paclitaxel 200 mg/m^2 administered over 3 h on day 1 plus carboplatin dosed with an area under the curve of 6 on day 1 of a 21-day cycle (28 patients). We used the standard criteria to evaluate the response to chemotherapy. Complete response was defined as the disappearance of all clinically detectable disease for at least 4 weeks. Partial response required a minimum of a 50% reduction in the sum of the products of the greatest perpendicular diameters of all measurable lesions for a minimum of 4 weeks. Progressive disease was defined as the appearance of new lesions or an increase in disease >25% measured in the same manner as for partial response. All other results were classified as "no change." The response rate was defined as the sum of the complete responses and partial responses cases expressed as a percentage of the total number of cases.

2.3. Immunohistochemistry

Immunohistochemical staining was performed on 4 μm formalin-fixed, paraffin-embedded tissue sections. The slides were deparaffinized in xylene and dehydrated in a graded ethanol series. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 15 min. For antigen retrieval, the slides for BCRP (clone BXP21, dilution 1:20, Sanbio, Uden, Netherlands), MRP2 (clone M2III-6, dilution 1:20, Sanbio, Uden, Netherlands), ERCC1 (clone 8F1,dilution 1:100, Thermo Fisher, Scientific Inc., Fremont, USA), and BRCA1 (clone MS110, dilution 1:150, EMD chemicals Inc., Darmstadt, Germany) were immersed in 10 mm citric buffer solution (pH 6.0). The slides for BCRP, MRP2, and BRCA1 were heated to 95 °C by exposure to microwave irradiation for 20 min, and the slides for ERCC1 were heated to 125 °C by exposure to autoclave irradiation for 15 min. The slides were then allowed to cool for 1 h at room temperature and washed in water and PBS. Next, nonspecific binding was blocked by preincubation with 2% BSA plus 0.1% NaN_3 for 30 min. The blocking solution was drained off, and the slides were incubated overnight at 4 °C with the primary antibodies. Staining with an irrelevant mouse IgG1 or IgG2a was routinely performed as a negative control procedure. After washing three times in PBS, the slides were incubated with a labeled polymer, EnVision + Peroxidase Mouse (DAKO, Glostrup, Denmark), for 30 min. The chromogen used was 2% 3,3′-diaminobenzidine in 50 mM Tris buffer (pH 7.6) containing 0.3% hydrogen. Slides were counterstained with hematoxylin. Normal liver and lung tissue was used as a positive control. Staining with all antibodies was considered positive if >10% of the tumor cells stained, because a 10% cutoff level has been used in several studies using these antibodies. All of the slides were examined and scored independently by two observers (S.O and G.I) without any knowledge of the patient's clinical data. When their staining evaluations differed, the examiners discussed then, with or without reevaluating the slides, until agreement was reached.

2.4. Statistical analysis

The correlations between immunohistochemical expression and the clinical variables and response to chemotherapy were evaluated by the χ^2 test or Fisher's exact test, as appropriate. Overall survival was measured from the start of chemotherapy to the date of death from any cause or the date the patient was last known to be alive. Survival curves were estimated by the Kaplan–Meier method. The Cox proportional hazards model was used for multivariate

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analysis. P values < 0.05 were considered significant. Two-sided statistical tests were used in all of the analyses. Statistical analysis software (Dr SPSSII, Windows) was used to perform the analyses

3. Results

3.1. Expression of ABC transporter and DNA damage repair proteins in NSCLC

Eighty (51%) of the 156 tumors were BCRP-positive, 26 (17%) were MRP2-positive, 100 (64%) were ERCC1-positive, and 131 (84%) were BRCA1-positive. Median percentage of staining for BCRP, ERCC1, BRCA1, and MRP2 was 20%, 40%, 50%, and 10%, respectively (the range was 0–100%).

Most of the ABC-transporter-protein-positive tumors showed mixed membranous and cytoplasmic staining. An external positive control for BCRP was canalicular membrane in liver. BCRP in the apical membrane of the bronchial layer was used an internal control, and the endothelial cells of blood vessels also stained positive. An external positive control for ERCC1 was endothelial in the tonsil and an internal positive control was stroma cells. Representative immunohistochemical BCRP and ERCC1 staining is shown in Fig. 1. The relationship between expression of ABC transporter proteins and DNA damage repair proteins and the clinical variables is shown in Table 1. ERCC1 expression and BRCA1 expression were significantly greater in the patients with a smoking history (≧20 pack years) (P = 0.015). BRCA1 expression was significantly greater in the males than in the females (P = 0.027). BRCA1 expression correlated to ERCC1 expression (P = 0.003). BCRP expression correlated to ERCC1 (P = 0.012), MRP2 (P = 0.005), but not BRCA1 (P = 0.126) (data not shown).

3.2. Expression of ABC transporter and DNA damage repair proteins and clinical outcome

It was possible to assess all 156 patients for response to chemotherapy and to analyze their survival data. The relationships between clinical variables and response to chemotherapy and survival in this study are shown in Table 2. Only "smoking history" was significantly associated with both PFS (P = 0.05) and overall survival (P = 0.02). Table 3 shows the relationships between expression of ABC transporter proteins and DNA damage repair proteins and the response to chemotherapy and survival. No significant associations were found between MRP2 expression and response to chemotherapy (P = 0.63), PFS (P = 0.94), or survival (P = 0.96), and between BRCA1 expression and response to chemotherapy (P = 0.62), PFS (P = 0.67), or survival (P = 0.06). By contrast, BCRP expression was significantly associated with both PFS (P = 0.02) and survival (P = 0.02), but not with response to chemotherapy (P = 0.15). ERCC1 expression was associated with overall survival (P = 0.03) but not with response to chemotherapy (P > 0.09) or PFS (P = . 0.06).

3.3. Multivariate analysis for PFS and overall survival

Multivariate analysis was performed by using the Cox proportional hazards model to determine whether the prognostic value of BCRP or ERCC1 disappeared when other prognostic factors were considered (Tables 4 and 5). A multivariate analysis that included gender, age, smoking history, PS, histology, BCRP, and ERCC1, showed that BCRP was not a significant independent variable correlated with PFS (P = 0.13) but overall survival was marginal (P = 0.06). The BCRP-positive value for overall survival yielded a hazard ratio of 0.72, with a 95% confidence interval of 0.51–1.01. The results show that negativity for the expression of BCRP tends to be a prognostic factor in advanced NSCLC. The PFS and overall survival curves drawn by the Kaplan–Meier method are shown according to BCRP in Fig. 2. Median survival time in the BCRP-negative group was 412 days, as opposed to 214 days in the BCRP-positive group.

4. Discussion

In this study the BCRP-positive cases had a shorter overall survival time, and BCRP expression tend to be a prognostic factor overall survival in the multivariate analysis. Expression of MRP2, on the other hand, was not an independent prognostic factor, a finding that was consistent with previous studies [9,10]. MRP2 was studied within the IALT biologic program [23]. This was the largest group of NSCLC patients used for the study of MRP2 expression and the result was that MRP2 does not predict response to adjuvant cisplatin-based chemotherapy. Yoh et al. found that the expression of BCRP in stages III and IV NSCLC patients was a significant independent variable that correlated with PFS and tend to correlated

Table 1
Relationship between clinical variables and immunohistochemical expression

	n (%)	BCRP-positive patients	MRP2-positive patients	ERCC1-positive patients	BRCA1-positive patients
Total	156	80	26	100	131
Gender					
Male	112 (72)	60	22	76	99[b]
Female	44 (28)	20	4	24	32
Age					
≧70	34 (22)	22	7	25	32
<70	122 (78)	58	19	75	99
Histology					
Ad	100 (64)	50	20	61	81
Non-ad	56 (36)	30	6	39	50
PS					
0	38 (24)	15	4	23	29
1	118 (76)	65	22	77	102
Smoking history					
≧20 pack year	99 (63)	56	19	71[a]	89[c]
<20 pack year	57 (37)	24	7	29	42

[a] P = 0.015.
[b] P = 0.027.
[c] P = 0.012.

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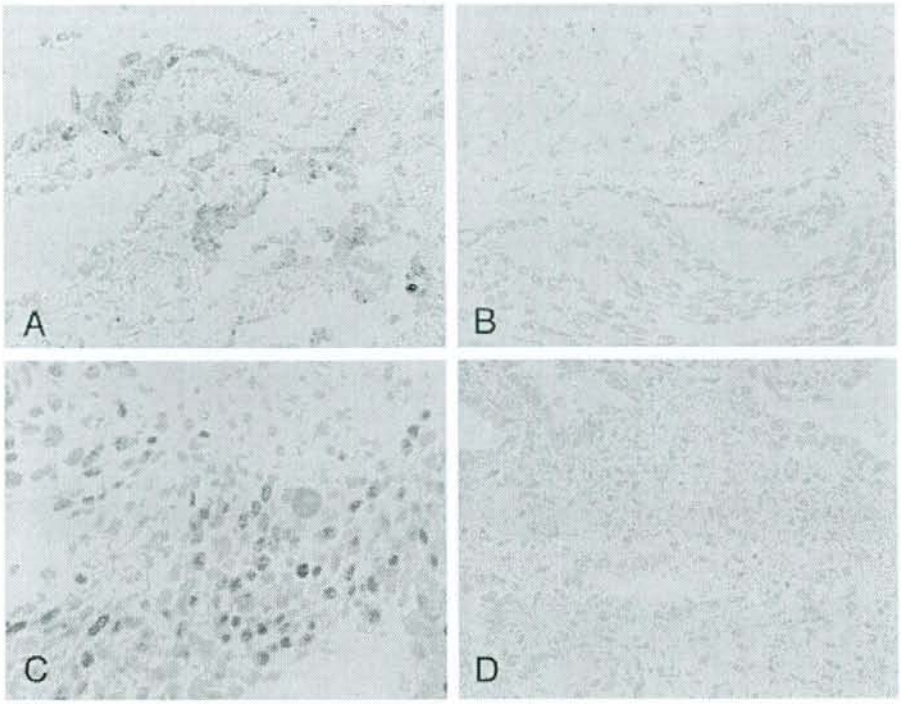

Fig. 1. Typical immunohistochemical staining patterns of NSCLC tumor biopsy specimens for BCRP (A and B) and ERCC1 (C and D). (A) Adenocarcinoma showing membrane staining for BCRP. (B) BCRP-negative adenocarcinoma. (C) Squamous cell carcinoma with positive nuclear staining for ERCC1. (D) ERCC1-negative adenocarcinoma.

with response to chemotherapy or overall survival in a multivariate analysis [9]. We think that the results of the present study reinforce the reliability of the prognostic significance of BCRP expression in stage IV NSCLC patients.

ERCC1 expression and BRCA1 expression were significantly greater in the patients with a smoking history ($P=0.015$, $P=0.012$ respectively). The correlation between smoking history and ERCC1, BRCA1 expression is often a lack in previous studies such as in the IALT-bio study. Fujii et al. [24] and Lee et al. [25] reported relationship between ERCC1, BRCA1 expression and smoking history, which tended to be greater in the patients with a smoking history but it was not significant statistically. Relationship between DNA repair gene protein expression and DNA damage arised from smoking could only be presumed. Interestingly, we also noticed in the present study that patients with high ERCC1 and BRCA1 double expression in tumors had shorter survival than patients who have

Table 2
Summary of relationship between clinical variables and response to chemotherapy or survival

	n	Response rate (%)	P	PFS (day)	P	MST (day)	P
Total	156	26		163		317	
Gender							
Male	112	24	0.32	155	0.17	307	0.23
Female	44	32		223		324	
Age							
≧70	34	18	0.27	119	0.10	261	0.14
<70	122	29		171		333	
Histology							
Ad	100	22	0.13	148	0.32	366	0.55
Non-ad	56	34		180		261	
PS							
0	38	26	>0.99	184	0.35	386	0.23
1	118	26		153		274	
Smoking history							
≧20 pack year	99	23	0.26	151	0.05	256	0.02
<20 pack year	57	32		223		426	

PFS: progression free survival, MST: median survival time.

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Table 3
Relationship between immunohistochemical expression and response to chemotherapy or survival

	n	Response rate (%)	P	PFS (day)	P	MST (day)	P
BCRP							
Positive	80	21	0.15	148	0.02	214	0.02
Negative	76	32		211		412	
MRP2							
Positive	26	31	0.63	161	0.94	344	0.96
Negative	130	25		165		304	
ERCC1							
Positive	100	26	>0.99	148	0.06	237	0.03
Negative	56	27		187		453	
BRCA1							
Positive	131	27	0.62	161	0.67	261	0.06
Negative	25	20		184		461	

PFS: progression free survival, MST: median survival time.

Table 4
Multivariate analysis for overall survival of 156 patients

Variables	Category	Risk ratio	95% CI	P
Gender	Male vs. female	1.08	0.68–1.72	0.74
Age	≧70 vs. <70	1.18	0.75–1.82	0.50
PS	0 vs. 1	1.16	0.77–1.75	0.48
Histology	Ad vs. non-ad	1.33	0.93–1.90	0.12
Smoking history	≧20 vs. <20	1.47	0.96–2.27	0.08
BCRP	(−) vs. (+)	0.72	0.51–1.01	0.06
ERCC1	(−) vs. (+)	0.75	0.52–1.07	0.12

other expression pattern for those two markers ($p = 0.0027$) (data not shown).

A relation between expression of ERCC1 mRNA and resistance to platinum-based chemotherapy has been corroborated by small, retrospective studies in patients with advanced gastric, ovarian, colorectal, and esophageal cancer and in NSCLC patients [16–20]. Simon et al. reported that patients who have undergone complete resection of NSCLC with high ERCC1 mRNA expression have a better survival than patients with low ERCC1 mRNA expression [26]. Olaussen et al. found that patients who underwent complete resection of ERCC1-negative NSCLC appeared to benefit from adjuvant cisplatin-based chemotherapy, and, showed that in the group that had not received adjuvant therapy patients with ERCC1-positive tumors had a longer overall survival than patients with ERCC1-negative tumors [27]. Our findings showed that expression of ERCC1 was significantly associated with overall survival but not with response to chemotherapy or PFS. Thus, ERCC1 expressing tumor may become a poor prognostic tumor by other factors induced by administered chemotherapy (e.g., tolerance to DNA damage).

Rosell's group in Barcelona reported overexpression of BRCA1 mRNA was strongly associated with poor survival in NSCLC patients [28]. In this study, BRCA1 protein expression was borderline significance for correlation with overall survival. The reason of this discrepantly result might be explained by the difference of study population. Rosell's group study targeted for operable early stage NCSLC. To solve this problem, further investigation was needed.

In our study, BCRP expression, alone not ERCC1 expression, was of marginal significant prognostic value for MST in the multivariate analysis. Liedert et al. reported overexpression of ABC transporter protein in a cisplatin-resistant melanoma cell line [29], and this observation was accompanied by reduced formation of platinum-DNA adducts measured by an immunocytologic assay. The overexpression of ABC transporter may regulate the formation of platinum-DNA adducts in tumor cells, and BCRP may act upstream during the chemoresistance process. This mechanism may have been responsible for BCRP expression alone, not ERCC1 expression, being of marginal significant prognostic value for MST in the multivariate analysis in this study.

It is noteworthy that there was no significant association between any of the markers and response to chemotherapy. One explanation might be that response rate by itself did not correlate to survival in our study. Another consideration to answer to the discrepancy between response, PFS and overall survival, is that ERCC1 expressing tumor may become a poor prognostic tumor due to other factors than those induced by chemotherapy. Illustration of this notion came from a recent study by Hadnagy et al. [30] who recently reported a relationship between BCRP expression and cancer stem cells that seemed to play a pivotal role in tumor progression. BCRP expression by itself may be poor prognostic factor regardless of chemotherapy. A widely used flow cytometry assay for identifying cancer stem cells defines a "side-population" (SP) of cells that display Hoechst 33342 [31]. Cancer stem cells can be puri-

Table 5
Multivariate analysis for progression free survival of 156 patients

Variables	Category	Risk ratio	95% CI	P
Gender	Male vs. Female	0.90	0.56–1.49	0.70
Age	≧70 vs. < 70	1.24	0.78–1.98	0.37
PS	0 vs. 1	1.10	0.70–1.74	0.68
Histology	Ad vs. Non-ad	1.39	0.93–2.07	0.11
Smoking history	≧20 vs. <20	1.41	0.91–2.21	0.12
BCRP	(−) vs.(+)	0.72	0.48–1.10	0.13
ERCC1	(−) vs.(+)	0.82	0.54–1.26	0.37

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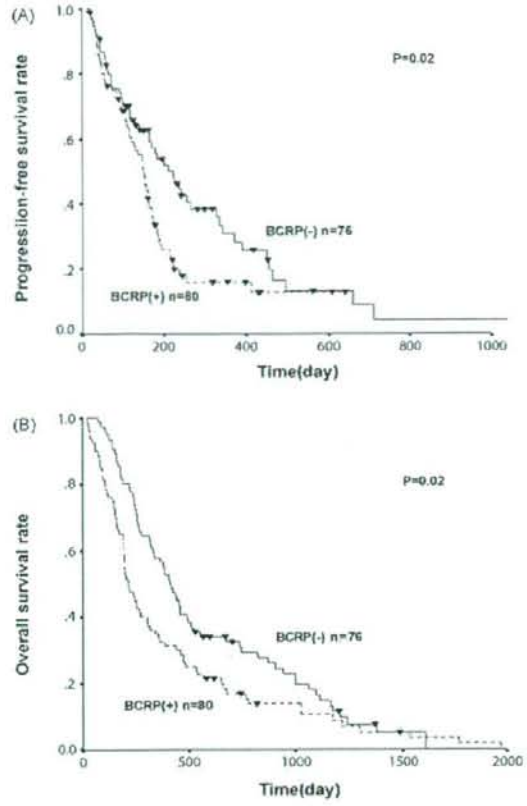

Fig. 2. (A) Progression-free survival curve of 156 patients with advanced non-small-cell lung cancer, according to BCRP expression. The median progression-free survival period of the BCRP-negative patients and BCRP-positive patients was 211 and 148 days, respectively. (B) Overall survival curves of 156 patients with advanced non-small-cell lung cancer, according to BCRP expression. Patients with BCRP-negative tumors survived longer than those with BCRP-positive tumors, and the difference was statistically significant ($P = 0.02$).

fied based on the efflux of dyes and Hoechst 33342 [32,33]. Zhou et al. reported that BCRP mRNA is expressed in a wide variety of stem cells and is a molecular determinant of the SP cells, and moreover, dyes and Hoechst 33342 efflux activity was provided by BCRP expression in mice [34]. Haraguchi et al. [35] reported significantly increased BCRP expression in SP cells in human gastrointestinal system cancer cell lines and that SP cells exhibited greater resistance to chemotherapy. The self-renewal and chemoresistance capacities of these cancer SP cells may also play important roles in maintaining cancer foci to proliferate after chemotherapy and radiotherapy. Investigating whether BCRP-positive cells have the characteristics, as cancer stem cells in NSCLC will be a future task.

We speculate, that patients with tumors that are positive for BCRP expression show drug resistance to platinum-based chemotherapy. We suggest that BCRP serve as molecular target for reducing drug resistance. Kuppens et al. reported a phase I study of Elacridar (GF120918) [36,37]. Minderman et al. [38] reported Bricodar (VX710) increases drug retention and enhances chemosensitivity in resistant cells expressing BCRP. Another approach to solving platinum-based chemotherapeutic resistance, non-platinum chemotherapy will be alternative regimen for the subgroup which over express BCRP protein.

Conflict of interest

The authors certify that there are no potential conflicts of interest.

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Marital status and non-small cell lung cancer survival: the Lung Cancer Database Project in Japan

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Abstract

***Objective*: Previous studies have suggested that marital status is associated with survival from lung cancer; however, its association is not conclusive. The association between marital status and survival in Japanese patients with non-small cell lung cancer (NSCLC) was prospectively investigated.**

***Methods*: Between July 1999 and July 2004, a total of 1230 NSCLC patients were enrolled. The baseline survey consisted of the collection of clinical information and various demographic data, including marital status. A Cox regression model was used to estimate the hazards ratio (HR) of all-cause mortality adjustments for age, BMI, education level, performance status, histology type, clinical stage, smoking status, choice of definitive treatment, and depression.**

***Results*: The multivariable adjusted HR of male widowed patients versus male married patients was 1.7 (95% confidence interval = 1.2–2.5, $p = 0.005$). However, no significant increased risk of death in female widowed patients compared with female married patients was observed (HR = 0.7, 95% confidence interval = 0.5–1.1, $p = 0.15$). With regard to separated/divorced and single patients no significant increased risk of death in male and/or female compared with married patients was observed.**

***Conclusions*: The present data suggest that male widowed patients with NSCLC have a higher mortality rate than male married patients with NSCLC, after controlling for various factors.**

Keywords: marital status; non-small cell lung cancer; prospective study; survival

Introduction

Lung cancer is among the most common forms of cancer and is the most common cause of cancer-related death in the world [1,2]. Many studies have suggested that marital status is associated with survival from lung cancer; however, its association is not conclusive. Having a spouse die can significantly increase a person's risk of death; this 'widow/widower effect' is especially pronounced in men [3–6]. Therefore, the association between marital status and lung cancer survival should be clarified according to sex and subdivided marital status, such as married, widowed, separated/divorced, or single. However, only two studies have examined the association between marital status and lung cancer survival according to sex and subdivided marital status [7,8]. One study suggested that separated/divorced, single, and widowed patients had a higher risk of death compared with married patients, for both sexes [7]. The other one found no association between marital status and survival among divorced and widowed patients [8]. However, these studies were limited by small sample sizes [8] and a lack of differentiation between small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [7,8].

Possible associations between marital status and survival from lung cancer may be mediated by several factors. An unmarried status has been associated with an increased frequency of unhealthy life-style behaviors (especially with regard to smoking habits), maladjustment to the cancer diagnosis (especially among subjects who continue smoking even after they have been diagnosed as having cancer), psychological reactions (especially depression), delays in seeking treatment (more