The major toxicity of amrubicin was hematologic. Grade 3 or 4 leukopenia was frequently observed in 51.5% of patients and grade 3 or 4 neutropenia in 84.8% of patients. Despite such severe hematologic toxicity, 88% of the total treatment cycles could be delivered without dose reduction and non-hematologic toxicities were mild. Although anorexia (54.5%) and nausea and vomiting (57.6%) were frequently observed, there were no episodes of grade 3 or 4 toxicity, except for 3 patients (9.1%) with grade 3 anorexia and 1 patient (3.0%) with grade 3 alopecia. A single patient developed interstitial pneumonia; however, this was reversible with steroid therapy. ECG abnormalities were observed in 3 patients, but they were each reviewed by a medical cardiologist and judged not to be clinically significant. No LVEF decrease was observed. Results show that the toxic profiles of amrubicin are acceptable and favorable in the treatment of extensive-disease SCLC, although due to its hematologic toxicity, in particular neutropenia, G-CSF support is needed.

In conclusion, amrubicin is a very active and promising agent with acceptable toxicity for patients with SCLC. Further studies are warranted in combination with other agents for this disease.

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# Performance Status and Sensitivity to First-line Chemotherapy Are Significant Prognostic Factors in Patients With Recurrent Small Cell Lung Cancer Receiving Second-line Chemotherapy

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**BACKGROUND.** To the authors' knowledge, the prognostic factors in recurrent small cell lung cancer (SCLC) patients treated with second-line chemotherapy have not yet been clearly identified to date.

**METHODS.** Between July 1992 and December 2003, 232 of 515 patients who were diagnosed to have SCLC at the National Cancer Center Hospital East were administered second-line chemotherapy for recurrent disease. The authors retrospectively analyzed the relation between clinical factors evaluated at the time of recurrence and the response to second-line chemotherapy or survival in these patients.

**RESULTS.** The results of univariate analyses revealed that response was significantly associated with the performance status (PS) alone, whereas survival was significantly associated with the PS, disease extent, and sensitivity to first-line chemotherapy. Multivariate analysis identified PS ($P < .0001$) and sensitivity to first-line chemotherapy ($P = .0024$) as the independent prognostic factors for survival. When the patients were grouped according to these 2 significant prognostic factors, the survival of patients with a PS of 0 to 1 was significantly better than that of the patients with a PS of 2 to 4 both among cases that were sensitive and those that were refractory to first-line chemotherapy. Although the survival of sensitive recurrent cases was significantly better than that of the refractory recurrent cases among the patients with a PS of 0 to 1 patients, no survival difference was observed between the sensitive and refractory recurrent cases in the patients with a PS of 2 to 4.

**CONCLUSIONS.** Both PS and sensitivity to initial chemotherapy were found to be significant prognostic factors for survival in recurrent SCLC patients treated with second-line chemotherapy. These 2 factors should therefore be used as stratification factors in future clinical trials. ***Cancer* 2008;113:2518–23.** © *2008 American Cancer Society.*

**KEYWORDS: small cell lung cancer, second-line chemotherapy, prognostic factor, performance status, sensitive recurrence, refractory recurrence.**

Although the proportion of small cell lung cancer (SCLC) among cases of lung cancer has been decreasing in recent years, it still accounts for 14% of all new lung cancer cases, and the actual number of patients was estimated to be 77,000 in the US and Europe in 2004.[1] In general, SCLC is an exceedingly aggressive cancer, and greater than 66% of patients have clinically obvious metastatic disease at the time of diagnosis.[2] SCLC is also extremely sensitive to chemotherapy; therefore, the main treatment strategy for SCLC is


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systemic chemotherapy. Currently, both cisplatin plus etoposide (PE) and cisplatin plus irinotecan (IP) are considered as standard chemotherapeutic regimens for SCLC.[3,4] Despite the high initial sensitivity to chemotherapy, the majority of patients develop disease recurrence. The prognosis of patients with recurrent SCLC is usually abysmal, and the overall survival time after recurrence is reportedly 2 to 4 months.[5]

In general, second-line chemotherapy is considered for cases with recurrent SCLC, and a few studies have reported on the efficacy of some second-line treatments.[6,7] For example, a prospective randomized trial comparing oral topotecan with best supportive care (BSC) revealed the benefits of treatment with oral topotecan in terms of the survival and quality of life.[7]

Although some studies have shown the importance of both response and the duration of the response to initial chemotherapy in predicting the efficacy of second-line chemotherapy,[8-10] the number of studies conducted to identify the prognostic factors in recurrent SCLC patients is quite limited. In this retrospective study, we investigated the prognostic factors in recurrent SCLC patients administered second-line chemotherapy to determine the factors that need to be used for stratifying the patients in future clinical trials.

## MATERIALS AND METHODS

### Patient Flow

Between July 1992 and December 2003, 515 patients were diagnosed to have SCLC at the National Cancer Center Hospital East, and 474 of these patients received initial chemotherapy with or without thoracic radiotherapy. Of 474 patients, radiographic response was observed in 409 patients, with 98 demonstrating complete response and 311 demonstrating partial response. An evaluation in April 2007 revealed that among these responders, 322 had developed disease recurrence, 75 had maintained responses, and 12 patients could not be evaluated for disease recurrence. Thus, 387 patients (including the 322 with disease recurrence and the 65 nonresponders) were considered potential candidates for second-line chemotherapy. Of these, 232 received second-line chemotherapy, whereas the remaining 155 did not. There were no distinct eligibility criteria for second-line chemotherapy, and the decision to administer chemotherapy was based on the patient's general condition and willingness to undergo second-line therapy. The patient flow is shown in Figure 1. Among patients who received second-line chemotherapy, those who deemed to have stable disease or not to be evaluable to first-line chemotherapy were treated right after completion of front-line therapy. All patients' data were obtained from our database.

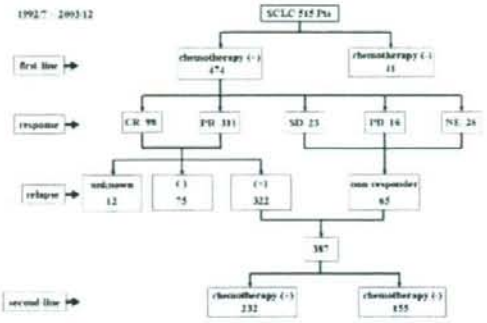


**FIGURE 1.** Patient flow is depicted. CR indicates complete response; NE, not evaluable; PD, progressive disease; PR, partial response; Pts, patients; SCLC, small-cell lung cancer; SD, stable disease; +, positive; −, negative.

### Analyzed Clinical Factors

The correlations between clinical factors evaluated at the time of disease recurrence, such as the age (<70/≥70), sex (women/men), Eastern Cooperative Oncology Group performance status (PS) (0-1 or 2-4), disease extent (limited disease [LD]/extensive disease), sensitivity to first-line chemotherapy (sensitive/refractory), and response to second-line chemotherapy or survival after disease recurrence were retrospectively investigated in the 232 patients. In this study, patients who responded to initial chemotherapy and developed disease recurrence more than 3 months after the completion of chemotherapy were defined as sensitive recurrence cases, whereas patients who did not respond to initial chemotherapy or developed disease recurrence within 3 months were defined as refractory recurrence cases.

### Tumor Evaluation and Statistical Analysis

Tumor response was re-evaluated by 2 physicians (Y.H.K. and K.G.) using the Response Evaluation Criteria in Solid Tumors (RECIST).[11] The survival time was measured from the date of disease recurrence. The survival curve was estimated by the Kaplan-Meier method, and compared by the log-rank test. Comparison between each clinical factor and response was performed by the chi-square test. Multivariate analysis was conducted according to the Cox proportional hazard model. $P < .05$ was considered to denote statistical significance. All statistical analyses were performed using StatView statistical

TABLE 1
Characteristics of All Patients at the Time of Disease Recurrence (N = 387)

| Characteristics | Second-line Chemotherapy (+) (n=232) | (−) (n=155) | P |
|---|---|---|---|
| Age at recurrence, y | | | <.0001 |
| Median | 65 | 68 | |
| Range | 30-80 | 28-87 | |
| Gender | | | .9867 |
| Women | 38 (16%) | 25 (16%) | |
| Men | 194 (84%) | 130 (84%) | |
| PS at recurrence | | | <.0001 |
| 0-1 | 162 (70%) | 43 (28%) | |
| 2-4 | 70 (30%) | 112 (72%) | |
| Disease extent at recurrence | | | .0476 |
| LD | 65 (28%) | 30 (19%) | |
| ED | 167 (72%) | 125 (81%) | |
| Response to first-line chemotherapy | | | <.0001 |
| CR/PR | 216 (93%) | 108 (70%) | |
| SD/PD | 16 (7%) | 47 (30%) | |
| Sensitivity to first-line chemotherapy | | | .1661 |
| Sensitive | 146 (63%) | 63 (41%) | |
| Refractory | 86 (37%) | 92 (59%) | |

+ indicates positive; −, negative; PS, performance status; LD, limited disease; ED, extensive disease; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

software (version 5.0; Abacus Concepts, Berkley, Calif).

## RESULTS

### Patient Characteristics

The characteristics of the 387 patients who were believed to be potential candidates for second-line chemotherapy (of whom only 232 eventually received second-line chemotherapy, designated as the chemotherapy group) are listed in Table 1. The patients in the chemotherapy group were significantly younger ($P < .0001$), had better PS ($P < .0001$), and had a higher frequency of LD ($P = .0476$) than the nonchemotherapy group. Whereas the response to first-line chemotherapy was significantly different ($P < .0001$), the sensitivity to first-line chemotherapy was not significantly different ($P = .1661$) between the 2 groups, and approximately 33% of the patients who received second-line chemotherapy were refractory recurrence cases. As first-line chemotherapy, 156 patients (67%) had received platinum plus etoposide combination chemotherapy, and 24 (10%) had received the IP regimen. The second-line chemotherapy regimens administered to the 232 patients are listed in Table 2. At our hospital, the vast majority of the patients had received some kind of platinum-based combination chemotherapy, such as cisplatin, vincristine, doxorubicin, and etoposide; cisplatin, etoposide, and irinotecan (PEI); IP; PE; or carboplatin plus etoposide. The distribution of these regimens was similar in the sensitive and refractory recurrence patients.

TABLE 2
Second-line Chemotherapy Regimens Administered to 232 Patients

| Regimen | No. of Patients | No. Sensitive (%) | No. Refractory (%) |
|---|---|---|---|
| CODE | 80 | 50 (34) | 30 (35) |
| PEI | 44 | 17 (12) | 27 (31) |
| IP | 34 | 28 (19) | 6 (7) |
| PE | 19 | 13 (9) | 6 (7) |
| CE | 14 | 12 (8) | 2 (2) |
| TOP | 14 | 9 (6) | 5 (6) |
| CPT-11 | 13 | 9 (6) | 4 (5) |
| AMR | 6 | 5 (4) | 1 (1) |
| Others | 8 | 3 (2) | 5 (6) |
| Total | 232 | 146 (100) | 86 (100) |

CODE indicates cisplatin, vincristine, doxorubicin, and etoposide; PEI, cisplatin, etoposide, and irinotecan; IP, cisplatin and irinotecan; PE, cisplatin and etoposide; CE, carboplatin and etoposide; TOP, topotecan; CPT-11, irinotecan; AMR, amrubicin.

TABLE 3
Univariate Analysis for Response and Survival

| Characteristics | No. of Patients | Response Rate, % | P | MST, Months | P |
|---|---|---|---|---|---|
| Age at recurrence, y | | | | | |
| <70 | 167 | 56 | .5058 | 9.0 | .6347 |
| ≥70 | 65 | 62 | | 8.8 | |
| Gender | | | | | |
| Women | 38 | 68 | .1826 | 10.0 | .5672 |
| Men | 194 | 55 | | 8.7 | |
| PS at recurrence | | | | | |
| 0-1 | 162 | 63 | .0126 | 11.0 | <.0001 |
| 2-4 | 70 | 44 | | 4.9 | |
| Disease extent at recurrence | | | | | |
| LD | 65 | 62 | .5085 | 12.6 | .0043 |
| ED | 167 | 56 | | 7.3 | |
| Sensitivity to first-line chemotherapy | | | | | |
| Sensitive | 146 | 60 | .4413 | 10.6 | .0016 |
| Refractory | 86 | 53 | | 6.8 | |

MST indicates median survival time; PS, performance status; LD, limited disease; ED, extensive disease.

### Predictive and Prognostic Factors

According to the results of the univariate analyses, response was significantly associated with the PS alone, whereas survival was significantly associated with the PS, disease extent, and sensitivity to first-line chemotherapy (Table 3). Survival curves drawn according to the PS and sensitivity to first-line chemotherapy are shown in Figure 2 and 3, respectively. Multivariate analysis identified PS ($P < .0001$) and

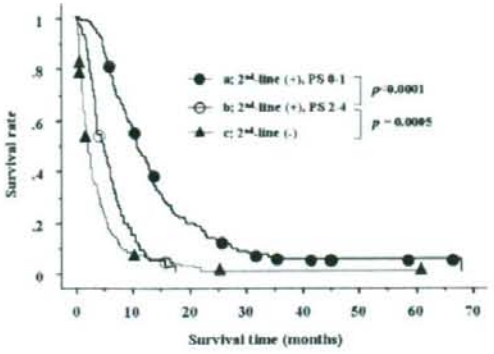


**FIGURE 2.** Survival curves according to the performance status (PS) at the time of disease recurrence. + indicates positive; −, negative.

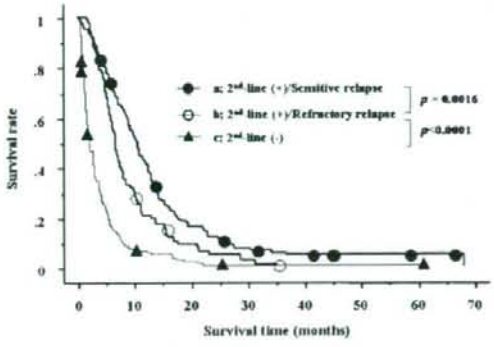


**FIGURE 3.** Survival curves according to sensitivity to first-line chemotherapy. + indicates positive; −, negative.

sensitivity to first-line chemotherapy ($P = .0024$) as the independent prognostic factors for survival (Table 4). The survival of patients with a PS of 2 to 4 ($P = .005$) (Fig. 2) and refractory disease recurrences ($P < .0001$) (Fig. 3) was significantly better than that of those who did not receive second-line chemotherapy.

In addition, we performed further analysis, in which all patients who received second-line chemotherapy were divided into 4 groups according to the combination of the 2 identified independent prognostic factors for survival: Group A (PS of 0-1/sensitive recurrence), Group B (PS of 0-1/refractory recurrence), Group C (PS of 2-4/sensitive recurrence), and Group D (PS of 2-4/refractory recurrence). The survival curves for each group are shown in Figure 4. The survival of patients with a PS of 0 to 1 was significantly better than that of the patients with a PS of 2 to 4 among both cases with sensitive

TABLE 4
Multivariate Analysis for Survival

| Variables | Odds Ratio | 95% CI | P |
|---|---|---|---|
| PS at recurrence, 0-1 | 3.171 | 2.307-4.357 | <.0001 |
| Disease extent at recurrence, LD | 1.308 | 0.956-1.790 | .093 |
| Sensitivity to first-line chemotherapy, sensitive | 1.544 | 1.166-2.043 | .0024 |

95% CI indicates 95% confidence interval; PS, performance status; LD, limited disease.

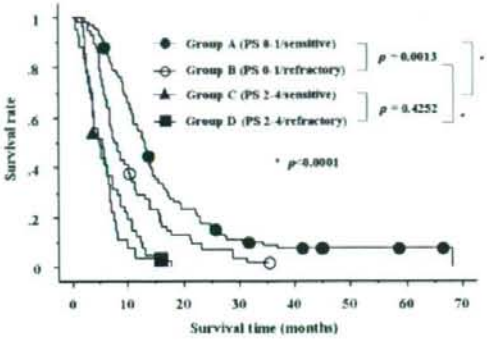


**FIGURE 4.** Survival curves according to the 2 independent prognostic factors. PS indicates performance status.

(Group A vs Group C; $P < .0001$) and those with refractory recurrence (Group B vs Group D; $P = .0001$). Whereas the survival of the sensitive recurrence cases was significantly better than that of the refractory recurrence cases among the patients with a PS of 0 to 1 (Group A vs Group B; $P = .0013$), no survival difference was observed between the sensitive and refractory recurrence cases among the patients with a PS of 2 to 4 patients (Group C vs Group D; $P = .4252$).

Among the 232 patients who received second-line chemotherapy, 29 received the same regimen as first-line chemotherapy, and the rest received a regimen different from first-line chemotherapy. However, these differences did not appear to have an impact on either response ($P = .7519$) or survival ($P = .5873$).

## DISCUSSION

Some studies have shown the importance of both response and the duration of the response to initial chemotherapy in predicting the survival of recurrent SCLC patients receiving second-line chemotherapy,[8-10] and currently it is widely accepted that recurrent SCLC patients should be classified into 2 groups: cases with sensitive recurrence and those with refrac-

tory recurrence.[12] In contrast, Sundstrom et al, who recently analyzed 19 clinical factors at both the time of initial diagnosis and the time of recurrence, have suggested that the PS at the time of disease recurrence, and not the sensitivity status to first-line chemotherapy, was the only significant prognostic indicator for survival after second-line chemotherapy.[13] In this study, we investigated the relation between clinical factors evaluated at the time of disease recurrence and survival after recurrence, and identified both PS and sensitivity to first-line chemotherapy as being significant prognostic factors for survival.

Some may argue that the survival time of the patients with a PS $\geq$3 in this study was too short, which might have strongly influenced the inferior survival of the patients with a PS of 2 to 4 as compared with that of the patients with a PS of 0 to 1. Although our study included 18 cases with a PS $\geq$3 among the patients administered second-line chemotherapy, the results of the analyses were found to be the same even after exclusion of these patients with a PS $\geq$3 (data not shown). This finding suggests that the prognosis of the patients with a PS of 2 is clearly different from that of the patients with a PS of 0 to 1 patients. The diversity of our second-line regimens may be criticized as well, because the differences in the regimens could have affected the patients' outcomes. However, to our knowledge, there are no comparative studies suggesting the superiority of any particular regimen for second-line chemotherapy. At our hospital, as shown in Table 2, mainly platinum-based combination chemotherapy is used even for second-line chemotherapy, and various agents are combined with platinum agents.

The results of the current study indicate that the prognosis of patients with impaired PS is inevitably poor. In such patients, no survival difference was found between the cases with sensitive and those with refractory recurrence. Does this mean that patients with a PS $\geq$2 should not receive second-line chemotherapy? A phase 3 trial comparing oral topotecan with BSC demonstrated a significant survival advantage of oral topotecan, and such survival benefit was also found to be preserved for patients with a PS of 2 who accounted for approximately 30% of the enrolled patients.[7] Conversely, with regard to the patients with a PS $\geq$3, there is no evidence as yet to suggest the clinical benefit of administering second-line chemotherapy. In our study, however, response rates of 64% in patients with a PS of 3 (n = 14) and 25% in patients with a PS of 4 (n = 4) were observed. These results suggest that second-line chemotherapy might be beneficial for adequately selected patients with a PS of $\geq$2, although the survival benefit is limited as compared with that for the patients with a PS of 0 to 1. Further studies are required for precise selection of criteria for second-line chemotherapy.

In this study, the survival of patients who received second-line chemotherapy with a PS of 2 to 4 or refractory recurrences was still significantly better than that of those who did not receive second-line chemotherapy. However it was not surprising, because the patient selection for second-line chemotherapy was performed pragmatically, and patients who were thought to be unfit for chemotherapy were not administered second-line chemotherapy. The finding that the nonchemotherapy group had more patients with a PS of 2 to 4 and refractory recurrence, the 2 independent prognostic factors identified in this study, suggests that our patient selection was reasonable.

The prognosis of recurrent SCLC patients is generally poor, and to our knowledge no standard treatment has been established for these patients. In addition to the randomized trial comparing oral topotecan with BSC mentioned above, 2 phase 3 trials for recurrent SCLC have been reported to date.[14,15] A trial comparing intravenous topotecan with the combination of cyclophosphamide, doxorubicin, and vincristine demonstrated comparable response rates and survival; however, intravenous topotecan yielded greater symptomatic improvement for 4 of the 8 symptoms evaluated.[14] In the other trial, comparing oral topotecan with intravenous topotecan, no survival difference was observed.[15] Currently, topotecan is the only drug approved by the US Food and Drug Administration for recurrent SCLC. Recently, however, promising results of phase 2 studies have been reported for drugs other than topotecan for recurrent SCLC. In particular, amrubicin[16,17] and PEI[18,19] have been shown to yield excellent response rates and survival in not only sensitive but also refractory recurrent cases. In Japan, a phase 3 randomized trial comparing topotecan with PEI is now ongoing.

In conclusion, we identified PS and sensitivity to initial chemotherapy as being significant prognostic factors for survival in patients with recurrent SCLC treated with second-line chemotherapy. PS was also found to be predictive in terms of response. In future clinical trials of second-line chemotherapy, both PS and sensitivity to initial chemotherapy should be incorporated as stratification factors. The survival benefit of second-line chemotherapy is limited in patients with impaired PS, even among sensitive recurrence cases. Therefore, careful consideration of the potential risks and benefits is required in the treatment of these patients.

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# Expression of breast cancer resistance protein is associated with a poor clinical outcome in patients with small-cell lung cancer

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ABSTRACT

*Background:* ATP-binding cassette (ABC) transporter and DNA excision repair proteins play a pivotal role in the mechanisms of drug resistance. The aim of this study was to investigate the expression of ABC transporter and DNA excision repair proteins, and to elucidate the clinical significance of their expression in biopsy specimens from patients with small-cell lung cancer (SCLC).
*Methods:* We investigated expression of the ABC transporter proteins, P-glycoprotein (Pgp), multidrug resistance associated-protein 1 (MRP1), MRP2, MRP3, and breast cancer resistance protein (BCRP), and the DNA excision repair proteins, excision repair cross-complementation group 1 (ERCC1) protein and breast cancer susceptibility gene 1 (BRCA1) protein, in tumor biopsy specimens obtained before chemotherapy from 130 SCLC patients who later received platinum-based combination chemotherapy, and investigated the relationship between their expression and both response and survival.
*Results:* No significant associations were found between expression of Pgp, MRP1, MRP2, MRP3, ERCC1, or BRCA1 and either response or survival. However, there was a significant association between BCRP expression and both response ($p = 0.026$) and progression-free survival (PFS; $p = 0.0103$).
*Conclusions:* BCRP expression was significantly predictive of both response and progression-free survival (PFS) in SCLC patients receiving chemotherapy. These findings suggest that BCRP may play a crucial role in drug resistance mechanisms, and that it may serve as an ideal molecular target for the treatment of SCLC.



## 1. Introduction

Lung cancer is the leading cause of cancer-related deaths in many industrialized countries. Although the proportion of patients with small-cell lung cancer (SCLC) has been decreasing, it still accounts for approximately 15% of all cases of lung cancer. SCLC is one of the most chemo-sensitive solid tumors, but the vast majority of patients eventually experience a relapse, and as a result the median survival time is 14–20 months for limited disease (LD) and 7–10 months for extensive disease (ED) [1].

Intrinsic or acquired drug resistance is considered to be a major factor limiting the effectiveness of chemotherapy. Drug resistance by tumors occurs not only to a single cytotoxic agent, but in the form of cross-resistance to other cytotoxic agents, called multidrug resistance (MDR). One of the major mechanisms of MDR is increased ability of tumor cells to actively efflux drugs, which leads to a decrease in intracellular drug accumulation, and the mechanism is mediated by ATP-dependent drug efflux pumps that are known as ATP-binding cassette (ABC) transporters [2,3]. To date, at least 48 human ABC transporters have been identified, and they have been divided into seven subfamilies, ABC-A through ABC-G. Five of them, P-glycoprotein (Pgp), multidrug resistance associated-protein 1 (MRP1), MRP2, MRP3, and breast cancer resistance protein (BCRP), have been most intensively investigated, and *in vitro* studies have demonstrated associations between their expression and resistance to cytotoxic drugs commonly used in the treatment of SCLC, including etoposide, irinotecan, and topotecan [4].

Another important mechanism of drug resistance is increased repair of DNA damage mediated by the DNA excision repair gene. Resistance to platinum is associated with increased removal of platinum-DNA adducts, and DNA excision repair plays a pivotal role in this process [5]. Nucleotide excision repair (NER) is a major

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**Table 1**
Panel of primary antibodies.

| Antibody | Clone | Pretreatment | Dilution | City/nation | Source |
|---|---|---|---|---|---|
| Pgp (mono) | JSB-1 | Autoclave | 1:20 | Newcastle/United Kingdom | Novocastra |
| MRP1 (mono) | MRPm6 | Autoclave | 1:50 | Uden/Netherlands | Sanbio |
| MRP2 (mono) | M2III-6 | Autoclave | 1:20 | Uden/Netherlands | Sanbio |
| MRP3 (mono) | DTX1 | Autoclave | 1:100 | Newcastle/United Kingdom | Novocastra |
| BCRP (mono) | BXP21 | Autoclave | 1:20 | Uden/Netherlands | Sanbio |
| ERCC1 (mono) | 8F1 | Autoclave | 1:100 | Warm Springs/United States | Lab vision |
| BRCA1 (mono) | MS110 | Microwave | 1:100 | San Diego/United States | Carbiochem |

mechanism for repairing platinum-DNA adducts, and it is now known that there are two pathways in NER: transcription-coupled NER (TC-NER) and global genomic NER (GG-NER) [5]. Among NER proteins, excision repair cross-complementation group 1 (ERCC1) protein, which is involved in the GG-NER pathway, has been most intensively investigated. Expression of ERCC1 has recently been shown to be a significant negative predictive factor for survival of non-small cell lung cancer (NSCLC) patients receiving cisplatin-based adjuvant chemotherapy [6]. On the other hand, the results of an *in vitro* study have suggested the superiority of TC-NER pathway, in which breast cancer susceptibility gene 1 (BRCA1) protein is involved, to GG-NER pathway in predicting platinum resistance [7]. Since platinum agents are considered to be key drugs in the treatment of SCLC as well as NSCLC [8–10], it is of great interest to determine whether there is an association between the expression of DNA excision repair genes and the effectiveness of platinum-based chemotherapy in SCLC patients.

In this retrospective study we investigated the immunohistochemical expression of the ABC transporter proteins, Pgp, MRP1, MRP2, MRP3, and BCRP, and the DNA excision repair proteins, ERCC1 protein and BRCA1 protein, in tumor biopsy specimens obtained before chemotherapy from 130 SCLC patients who later received platinum-based combination chemotherapy, and we investigated the relationship between their expression and the patients' clinical outcome.

## 2. Materials and methods

### 2.1. Subjects

A total of 626 patients were diagnosed with SCLC at the National Cancer Center Hospital East between July 1992 and December 2005, and 578 of them received platinum-based combination chemotherapy as an initial treatment. After excluding the 246 patients who received thoracic radiotherapy and 2 patients who received surgery in order to eliminate the effects of treatment other than chemotherapy, the 191 patients of the remaining 330 patients diagnosed only cytologically, and therefore with no specimens available for analysis, and the nine patients whose specimens were unsuitable for immunohistochemistry. In this study, we analyzed biopsy specimens from 130 patients consisting of 104 responders and 26 non-responders. Institutional Review Board-approved informed consent was obtained from all patients.

### 2.2. Clinical evaluation

The classification system proposed by the Veterans' Administration Lung Study Group was used to stage SCLC as limited disease (LD) or extensive disease (ED) [11]. LD is defined as disease confined to one hemithorax that can be encompassed within a single radiation field, and ED is defined as disease that extends beyond these confines. Performance status (PS) was determined based on the Eastern Cooperative Oncology Group (ECOG) scale. Patient response was evaluated by using the Response Evaluation Criteria in Solid Tumors (RECIST) [12].

### 2.3. Immunohistochemistry

Tissue blocks were cut into 4-μm sections and mounted on silane-coated slides (Matsunami, Tokyo, Japan). The slides were then deparaffinized in xylene and dehydrated in a graded alcohol series. For antigen retrieval, the slides for Pgp, MRP1, MRP2, BCRP, ERCC1, and BRCA1 were immersed in 10 mM citric buffer solution (pH 6.0) at 120 °C for 20 min and the slides for MRP3 were immersed in 1 mM EDTA retrieval fluid (pH 8.0) at 95 °C for 20 min. The slides were then allowed to cool for 1 h at room temperature and washed in PBS. Nonspecific binding was blocked by incubation with 2% BSA plus 0.1% $NaN_3$ for 30 min, and after draining off the blocking solution, the slides were incubated overnight at 4 °C with the primary antibodies listed in Table 1. Endogenous peroxidase was then blocked with 0.3% $H_2O_2$ in methanol for 10 min, and after washing three times in PBS, the slides were incubated for 60 min with a labeled polymer En Vision+, peroxidase Mouse (DAKO, Glostrup, Denmark). The chromogen used was 2% 3,3'-diaminobenzidine in 50 mM Tris buffer (pH 7.6) containing 0.3% hydrogen, and the slides were counterstained with hematoxylin. Normal human liver tissue was used as a positive control for Pgp, MRP2, MRP3, and BCRP, normal human lung tissue for MRP1, normal human tonsil tissue for ERCC1, and breast cancer tissue human for BRCA1. Negative controls for each antibody were prepared by using non-immune serum instead of the primary antibodies. Membranous or cytoplasmic staining was evaluated for ABC transporter proteins [13], while nuclear staining was evaluated for DNA excision repair proteins [6,14]. Staining of each antibody was considered positive if >10% of the tumor cells stained. All of the slides were examined and scored independently by two observers (Y.K. and G.I.) without knowledge of the patients' clinical data. When judgments differed between two observers, they discussed it until an agreement was reached.

### 2.4. Statistical analysis

The significance of the relationship between immunohistochemical expression and clinical variables or response to chemotherapy was evaluated by using the $\chi^2$ test or Fisher's exact test, as appropriate. The logistic regression model was used for multivariate analysis of response. Progression-free survival (PFS) was used as a clinical marker for duration of response to chemotherapy. Overall survival (OS) was measured from the start of chemotherapy to the date of death from any cause or the date patients were last known to be alive. Survival rates were calculated by the Kaplan–Meier method, and the statistical significance of any differences in PFS and OS were evaluated by a log-rank test. The Cox proportional hazards model was used for multivariate analysis of survival. $p$ values less than 0.05 were considered significant. All statistical analyses were performed using

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**Table 2**
Patient characteristics ($n = 130$).

| Characteristics | No. of patients (%) |
|---|---|
| Age | |
| Median | 67 |
| Range | 28–83 |
| Gender | |
| Male | 108 (83) |
| Female | 22 (17) |
| Disease extent | |
| LD | 18 (14) |
| ED | 112 (86) |
| Performance status | |
| 0 | 2 (2) |
| 1 | 93 (71) |
| 2 | 25 (19) |
| 3 | 8 (6) |
| 4 | 2 (2) |
| Chemotherapy regimen | |
| CE | 36 (28) |
| PE | 35 (27) |
| PI | 25 (19) |
| CODE | 18 (14) |
| CAV/PE | 7 (5) |
| PEI | 7 (5) |
| PT | 2 (2) |

LD, limited disease; ED, extensive disease; CE, Carboplatin + Etoposide; PE, Cisplatin + Etoposide; PI, Cisplatin + Irinotecan; CODE, Cisplatin + Vincristine + Doxorubicin + Etoposide; CAV/PE, Cyclophosphamide + Doxorubicin + Vincristine/Cisplatin + Etoposide; PEI, Cisplatin + Etoposide + Irinotecan; PT, Cisplatin + Topotecan.

the statistical program StatView, Version 5.0 (Abacus Concepts, Berkley, CA).

## 3. Results

### 3.1. Patient characteristics

The patient characteristics are summarized in Table 2. The median age of the patients was 67 years (range: 28–83 years). More than 80% of the patients were male, and more than 80% had ED. Despite excluding patients who had received thoracic radiotherapy or surgery, our study included 18 LD patients. The major reasons for omitting thoracic radiotherapy in these LD patients were the presence of a malignant pleural effusion (9 patients) and interstitial pneumonia (5 patients). PS was generally good; approximately 70% of the patients were PS 0 or 1. All patients received chemotherapy containing etoposide, irinotecan, or topotecan. The details of administered chemotherapy are shown in Table 3.

### 3.2. Expression of ABC transporter and DNA excision repair proteins in SCLC

The immunostaining of ABC transporter proteins was both membranous and cytoplasmic, whereas the immunostaining of the DNA excision repair proteins was mostly restricted to the nucleus. Forty-two (33%) of the 130 tumors were Pgp-positive, 29 (22%) were MRP1-positive, 25 (19%) were MRP2-positive, 9 (7%) were MRP3-positive, 48 (37%) were BCRP-positive, 36 (27%) were ERCC1-positive, and 109 (83%) were BRCA1-positive. The relationships between expression of the ABC transporter and DNA excision repair proteins and the clinical variables are shown in Table 4. BCRP expression was significantly greater in the PS 2–4 cases than in the PS 0-1 cases ($p = 0.0223$). There were no significant correlations between expression of Pgp, MRP1, MRP2, MRP3, ERCC1, or BRCA1 and the clinical variables.

### 3.3. Association between expression of ABC transporter and DNA excision repair proteins and clinical outcome

The relationships between clinical variables and response to chemotherapy and survival are shown in Table 5. Response rate was not associated with any clinical variables, but PFS ($p = 0.0199$) and OS ($p = 0.0159$) were significantly associated with PS. Table 6 shows the associations between expression of ABC transporter and DNA excision repair proteins and response to chemotherapy and survival. BCRP expression was significantly predictive of response to chemotherapy ($p = 0.026$), and MRP2 expression was marginally predictive ($p = 0.0515$).

The median follow-up time was 8.3 years, and 119 patients had been dead until the time of analysis. The results for survival showed that BCRP expression was significantly associated with PFS ($p = 0.0103$), but not with OS ($p = 0.1427$). No significant associations were observed between expression of Pgp, MRP1, MRP3, ERCC1, or

**Table 3**
Details of administered chemotherapy.

| Regimen | Dosage of each agent | | Schedule | | Median number of treatment cycles (range) |
|---|---|---|---|---|---|
| CE | Carboplatin | AUC 6 | Day 1 | q3w | 4 (1–4) |
| | Etoposide | 100 mg/m² | Days 1–3 | | |
| PE | Cisplatin | 60 mg/m² | Day 1 | q3w | 4 (1–4) |
| | Etoposide | 100 mg/m² | Days 1–3 | | |
| PI | Cisplatin | 60 mg/m² | Day 1 | q4w | 4 (1–4) |
| | Irinotecan | 60 mg/m² | Days 1, 8, 15 | | |
| CODE | Cisplatin | 25 mg/m² | Day 1 (1, 2, 3, 4, 5, 6, 7, 8, 9 weeks) | Weekly | 9 (2–9) |
| | Vincristine | 1 mg/m² | Day 1 (2, 4, 6, 8 weeks) | | |
| | Doxorubicin | 40 mg/m² | Day 1 (1, 3, 5, 7 weeks) | | |
| | Etoposide | 80 mg/m² | Day 1–3 (1, 3, 5, 7 weeks) | | |
| CAV/PE | Cyclophosphamide | 800 mg/m² | Day 1 | Alternatively | 6 (3–6) |
| | Doxorubicin | 50 mg/m² | Day 1 | | |
| | Vincristine | 1.4 mg/m² | Day 1 | | |
| | Cisplatin | 80 mg/m² | Day 1 | | |
| | Etoposide | 100 mg/m² | Day 1, 3, 5 | | |
| PEI | Cisplatin | 25 mg/m² | Day 1 (1, 2, 3, 4, 5, 6, 7, 8, 9 weeks) | Weekly | 4 (2–9) |
| | Etoposide | 60 mg/m² | Days 1–3 (1, 3, 5, 7 weeks) | | |
| | Irinotecan | 90 mg/m² | Day 1 (2, 4, 6, 8 weeks) | | |
| PT | Cisplatin | 60 mg/m² | Day 5 | q3w | 4.5 (4–5) |
| | Topotecan | 1 mg/m² | Days 1–5 | | |

AUC, area under the curve.

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**Table 4**
Relationship between clinical variables and expression of ABC transporter and DNA excision repair proteins.

| | n | Pgp-positive (%) | MRP1-positive (%) | MRP2-positive (%) | MRP3-positive (%) | BCRP-positive (%) | ERCC1-positive (%) | BRCA1-positive (%) |
|---|---|---|---|---|---|---|---|---|
| Total | 130 | 42 (33) | 29 (22) | 25 (19) | 9 (7) | 48 (37) | 36 (27) | 109 (83) |
| Age | | | | | | | | |
| <70 | 83 | 29 (35) | 16 (19) | 15 (18) | 5 (6) | 29 (35) | 24 (29) | 70 (84) |
| ≥70 | 47 | 13 (28) | 13 (28) | 10 (21) | 4 (9) | 19 (40) | 12 (26) | 39 (83) |
| Gender | | | | | | | | |
| Male | 108 | 36 (33) | 23 (21) | 19 (18) | 9 (8) | 41 (38) | 30 (28) | 93 (86) |
| Female | 22 | 6 (27) | 6 (27) | 6 (27) | 0 (0) | 7 (32) | 6 (27) | 16 (73) |
| Disease extent | | | | | | | | |
| LD | 18 | 8 (44) | 3 (17) | 6 (33) | 3 (17) | 8 (44) | 4 (22) | 16 (89) |
| ED | 112 | 34 (30) | 26 (23) | 19 (17) | 6 (5) | 40 (36) | 32 (29) | 93 (83) |
| PS | | | | | | | | |
| 0–1 | 95 | 33 (35) | 20 (21) | 21 (22) | 8 (8) | 29 (31)[a] | 27 (28) | 80 (84) |
| 2–4 | 35 | 9 (26) | 9 (26) | 4 (11) | 1 (3) | 19 (54) | 9 (26) | 29 (83) |

ABC, ATP-binding cassette; Pgp, P-glycoprotein; MRP, multidrug resistance protein; BCRP, breast cancer resistance protein; ERCC, excision repair cross-complementation group; BRCA, breast cancer susceptibility gene; LD, limited disease; ED, extensive disease; PS, performance status.
[a] $p = 0.0223$.

**Table 5**
Summary of relationship between clinical variables and response to chemotherapy and survival.

| | n | Response rate (%) | p | PFS (mo) | p | MST (mo) | p |
|---|---|---|---|---|---|---|---|
| Total | 130 | 79 | | 5.2 | | 9.0 | |
| Age | | | | | | | |
| <70 | 83 | 80 | >0.9999 | 5.1 | 0.1296 | 9.4 | 0.3493 |
| ≥70 | 47 | 81 | | 5.4 | | 10.9 | |
| Gender | | | | | | | |
| Male | 108 | 81 | 0.7715 | 5.1 | 0.5496 | 9.4 | 0.6528 |
| Female | 22 | 77 | | 5.7 | | 13.2 | |
| Disease extent | | | | | | | |
| LD | 18 | 67 | 0.2277 | 5.6 | 0.4838 | 9.4 | 0.8856 |
| ED | 112 | 82 | | 5.2 | | 10.4 | |
| PS | | | | | | | |
| 0–1 | 95 | 82 | 0.4584 | 5.5 | 0.0199* | 10.8 | 0.0159* |
| 2–4 | 35 | 74 | | 4.2 | | 8.1 | |

LD, limited disease; ED, extensive disease; PS, performance status; PFS, progression-free survival; MST, median survival time.
* $p < 0.05$.

**Table 6**
Association between expression of ABC transporter and DNA excision repair proteins and response to chemotherapy and survival ($n = 130$).

| | n | Response rate (%) | p | PFS (mo) | p | MST (mo) | p |
|---|---|---|---|---|---|---|---|
| Pgp | | | | | | | |
| Positive | 42 | 83 | 0.6730 | 5.5 | 0.7257 | 10.5 | 0.3006 |
| Negative | 88 | 78 | | 5.1 | | 9.9 | |
| MRP1 | | | | | | | |
| Positive | 29 | 90 | 0.1902 | 5.3 | 0.8141 | 11.0 | 0.2249 |
| Negative | 101 | 77 | | 5.2 | | 9.4 | |
| MRP2 | | | | | | | |
| Positive | 25 | 64 | 0.0515 | 5.6 | 0.5832 | 12.6 | 0.1261 |
| Negative | 105 | 84 | | 5.2 | | 9.3 | |
| MRP3 | | | | | | | |
| Positive | 9 | 78 | >0.9999 | 5.2 | 0.3181 | 11.9 | 0.1326 |
| Negative | 121 | 80 | | 5.3 | | 9.4 | |
| BCRP | | | | | | | |
| Positive | 48 | 69 | 0.0260* | 4.0 | 0.0103* | 9.1 | 0.1427 |
| Negative | 82 | 87 | | 5.6 | | 10.6 | |
| ERCC1 | | | | | | | |
| Positive | 36 | 89 | 0.1452 | 5.4 | 0.5383 | 11.9 | 0.6250 |
| Negative | 94 | 77 | | 4.3 | | 9.3 | |
| BRCA1 | | | | | | | |
| Positive | 109 | 79 | 0.5666 | 5.3 | 0.8404 | 10.5 | 0.4611 |
| Negative | 21 | 86 | | 4.7 | | 8.1 | |

ABC, ATP-binding cassette; Pgp, P-glycoprotein; MRP, multidrug resistance protein; BCRP, breast cancer resistance protein; ERCC, excision repair cross-complementation group; BRCA, breast cancer susceptibility gene; PFS, progression-free survival; MST, median survival time.
* $p < 0.05$.

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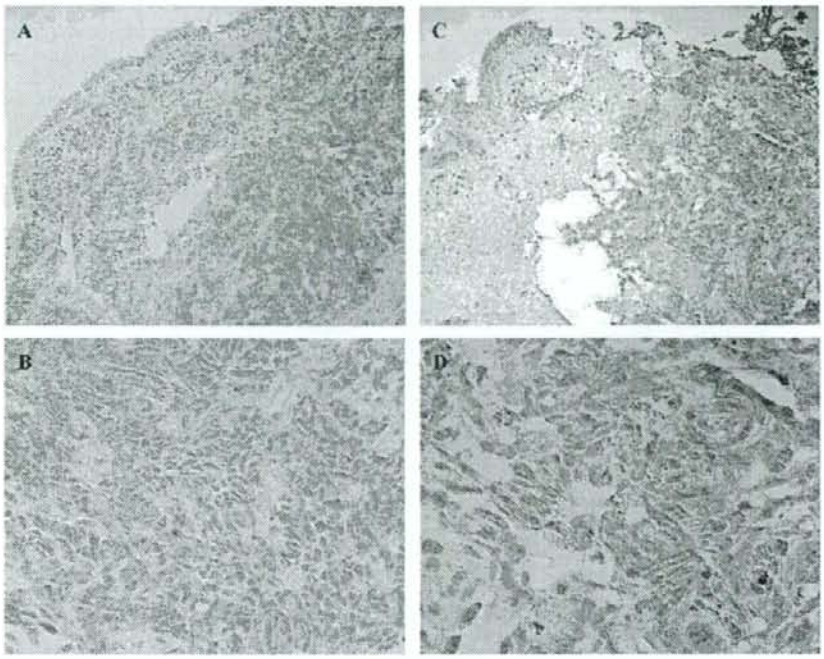


**Fig. 1.** Representative cases of positive immunostaining for BCRP (A, ×100; B, ×400) and MRP2 (C, ×100; D, ×400). BCRP and MRP2 in the apical membrane of the bronchial layer have been immunostained as a positive control.

BRCA1 and either response to chemotherapy or survival. Representative immunohistochemical staining of BCRP and MRP2 is shown in Fig. 1.

### 3.4. *Multivariate analysis for response and survival*

A multivariate analysis revealed that BCRP expression was significantly predictive of response to chemotherapy (Table 7). PFS was significantly associated with both PS ($p = 0.0299$) and BCRP expression ($p = 0.0138$), whereas OS was significantly associated with PS alone ($p = 0.0295$; Table 8). The PFS and OS curves according to BCRP expression are shown in Fig. 2.

## 4. Discussion

Although initial chemotherapy succeeds in 80–90% of SCLC patients, most patients eventually experience a relapse and their survival time is quite limited. Unfortunately, little progress in the chemotherapy of SCLC has been made during the past 30 years [15]. If drug resistance could be overcome, it would no doubt lead to an improved prognosis of this challenging disease, because drug resistance is considered a major obstacle to successful treatment. In this study we investigated expression of the five ABC transporter proteins that are thought to be the most important in the drug resistance mechanisms of SCLC, and the results showed that BCRP expression alone was significantly associated with either response to chemotherapy or PFS. Expression of BCRP was significantly correlated with impaired PS, but the multivariate analysis revealed BCRP to be an independent prognostic factor for PFS.

BCRP, which is classified as ABCG2 and known as the mitoxantrone resistance gene (MXR) or ABC transporter in placenta (ABC-P), is expressed in a variety of normal tissues, with the highest levels having been found in the placenta, and lower levels in the liver, small intestine, brain, and ducts and lobules of the breast [2,16]. BCRP was initially isolated from doxorubicin-resistant breast

**Table 7**
Multivariate analysis for response ($n = 130$).

| Variables | Category | Risk ratio | 95% CI | p |
|---|---|---|---|---|
| Age | <70 vs. ≥70 | 0.701 | 0.263–1.869 | 0.4776 |
| Gender | Female vs. Male | 0.857 | 0.258–2.848 | 0.8014 |
| Disease extent | LD vs. ED | 1.81 | 0.545–6.018 | 0.3329 |
| PS | 0–1 vs. 2–4 | 1.315 | 0.471–3.676 | 0.6013 |
| MRP2 | (−) vs. (+) | 2.238 | 0.779–6.429 | 0.1346 |
| BCRP | (−) vs. (+) | 2.804 | 1.103–7.128 | 0.0303* |

* $p < 0.05$.

**Table 8**
Multivariate analysis for survival ($n = 130$).

| Variables | Category | Risk ratio | 95% CI | p |
|---|---|---|---|---|
| A. Progression-free survival | | | | |
| Age | <70 vs. ≥70 | 0.691 | 0.464–1.028 | 0.0682 |
| Gender | Female vs. Male | 1.062 | 0.650–1.733 | 0.8105 |
| Disease extent | LD vs. ED | 0.87 | 0.501–1.512 | 0.6251 |
| PS | 0–1 vs. 2–4 | 1.592 | 1.046–2.424 | 0.0299* |
| BCRP | (−) vs. (+) | 1.614 | 1.102–2.363 | 0.0138* |
| B. Overall survival | | | | |
| Age | <70 vs. ≥70 | 0.832 | 0.565–1.224 | 0.3496 |
| Gender | Female vs. Male | 1.067 | 0.658–1.729 | 0.7936 |
| Disease extent | LD vs. ED | 1.131 | 0.673–1.901 | 0.6430 |
| PS | 0–1 vs. 2–4 | 1.588 | 1.047–2.407 | 0.0295* |
| BCRP | (−) vs. (+) | 1.235 | 0.831–1.833 | 0.2962 |

LD, limited disease; ED, extensive disease; PS, performance status; BCRP, breast cancer resistance protein.
* $p < 0.05$.

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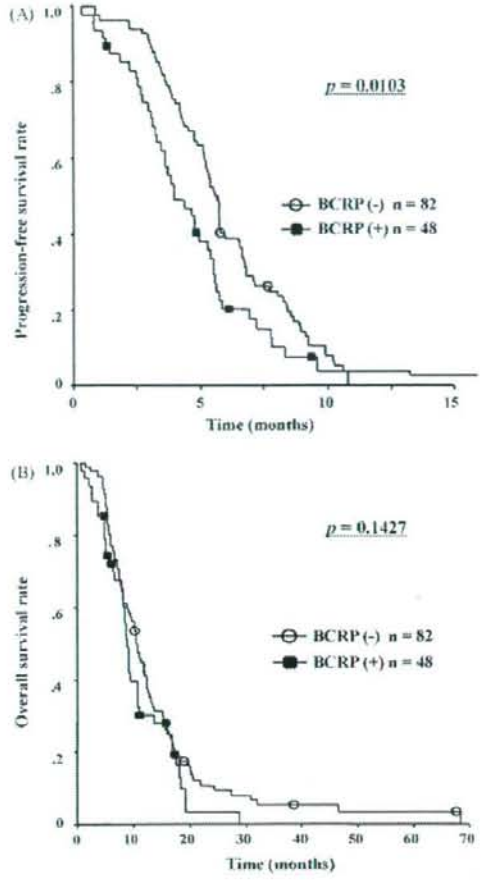


**Fig. 2.** Progression-free survival curves (A) and overall survival curves (B) for 130 SCLC patients, according to breast cancer resistance protein (BCRP) expression.

cancer cell line MCF-7, and its overexpression was found to promote resistance to topoisomerase I inhibitors, including irinotecan and topotecan [17]. We previously reported the finding that BCRP expression is a significant predictor of survival in advanced NSCLC [18], but to our knowledge no data have been reported regarding BCRP expression in SCLC.

No significant association was found between the expression of other ABC transporter proteins and clinical outcome in the present study. Some studies have shown a relationship between expression of Pgp or MRP1 and response or survival [19–23], however, their clinical usefulness as therapeutic targets is still obscure. In fact, two randomized phase III studies that incorporated modulators of Pgp and one phase II study of VX-710, an inhibitor of both Pgp and MRP1, failed to show any survival benefit in SCLC patients [24–26].

In this study we also investigated the expression of the DNA excision repair proteins ERCC1 and BRCA1 in SCLC, but neither of them was related to response or survival. Expression of DNA excision repair proteins has hardly ever been investigated in SCLC, and to our knowledge there has been only one study in regard to it. In that study high expression of ERCC1 was associated with poor survival, but when the cases were grouped according to stage, a significant decrease in survival was observed only in the LD patients, and the correlation between ERCC1 expression and response was not mentioned [27]. By contrast, expression of DNA excision repair proteins, especially ERCC1, has been intensively investigated in NSCLC recently, and expression of ERCC1 has been demonstrated to be related to platinum resistance in several studies [6,28,29]. We analyzed the ERCC1 expression also using the criterion by Olaussen et al. [6], but the results were similar and our conclusions did not change (data not shown). BRCA1 expression was also demonstrated to be significantly associated with chemoresistance in one study [30]. However, in other studies no significant association was observed between expression of ERCC1 or BRCA1 and either response or survival [14,31]. Their clinical significance in lung cancer including SCLC has yet to be determined, and further studies are awaited.

The concept of "cancer stem cells", a very small fraction of the whole cell population repeating self-renewal continues to supply cancer-constitute cells, has recently gained wide acceptance. Although the origin of cancer stem cells has not yet been elucidated, the idea that malignant transformation of a normal stem cell has been proposed [32]. Side population (SP) cells, defined by Hoechst 33342 dye exclusion in flow cytometry, are considered to be an enriched source of normal stem cells [33]. In addition, BCRP has been shown to be a molecular determinant of the SP phenotype, and it can be used as a marker for stem cell selection [34]. In a recent study, SP cells isolated from lung cancer displayed elevated expression of BCRP and showed resistance to multiple chemotherapeutic agents [35]. These findings indicate that it may be possible to use BCRP as a marker of cancer stem cells in certain types of lung cancer.

In conclusion, the results of the present study indicated that immunohistochemical expression of BCRP is significantly associated with response and PFS in SCLC patients treated with platinum-based chemotherapy. Our results should be tested in LD patients who received thoracic radiotherapy, and it is also desirable that our results will be validated in other methods, such as mRNA expression analysis. Although confirmatory studies are needed, BCRP may be an ideal therapeutic target for SCLC. A variety of BCRP inhibitors have already been identified [36–39]. Clinical trials of combination of these agents with conventional chemotherapy might be acceptable in SCLC.

## Conflict of interest statement

None declared.

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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

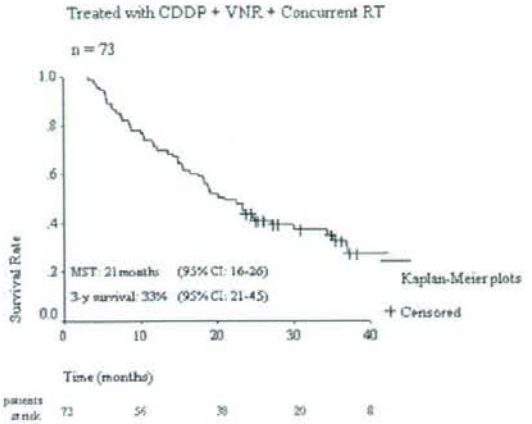


**FIGURE 1.** Overall survival of patients treated with CDDP + VNR + concurrent RT. CDDP, cisplatin; VNR, vinorelbine; RT, radiotherapy; MST, median survival time; 3-year survival, survival rate at 3 years.

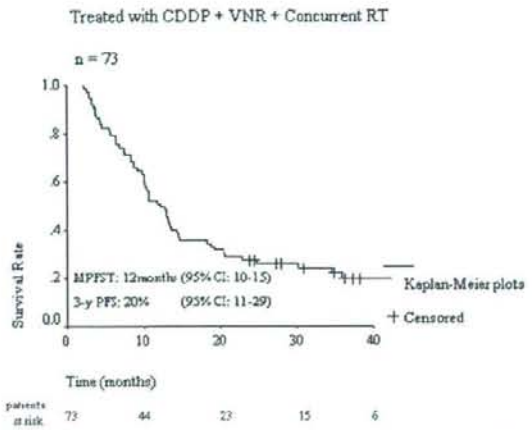


**FIGURE 2.** Progression-free survival of patients treated with CDDP + VNR + concurrent RT. CDDP, cisplatin; VNR, vinorelbine; RT, radiotherapy; MPFST, median progression-free survival time; 3-year survival, progression-free survival rate at 3 years.

survival. Multivariate analysis showed that no variables significantly affected the overall survival (Table 3).

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* |
| 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 |

* 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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