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III. 研究成果の刊行物・別刷

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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

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 chemo-

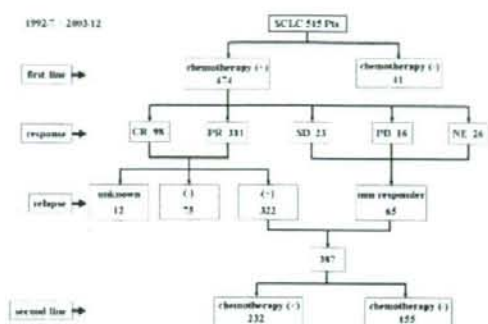


FIGURE 1. Patient flow is depicted. CR indicates complete response; NE, not evaluable; PD, progressive disease; PR, partial response; Pts, patients; SCLC, small-cell lung cancer; SD, stable disease; +, positive; -, negative.

therapy, 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.

Analyzed Clinical Factors

The correlations between clinical factors evaluated at the time of disease recurrence, such as the age (<70/ \geq 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		P
	(+) (n=232)	(-) (n=155)	
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, Berkeley, 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, doxor-

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.

bicin, 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.

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

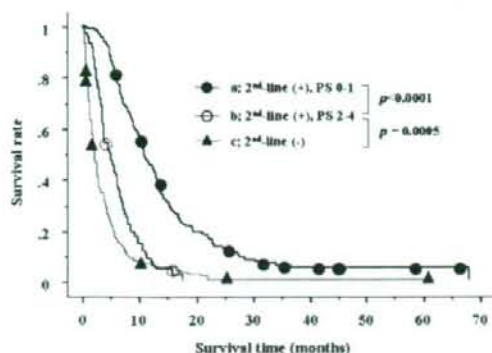


FIGURE 2. Survival curves according to the performance status (PS) at the time of disease recurrence. + indicates positive; -, negative.

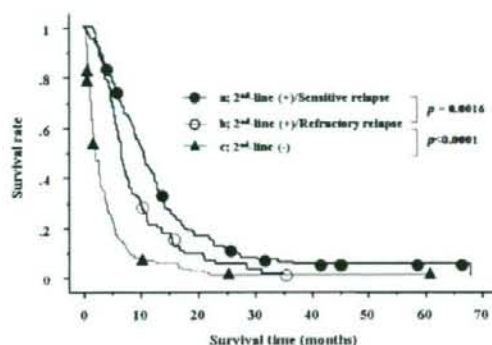


FIGURE 3. Survival curves according to sensitivity to first-line chemotherapy. + indicates positive; -, negative.

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.

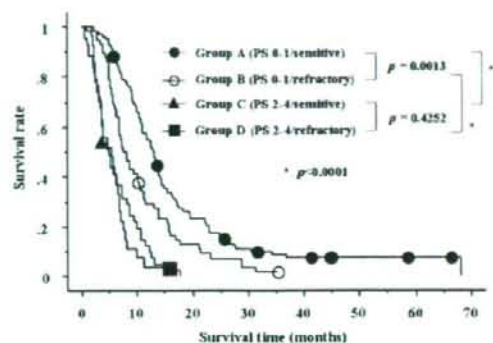


FIGURE 4. Survival curves according to the 2 independent prognostic factors. PS indicates performance status.

(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 ≥ 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 ≥ 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 ≥ 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 ≥ 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 ≥ 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 ≥ 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.

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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 mul-

tidrug 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)	J5B-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)	DX1	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- μ m 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% Na₂S₂O₈ 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% H₂O₂ 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.L.) 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 χ^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

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, Berkeley, 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	q3w
	Etoposide	100 mg/m ²	
PE	Cisplatin	60 mg/m ²	q3w
	Etoposide	100 mg/m ²	
PI	Cisplatin	60 mg/m ²	q4w
	Irinotecan	60 mg/m ²	
	Etoposide	60 mg/m ²	
CODE	Cisplatin	25 mg/m ²	Weekly
	Vincristine	1 mg/m ²	
	Doxorubicin	40 mg/m ²	
	Etoposide	80 mg/m ²	
CAV/PE	Cyclophosphamide	800 mg/m ²	Alternately
	Doxorubicin	50 mg/m ²	
	Vincristine	1.4 mg/m ²	
	Cisplatin	80 mg/m ²	
	Etoposide	100 mg/m ²	
PEI	Cisplatin	25 mg/m ²	Weekly
	Etoposide	60 mg/m ²	
	Irinotecan	90 mg/m ²	
	Cisplatin	60 mg/m ²	
PT	Cisplatin	60 mg/m ²	q3w
	Topotecan	1 mg/m ²	

AUC, area under the curve.

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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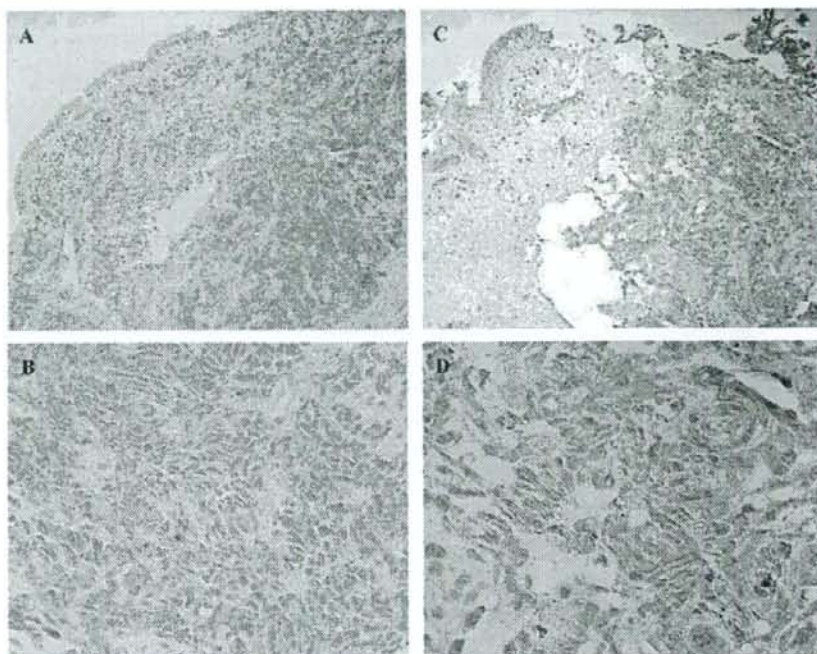


Fig. 1. Representative cases of positive immunostaining for BCRP (A, $\times 100$; B, $\times 400$) and MRP2 (C, $\times 100$; D, $\times 400$). BCRP and MRP2 in the apical membrane of the bronchial layer have been immunostained as a positive control.

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	<i>p</i>
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	<i>p</i>
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.

* $p < 0.05$.

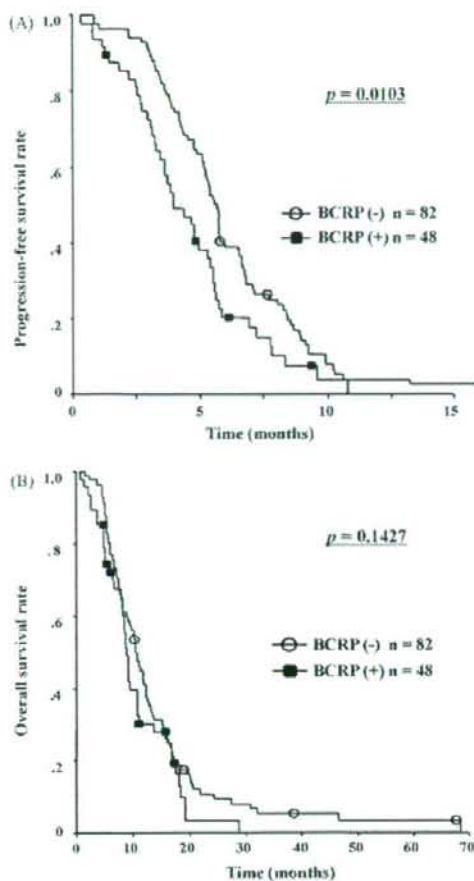


Fig. 2. Progression-free survival curves (A) and overall survival curves (B) for 130 SCLC patients, according to breast cancer resistance protein (BCRP) expression.

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 signifi-

cant 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 Olausson 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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