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Ⅲ. 研究成果の刊行物・別刷

研究代表者	後藤功一	国立がんセンター東病院
研究分担者	田村友秀	国立がんセンター中央病院
	森　清志	栃木県立がんセンター
	岡本浩明	横浜市立市民病院
	山本信之	静岡県立静岡がんセンター
	横山　晶	新潟県立がんセンター新潟病院
	樋田豊明	愛知県がんセンター中央病院
	今村文生	大阪府立成人病センター
	松井　薫	大阪府立呼吸器･アレルギー医療センター
	中川和彦	近畿大学医学部
	武田晃司	大阪市立総合医療センター
	木浦勝行	岡山大学大学院医歯薬学総合研究科
	河原正明	近畿中央胸部疾患センター
	安宅信二	近畿中央胸部疾患センター
	根来俊一	兵庫県立がんセンター
	西條長宏	近畿大学医学部
	瀬戸貴司	九州がんセンター

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

Young Hak Kim[a,b,c], Genichiro Ishii[a], Koichi Goto[b], Shuji Ota[a,b], Kaoru Kubota[b], Yukinori Murata[a], Michiaki Mishima[c], Nagahiro Saijo[b], Yutaka Nishiwaki[b], Atsushi Ochiai[a,*]

[a] Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan
[b] Division of Thoracic Oncology, National Cancer Center Hospital East, Chiba, Japan
[c] Department of Respiratory Medicine, Kyoto University Hospital, Kyoto, Japan

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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 mechanism for repairing platinum-DNA adducts, and it is

* Corresponding author. Tel.: +81 4 7134 6855; fax: +81 4 7134 6865.
E-mail address: aochiai@east.ncc.go.jp (A. Ochiai).

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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

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 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, 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/m^2	Days 1–3		
PE	Cisplatin	60 mg/m^2	Day 1	q3w	4 (1–4)
	Etoposide	100 mg/m^2	Days 1–3		
PI	Cisplatin	60 mg/m^2	Day 1	q4w	4 (1–4)
	Irinotecan	60 mg/m^2	Days 1, 8, 15		
CODE	Cisplatin	25 mg/m^2	Day 1 (1, 2, 3, 4, 5, 6, 7, 8, 9 weeks)	Weekly	9 (2–9)
	Vincristine	1 mg/m^2	Day 1 (2, 4, 6, 8 weeks)		
	Doxorubicin	40 mg/m^2	Day 1 (1, 3, 5, 7 weeks)		
	Etoposide	80 mg/m^2	Day 1–3 (1, 3, 5, 7 weeks)		
CAV/PE	Cyclophosphamide	800 mg/m^2	Day 1	Alternatively	6 (3–6)
	Doxorubicin	50 mg/m^2	Day 1		
	Vincristine	1.4 mg/m^2	Day 1		
	Cisplatin	80 mg/m^2	Day 1		
	Etoposide	100 mg/m^2	Day 1, 3, 5		
PEI	Cisplatin	25 mg/m^2	Day 1 (1, 2, 3, 4, 5, 6, 7, 8, 9 weeks)	Weekly	4 (2–9)
	Etoposide	60 mg/m^2	Days 1–3 (1, 3, 5, 7 weeks)		
	Irinotecan	90 mg/m^2	Day 1 (2, 4, 6, 8 weeks)		
PT	Cisplatin	60 mg/m^2	Day 5	q3w	4.5 (4–5)
	Topotecan	1 mg/m^2	Days 1–5		

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.
* $p < 0.05$.

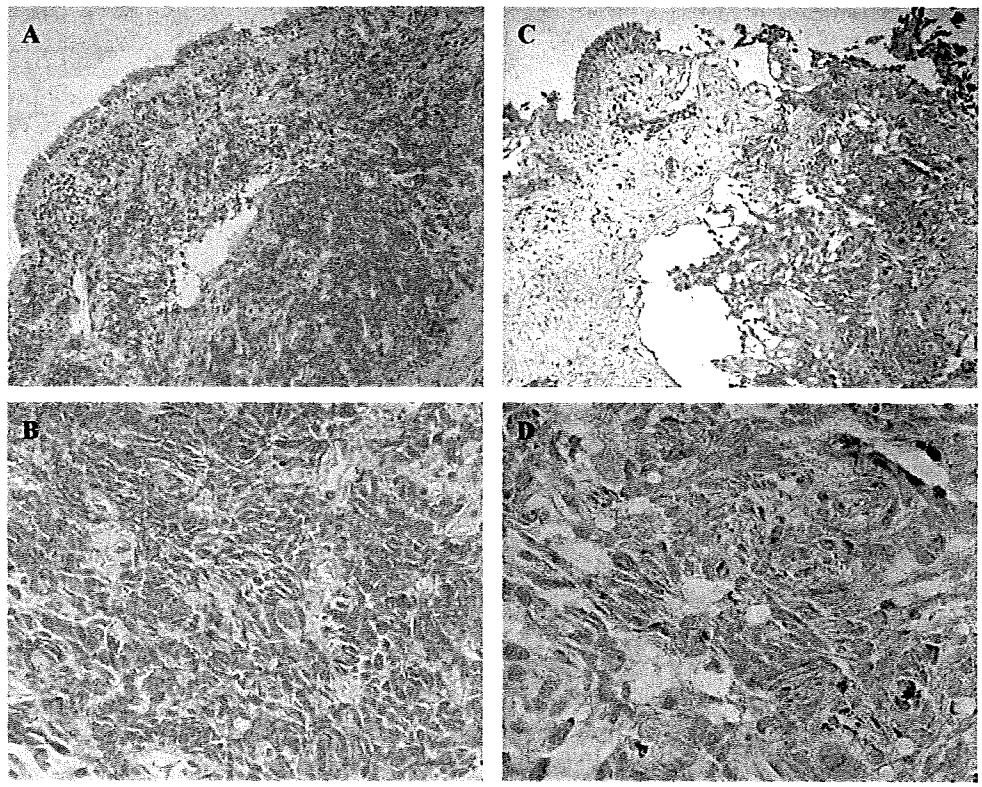

Fig. 1. Representative cases of positive immunostaining for BCRP (A, ×100; B, ×400) and MRP2 (C, ×100; D, ×400). BCRP and MRP2 in the apical membrane of the bronchial layer have been immunostained as a positive control.

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.
* $p < 0.05$.

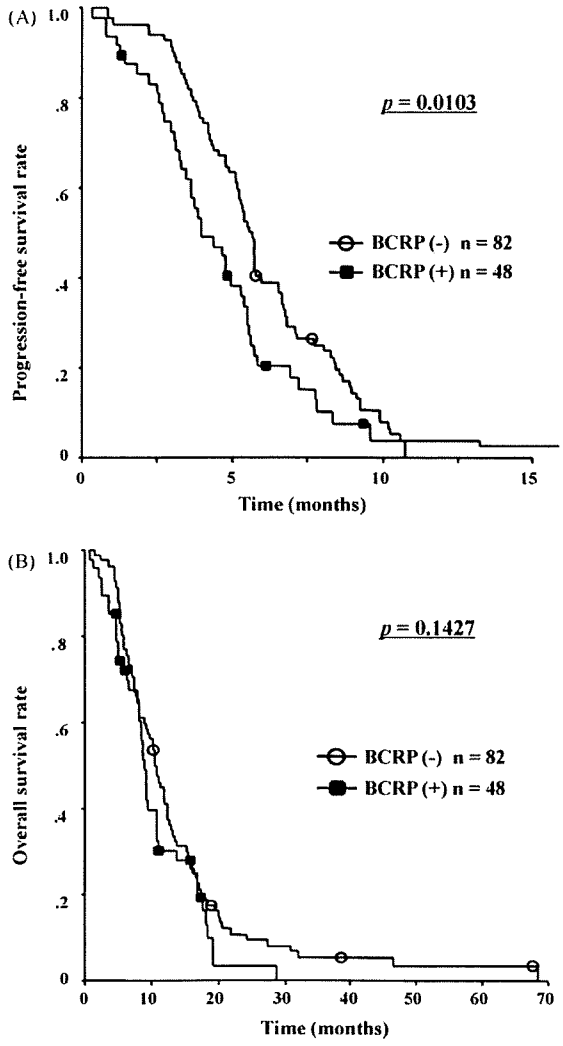

Fig. 2. Progression-free survival curves (A) and overall survival curves (B) for 130 SCLC patients, according to breast cancer resistance protein (BCRP) expression.

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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A Phase I Study of Gemcitabine and Carboplatin in Patients with Advanced Non-small Cell Lung Cancer and a Performance Status of 2

Young Hak Kim[1,2], Kaoru Kubota[1], Koichi Goto[1], Kiyotaka Yoh[1], Seiji Niho[1], Hironobu Ohmatsu[1], Nagahiro Saijo[1] and Yutaka Nishiwaki[1]

[1]Division of Thoracic Oncology, National Cancer Center Hospital East, Chiba and [2]Department of Respiratory Medicine, Kyoto University Hospital, Kyoto, Japan

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Objective: The aim of this study was to determine the maximum-tolerated dose (MTD) and the recommended dose of combination chemotherapy with gemcitabine (GEM) and carboplatin (CBDCA) in non-small cell lung cancer (NSCLC) patients with a performance status (PS) of 2.
Methods: Chemotherapy-naïve NSCLC patients with PS 2 were enrolled. Chemotherapy consisted of an escalated dose of GEM on days 1 and 8 and CBDCA on day 1 every 3 weeks. Patients were scheduled to receive GEM (mg/m^2)/CBDCA (area under the curve: AUC) at four dose levels: 800/4 (level 1), 1000/4 (level 2), 1000/4.5 (level 3) and 1000/5 (level 4), respectively.
Results: Between February 2004 and August 2006, 13 patients were enrolled in this study. Dose-limiting toxicities (DLTs) were thrombocytopenia, febrile neutropenia and hyponatremia. DLTs were observed in two of six patients at dose level 1 and in three of six patients at dose level 2. Dose level 2 was thus determined to be the MTD. Among 12 evaluable patients, 7 patients had stable diseases and 5 patients had progressive diseases, and the median survival time was 3.8 months.
Conclusions: The MTD and the recommended dose for Phase II studies of this regimen were determined to be GEM 1000 mg/m^2 and CBDCA AUC of 4. Additional objective measures are needed to evaluate patients' risk and benefit in future clinical trials for PS 2 patients.

Key words: non-small cell lung cancer – performance status 2 – gemcitabine – carboplatin – Phase I

INTRODUCTION

Platinum-based combination chemotherapy has been shown to improve survival and quality-of-life (QOL) in patients with advanced non-small cell lung cancer (NSCLC) (1,2). In the 1990s, new chemotherapeutic agents, such as gemcitabine (GEM), vinorelbine, docetaxel, paclitaxel (PTX) and irinotecan, were developed. Currently, platinum-based chemotherapy employing these new agents is accepted as the standard chemotherapy worldwide (3,4). In addition, a meta-analysis demonstrated significant longer progression-free survival of GEM and platinum combination compared with other new agents and platinum combinations (5). Thus, combination chemotherapy with GEM and platinum is now considered as one of the most active regimens for advanced NSCLC.

Like in other types of cancers, performance status (PS) has been shown to be one of the most important prognostic factors for survival in advanced NSCLC (6–8). Patients with impaired PS generally have lower response rate and shorter survival in spite of high risk for severe toxicities (9,10). Historically, clinical trials have excluded patients with Eastern Cooperative Oncology Group (ECOG) PS of 2 or worse. To date, it has not been fully elucidated whether platinum-based combination chemotherapy is feasible and effective in patients with PS 2.

Carboplatin (CBDCA), an analog of cisplatin (CDDP), has lower nephro- and gastrointestinal toxicity and has been widely used as a substitution of CDDP. Several randomized

For reprints and all correspondence: Young Hak Kim, Department of Respiratory Medicine, Kyoto University Hospital, 54 Shogoin-Kawaharacho, sakyo-ku, Kyoto 606-8507, Japan. E-mail: ckim@kuhp.kyoto-u.ac.jp

trials have shown the equivalence between GEM + CBDCA (GC) and GEM + CDDP (GP) in terms of response rate and survival (11,12). In those trials, toxicities, such as emesis, nephropathy and neuropathy were significantly mild in GC. Although recent meta-analysis disclosed slightly but significant survival advantage of CDDP (13,14), GC can be one of the treatment options, especially for patients who are not suitable to receive CDDP. In a randomized Phase III trial comparing GC with vinblastine + CDDP, GC showed better response rate and survival, and toxicities were similar between the two arms (15). Although 70% of all enrolled patients in the study had PS 2, overall response rate and median survival time (MST) were 27% and 11.6 months in GC arm. These survival data were comparable to those in patients with PS 0 or 1 who treated with platinum-based chemotherapy.

These results suggest the potential benefit of GC in patients with PS 2; however, the optimal dose of GC has not been investigated in patients with impaired PS. Therefore, we conducted a Phase I study to determine the maximum-tolerated dose (MTD) and the recommended dose for Phase II studies of GC in advanced NSCLC patients with PS 2.

PATIENTS AND METHODS

Eligibility

Patients with histologically or cytologically proven advanced NSCLC were eligible for the study. Each patient was required to meet the following criteria: (i) clinical stage IIIB or IV; (ii) ECOG PS of 2; (iii) aged 20–75 years; (iv) measurable lesion; (v) no prior chemotherapy; (vi) adequate hematological function (white blood cell $\geq$3500/mm^3, hemoglobin $\geq$9.5 g/dl and platelets $\geq$100 000/mm^3); (vii) adequate hepatic and renal function (total bilirubin $\leq$1.5 mg/dl, AST and ALT$<$100 IU/l and creatinine $\leq$1.5 mg/dl); (viii) PaO_2 $\geq$60 mmHg; and (ix) written informed consent. Patients with active concomitant malignancy, radiologically apparent interstitial pneumonia or pulmonary fibrosis, serious concurrent illness (e.g. uncontrolled diabetes mellitus, hypertension, angina pectoris, myocardial infarction within 3 months after onset or severe infection), history of severe drug allergy or pregnant/lactating women were excluded. The study protocol was approved by the institutional review board of the National Cancer Center.

Treatment Schedule

This was a Phase I, dose-escalation study planned for GEM on days 1 and 8 and CBDCA on day 1 of a 21-day course. The initial dose level of GEM was 800 mg/m^2 and CBDCA was an area under the concentration–time curve (AUC) of 4 mg min/ml. The actual dose of CBDCA was calculated based on Cockcroft–Gault equation (16) and Calvert formula (17) every course. CBDCA was infused over 60 min, and 60 min after the completion of CBDCA infusion, GEM was administered over 30 min. Prophylactic administration of granulocyte colony-stimulating factor (G-CSF) was not permitted. Administration of G-CSF was permitted for patients with grade 4 neutropenia and/or leukopenia and grade 3 febrile neutropenia. The administration of GEM was omitted on day 8 if patients met one of the following criteria: white blood cell $<$2000/mm^3, neutrophil $<$1000/mm^3, platelets $<$50 000/mm^3 and PS $\geq$3. No dose modification of GEM was permitted on day 8. If dose-limiting toxicity (DLT) was observed, the dose of each drug was reduced to 80% in the next course of chemotherapy. Treatment was to be performed for at least two courses, unless unacceptable toxicity or disease progression occurred.

The DLT was defined as follows: grade 4 thrombocytopenia, grade 3 or grade 4 febrile neutropenia, grade 3 non-hematological toxicity (except for nausea/vomiting and alopecia) and omission of the treatment on day 8. Dose-escalation schedule is shown in Table 1. Initially, three patients were treated at each dose level. If DLT was not observed in any of three patients, dose escalation was made. If DLT was observed in one or two of three patients, an additional three patients were entered in the same dose level. If DLT was observed in three or more of six patients or all of the initial three patients, we considered that the dose was the MTD. If DLT was observed in one or two of six patients, dose escalation was also made. Dose escalation was decided by the toxic data only in the first course of chemotherapy.

Baseline and Treatment Assessment

Pre-treatment evaluation consisted of complete medical history and physical examination, complete blood cell counts, blood chemistry studies, electrocardiograph, arterial blood gas analysis, chest radiography, computed tomography (CT) of the chest, CT or ultrasound study of the abdomen, CT or magnetic resonance imaging of the brain, and bone scintigraphy. Complete blood cell counts, blood chemistry studies and chest radiography were repeated every week. Creatinine clearance was estimated by the Cockcroft–Gault equation every course. Tumor response was assessed with the Response Evaluation Criteria in Solid Tumor (RECIST) criteria (18). Toxicity was evaluated according to the National Cancer Institute-Common Toxicity Criteria (version 2.0).

Table 1. Dose-escalation schedule

Dose level	Gemcitabine (mg/m^2)	Carboplatin (AUC)	No. of patients
1	800	4	3–6
2	1000	4	3–6
3	1000	4.5	3–6
4	1000	5	3–6

AUC, area under the curve.

RESULTS

Patient Characteristics

Between February 2004 and August 2006, 13 patients were enrolled in this study. However, one patient was excluded from the analysis because of the error in dose calculation. Table 2 shows the characteristics of 12 evaluable patients. Eleven patients were male and one was female. The median age of the patients was 68 years (range, 51–72 years). There were five adenocarcinomas, four squamous cell carcinomas, two large cell carcinomas and one pleomorphic carcinoma. Stage IIIB and IV patients were five and six, respectively, and one patient was a relapse after surgical resection.

Dose Escalation

At the dose level 1, DLT was observed in two of the first three patients: one experienced grade 3 hyponatremia and the other experienced grade 3 febrile neutropenia. Thereafter, we amended the protocol, and grade 3 hyponatremia was excluded from DLT criteria after that. Another three patients were treated at the same dose. Since these patients did not show any additional DLT, the dosage was then escalated to the next step. At the dose level 2, DLT was observed in two of the first three patients: one experienced grade 3 nausea/vomiting and omission on day 8 and the other experienced grade 3 febrile neutropenia and anorexia. Therefore, another three patients were assigned to receive the treatment at the same dose. Out of those three patients, one patient developed grade 4 febrile neutropenia and grade 3 anorexia. Thus, DLT was observed in three of six patients at the dose level 2. As a result, the dose level 2 (GEM, 1000 mg/m^2 and CBDCA, AUC of 4) was determined to be the MTD.

Table 2. Characteristics of evaluable patients ($n = 12$)

Characteristics	No. of patients
Gender	
Male	11
Female	1
Age (years)	
Median	68
Range	51–72
Histology	
Adenocarcinoma	5
Squamous cell carcinoma	4
Large cell carcinoma	2
Pleomorphic carcinoma	1
Stage	
IIIB	5
IV	6
Relapse after surgery	1

Toxicity

The worst grades for each patient in the first cycle are listed in Table 3. Grade 3/4 leukopenia or neutropenia was observed in one patient at level 1 and two patients at level 2. Febrile neutropenia was observed in one patient at level 1 and two patients at level 2. Two patients had grade 3/4 anemia at level 1 and one patient required red blood cell transfusion. No grade 3/4 anemia occurred at level 2. Thrombocytopenia was the principal toxicity of this combination chemotherapy. At level 1, grade 3/4 thrombocytopenias were observed in three patients, and two patients received platelet transfusion. At level 2, two patients experienced grade 3/4 thrombocytopenia requiring no platelet transfusions. Non-hematologic toxicities were generally mild at level 1, however, one patient experienced grade 3 nausea/

Table 3. Toxicities during the first cycle

NCI-CTC grade	Level 1 ($n = 6$)		Level 2 ($n = 6$)	
	G1/2	G3/4	G1/2	G3/4
Hematologic				
Leukopenia	1/2	0/1	2/1	2/0
Neutropenia	1/1	1/0	1/1	2/0
Febrile neutropenia	0/0	1/0	0/0	1/1
Anemia	1/3	1/1	2/3	0/0
Thrombocytopenia	1/2	2/1	1/1	4/0
Transaminase	2/0	0/0	4/2	0/0
Bilirubin	0/0	0/0	0/0	1/0
Creatinine	0/0	0/0	0/0	0/0
Hyponatremia	4/0	2/0	5/0	0/0
Non-hematologic				
Nausea/vomiting	2/0	0/0	3/1	1/0
Anorexia	4/1	0/0	2/1	3/0
Fatigue	1/0	0/0	1/2	1/0
Diarrhea	0/0	0/0	2/0	0/0
Constipation	0/0	0/0	0/1	0/0
Mucositis	0/0	0/0	0/0	0/0
Pneumonitis	0/0	0/0	0/0	0/0
Infection	0/0	0/0	0/0	0/0
Skin rash	1/0	0/0	1/0	0/0
Omission on day 8	0		1	
No. of patients with DLT	2		3	

NCI-CTC, National Cancer Institute-Common Toxicity Criteria; DLT, dose-limiting toxicity.