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Table 1. *In vitro* growth-inhibitory activity of SN-38, NK012, CPT-11, and CDDP in human SCLC cells

Cell line	IC_{50} (μmol/L)			
	SN-38	NK012	CPT-11	CDDP
SBC-3/VEGF	0.00330 ± 0.00210	0.00365 ± 0.00005	1.11 ± 0.29	2.21 ± 0.36
SBC-3/Neo	0.00872 ± 0.00063	0.0101 ± 0.0006	5.05 ± 0.08	12.8 ± 1.5
H69	0.0205 ± 0.0195	0.0417 ± 0.0052	22.2 ± 5.9	6.23 ± 0.33
H82	0.00716 ± 0.00079	0.00998 ± 0.00328	1.98 ± 0.55	4.08 ± 3.79

inflammation predominantly infiltrated with lymphocytes, and ++ indicating active inflammation infiltrated with lymphocytes and neutrophils.

Distribution of NK012 or CPT-11 in small intestine by fluorescence microscopy. NK012 or CPT-11 was administered to female BALB/c nude mice at 20 or 30 mg/kg on day 0, respectively. Mice were sacrificed 1, 6, 24, and 72 h after drug injection, and the small intestine was excised at the middle portion and embedded in an OCT compound (Sakura Finetechnochemical Co. Ltd.) and frozen at -80°C. Tissue sections (5 μm thick) were prepared using a cryostatic microtome (Tissue-Tek Cryo3, Sakura Finetechnochemical). Frozen sections were examined under a fluorescence microscope (Biorevo, Keyence) at a 358-nm excitation wavelength and a 461-nm emission wavelength to evaluate NK012 or CPT-11 distribution in the small intestine. Because formulations containing SN-38 bound via ester bonds possess a particular fluorescence, both NK012 and CPT-11 were detected under the same fluorescence conditions.

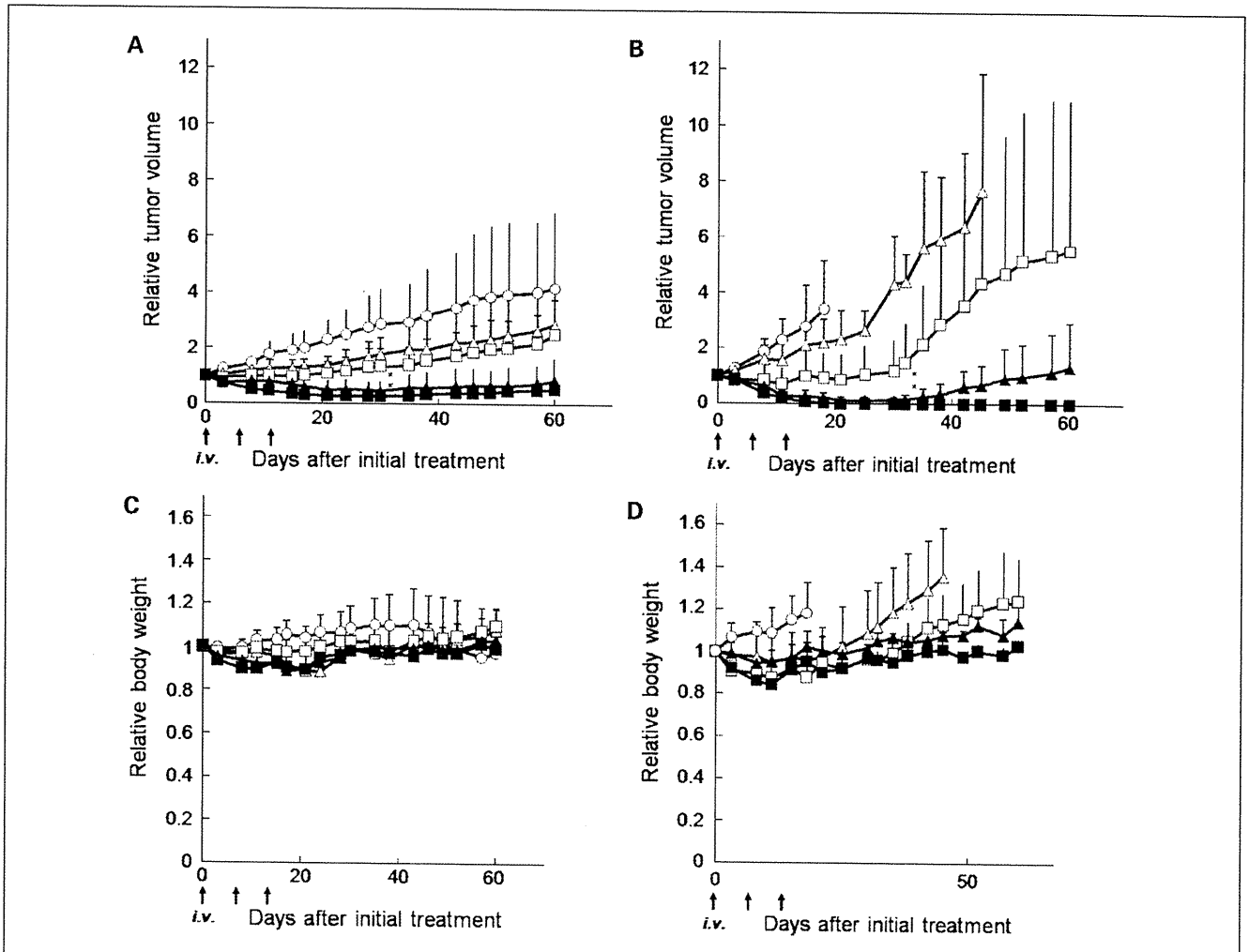

Fig. 1. Growth inhibitory effects of NK012/CDDP and CPT-11/CDDP on SBC-3/Neo and SBC-3/VEGF tumor xenografts. *A* and *B*, RTV in mice treated with NK012/CDDP or CPT-11/CDDP. SBC-3/Neo (*A* and *C*) and SBC-3/VEGF (*B* and *D*) tumors were inoculated s.c. into the flank of mice, as described in Materials and Methods. CPT-11 (10 mg/kg/d; Δ), CPT-11 (22 mg/kg/d; □), NK012 (5 mg/kg/d; ▲), or NK012 (10 mg/kg/d; ■) combined with CDDP (2.5 mg/kg/d) were i.v. administered on days 0, 7, and 14. ○, NaCl solution (0.9%) was i.v. administered as normal control. *Points,* mean; *bars,* SD. *, $P < 0.05$. *C* and *D*, treatment-related BW loss occurred in mice treated with NK012/CDDP and CPT-11/CDDP. *Points,* mean; *bars,* SD.

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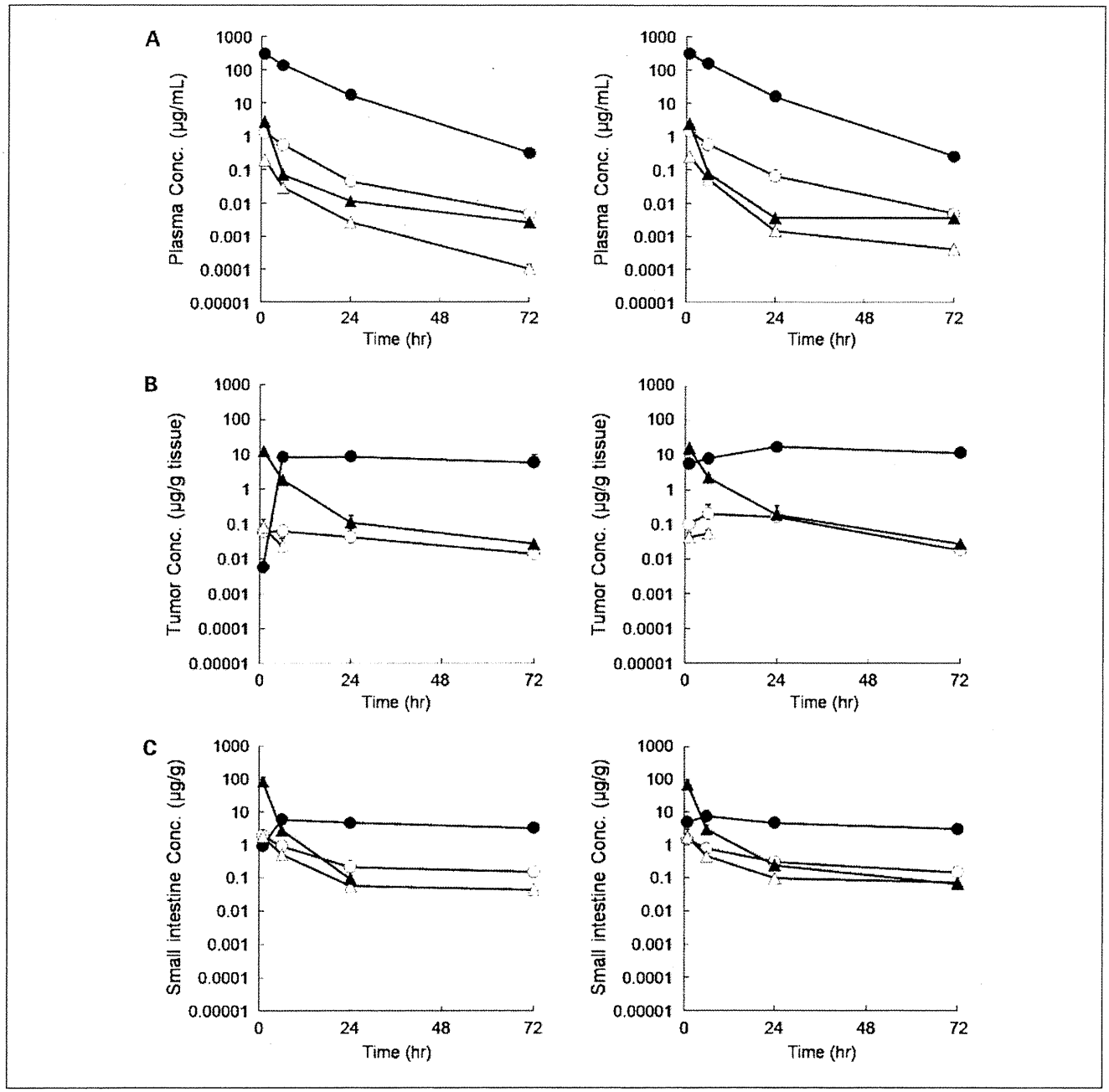

Fig. 2. Plasma, tumor, and small intestine concentrations of NK012, CPT-11, and free SN-38. Plasma (*A*), tumor (*B*), and small intestine (*C*) distribution of NK012, CPT-11, and free SN-38 after i.v. administration of CPT-11 (30 mg/kg) combined with CDDP (2.5 mg/kg) or NK012 (20 mg/kg) combined with CDDP (2.5 mg/kg). Left, SBC-3/Neo; right, SBC-3/VEGF. ●, polymer-bound SN-38; ○, free SN-38 (polymer-unbound SN-38); Δ, SN-38 converted from CPT-11; ▲, CPT-11.

Statistical analysis. Data were analyzed with Student's *t* test when groups showed equal variances (*F* test) or with Welch's test when they showed unequal variances (*F* test). $P < 0.05$ was considered significant. All statistical tests were two sided, and data were expressed as mean ± SD.

Results

Cellular sensitivity of SCLC cells to NK012, CPT-11, SN-38, and CDDP. The IC_{50} values of NK012 for the SCLC cell lines ranged from 0.004 μmol/L (SBC-3/VEGF) to 0.041 μmol/L (H69; Table 1). The cytotoxic effects of NK012 were 198- to 532-fold higher than those of CPT-11, whereas those of NK012 were 1.10- to 2.00-fold lower than those of SN-38. These features were comparable with those reported previously (12, 13).

The molar ratios of NK012 to CDDP of 1:600 in SBC-3/VEGF, 1:120 in SBC-3/Neo, 1:150 in H69, and 1:400 in H82 were used for the drug combination studies based on the IC_{50} values of NK012 and CDDP (Table 1). The synergic to additive

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effect between NK012 and CDDP was observed in these SCLC cell lines (data not shown).

Antitumor activity of NK012/CDDP and CPT-11/CDDP against SBC-3/Neo and SBC-3/VEGF tumors. SBC-3/Neo and SBC-3/VEGF tumors treated with 5 mg/kg/d NK012 plus 2.5 mg/kg/d CDDP were significantly smaller than those treated with 10 mg/kg/d CPT-11 plus 2.5 mg/kg/d CDDP on day 30 (P = 0.0024, SBC-3/Neo; P = 0.0437, SBC-3/VEGF). Moreover, both tumors treated with 10 mg/kg/d NK012 plus 2.5 mg/kg/d CDDP were significantly smaller than those treated with 22 mg/kg/d CPT-11 plus 2.5 mg/kg/d CDDP on day 30 (P = 0.0058, SBC-3/Neo; P = 0.0478, SBC-3/VEGF; Fig. 1A and B). Although treatment-related BW loss was observed in mice treated with each drug combination, BW recovered to the normal level in each group by day 30 (Fig. 1C and D). A stronger antitumor activity against SBC-3/VEGF tumors was observed than against SBC-3/Neo tumors. The complete response rates achieved with 10 mg/kg/d NK012 plus 2.5 mg/kg/d CDDP were 100% and 0% for SBC-3/VEGF and SBC-3/Neo, respectively. These results further confirm our previous findings that a more potent antitumor effect of NK012 is observed in highly vascularized tumors (12).

Pharmacokinetics of NK012 and CPT-11 after NK012/CDDP and CPT-11/CDDP administration in mice bearing SBC-3/Neo or SBC-3/VEGF tumors. After CPT-11/CDDP injection, the plasma concentrations of CPT-11 and SN-38 converted from CPT-11 decreased rapidly within 6 hours in a log-linear fashion (Fig. 2A). Those of NK012 (polymer-bound SN-38) and SN-38 released from NK012 decreased more gradually (Fig. 2A). As for the CPT-11 and free SN-38 concentrations in the SBC-3/Neo and SBC-3/VEGF tumors, they decreased rapidly within 6 hours, and almost no SN-38 converted from CPT-11 was detected at 24 hours in both tumors (Fig. 2B). In the case of NK012/CDDP administration, free SN-38 released from NK012 could be detected in the tumors even at 72 hours after administration (Fig. 2B). In contrast to the case of CPT-11/CDDP administration, the concentrations of free SN-38 released from NK012 were higher in the SBC-3/VEGF tumors than in the SBC-3/Neo tumors at any time point during the observation period (significant at 1 hour; P = 0.013).

Free SN-38 concentrations in the small intestine after NK012/CDDP or CPT-11/CDDP administration were still detectable up to 72 hours in a similar fashion. CPT-11 concentrations 1 hour after CPT-11/CDDP administration were significantly higher than NK012 concentrations after NK012/CDDP administration (P = 0.0056, SBC-3/Neo; P = 0.017, SBC-3/VEGF; Fig. 2C).

These kinetic profiles in liver, spleen, lung, and kidney of free SN-38 after NK012/CDDP or CPT-11/CDDP administration were almost similar to those of NK012 or CPT-11 when administered as a single agent, as described (data not shown; ref. 12).

Intestinal toxicity of NK012, NK012/CDDP, CPT-11, and CPT-11/CDDP. Pathologic findings and characteristic mucosal changes are shown in Table 2 and Fig. 3. The small intestinal mucosa of mice in the CPT-11 or CPT-11/CDDP treatment group showed fibrotic changes, and active inflammation with cellular invasion, healed erosion, deformed glandular alignment, and glandular duct disappearance were also found. On the other hand, the small intestinal mucosa of mice in the NK012/CDDP treatment group showed only mild shortening and decreased number of villi or mild inflammatory cell invasion.

We next analyzed the concentrations of NK012, CPT-11, and free SN-38 in the feces. CPT-11 concentrations at 1 hour were significantly higher than NK012 concentrations (P = 0.0021) and decreased rapidly within 24 hours but remained detectable up to 72 hours. On the other hand, NK012 (polymer-bound SN-38) could be detected at a low concentration from 72 hours (Fig. 4A). To evaluate drug distribution over time, sections of the small intestine treated with NK012 or CPT-11 were examined by fluorescence microscopy. In the sections of CPT-11–treated small intestine, strong fluorescence originating from CPT-11 was detected in the epithelium of the small intestine, whereas weaker fluorescence originating from NK012 was distributed uniformly in the mucosal interstitium (Fig. 4B).

Discussion

Here, we compared the antitumor activity of NK012/CDDP with CPT-11/CDDP, the latter being one of the most active regimens against SCLC and NSCLC. The present data showed that when NK012/CDDP was administered, NK012 effectively accumulated in SBC-3/VEGF tumors and sufficiently exerted antitumor effects. This suggests that CDDP did not affect the permeability of tumor vessels and NK012 retention in the tumors. Hasegawa et al. (29) reported that 17 of 24 patients showed positive immunoreactivity for the VEGF protein in tumor specimens and that elevated serum VEGF levels were

Table 2. Pathologic analysis of small intestine after i.v. administration of drugs

Case no.	Treatment group	Site	Fibrosis	Inflammation	
1	Control	Jejunum	-	-	
	Control	Ileum	-	-	
2	Control	Jejunum	-	-	
	Control	Ileum	-	-	
3	Control	Jejunum	-	-	
	Control	Ileum	-	-	
4	CPT-11	Jejunum	+	+	
	CPT-11	Ileum	+	++	Erosion
5	CPT-11	Jejunum	+	+	Edema
	CPT-11	Ileum	-	-	
6	CPT-11	Jejunum	-	-	
	CPT-11	Ileum	+	+	Erosion
7	CDDP + CPT	Jejunum	+	+	
	CDDP + CPT	Ileum	-	-	
8	CDDP + CPT	Jejunum	+	+	
	CDDP + CPT	Ileum	-	+	
9	CDDP + CPT	Jejunum	+	+	
	CDDP + CPT	Ileum	-	-	
10	NK012	Jejunum	-	-	
	NK012	Ileum	-	-	
11	NK012	Jejunum	-	-	
	NK012	Ileum	-	-	
12	NK012	Jejunum	-	-	
	NK012	Ileum	-	+	
13	CDDP + NK012	Jejunum	-	+	
	CDDP + NK012	Ileum	-	+	
14	CDDP + NK012	Jejunum	-	+	
	CDDP + NK012	Ileum	-	-	
15	CDDP + NK012	Jejunum	-	+	
	CDDP + NK012	Ileum	-	-	

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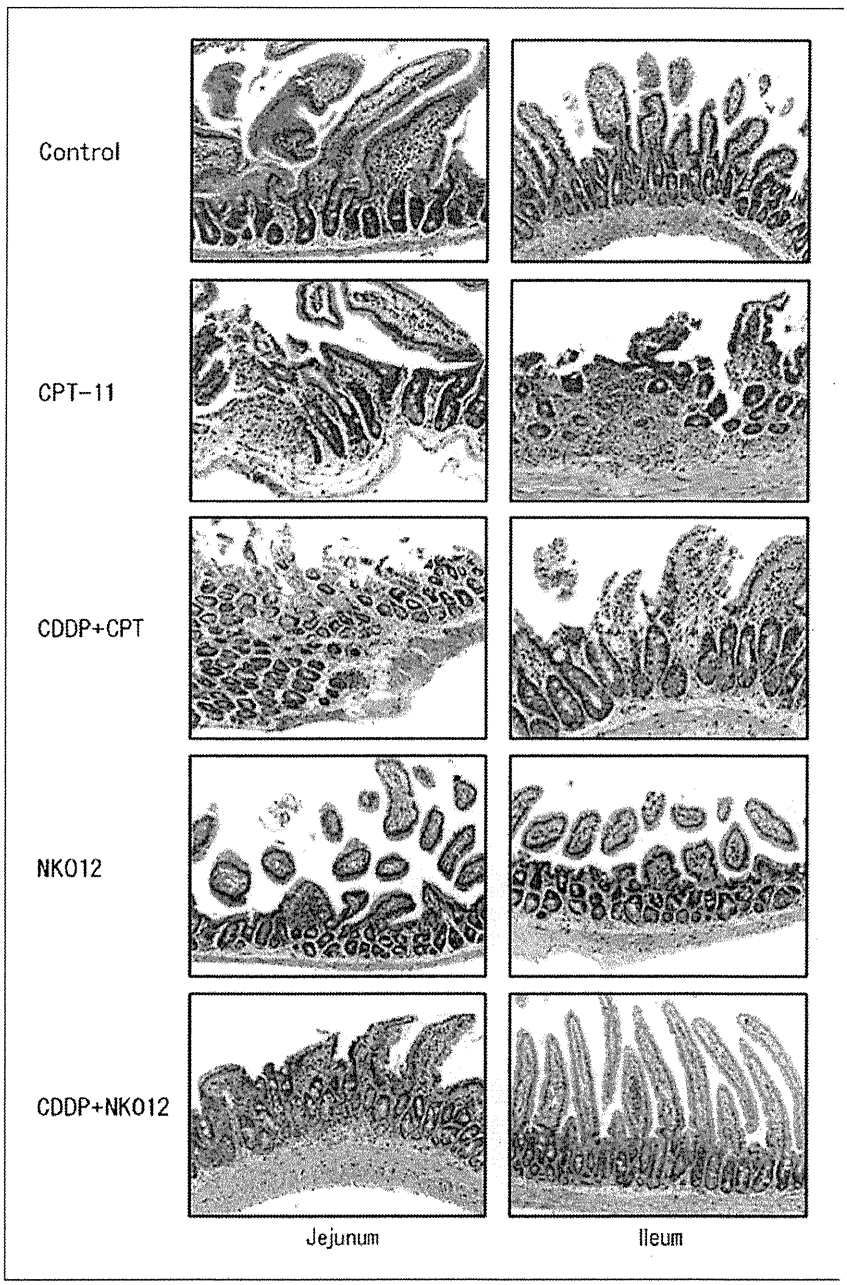

Fig. 3. Pathologic findings and characteristic mucosal changes in mouse. Jejunal and ileal mucosae from mice treated with NaCl solution (0.9%) as control, CPT-11 (22 mg/kg), CPT-11 (22 mg/kg) combined with CDDP (2.5 mg/kg), NK012 (10 mg/kg), or NK012 (10 mg/kg) combined with CDDP (2.5 mg/kg) on days 0, 7, and 14 were examined on day 28 after drug injections. The jejunal mucosa of mice in the CPT-11 treatment group showed healed erosion with fibrotic changes and lymphocytic invasion. Glandular arrangement was severely altered. Active inflammation with inflammatory cell invasion and disappearance of gland ducts were observed on the ileal mucosa in the CPT-11 treatment group. In the CPT-11/CDDP treatment group, the jejunal mucosa also showed healed erosion with scar-like fibrotic growth and mild inflammatory cell invasion into the ileal mucosa. The jejunal and ileal mucosae in the NK012 treatment group and the ileal mucosa in the NK012/CDDP treatment group were almost the same as those in the control group, that is, without inflammatory changes. The jejunal mucosa in the NK012/CDDP treatment group showed mild shortening and decreased number of villi or mild inflammatory cell invasion.

associated with poor outcome in SCLC. As for NSCLC, it was reported that the percentage of VEGF-positive cells was 52 ± 33% (95% confidence interval, 41-64%; median, 70%), and this value showed a positive association with high vascular grade (P = 0.008) and poor survival (P = 0.04; ref. 30). Taking all data together, NK012/CDDP may therefore be clinically effective against lung cancers, particularly those with high VEGF production.

Pathologic examinations were also conducted to evaluate changes in the small intestinal mucosa on day 14 after treatment. This is because diarrhea is one of the clinical dose-limiting toxicities of CPT-11, and epithelial apoptosis was reported as a mucosal change induced by CPT-11 (31). This pathologic change was observed on day 6 after i.p. administration of 100 mg/kg CPT-11 daily for 4 days. We found that the CPT-11–induced mucosal change was mainly fibrosis considered to be a form of recovery change from erosion. On the other hand, the small intestinal mucosa of the mice in the NK012/CDDP treatment group showed only mild shortening and decreased number of villi or mild inflammatory cell invasion. On comparison of these changes with those caused by CDDP (31), it was found that such alterations were mainly induced by CDDP rather than NK012.

A portion of SN-38 converted from CPT-11 undergoes subsequent conjugation as induced by UDP-glucuronyltransferase to form SN-38 β-glucuronide (SN-38-Glu; ref. 32). CPT-11, SN-38, and SN-38-Glu are excreted into the bile and then reach the small intestinal lumen (32, 33). SN-38-Glu is

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deconjugated in the cecum and colon to regenerate SN-38 through bacterial β-glucuronidase (34). In this study, CPT-11 was excreted into feces much more than NK012 and a high CPT-11 concentration was detected in the small intestinal epithelium. It is speculated that the highly excreted CPT-11 is reabsorbed in the small intestinal epithelium and converted to SN-38 to cause damage to the intestinal mucosa. On the other hand, NK012 was uniformly distributed in the mucosal interstitium at a lower concentration, which may be related to the less mucosal damage and diarrhea than those induced by CPT-11, although NK012 was observed for longer period than CPT-11. About other toxic effects including bone marrow, liver,

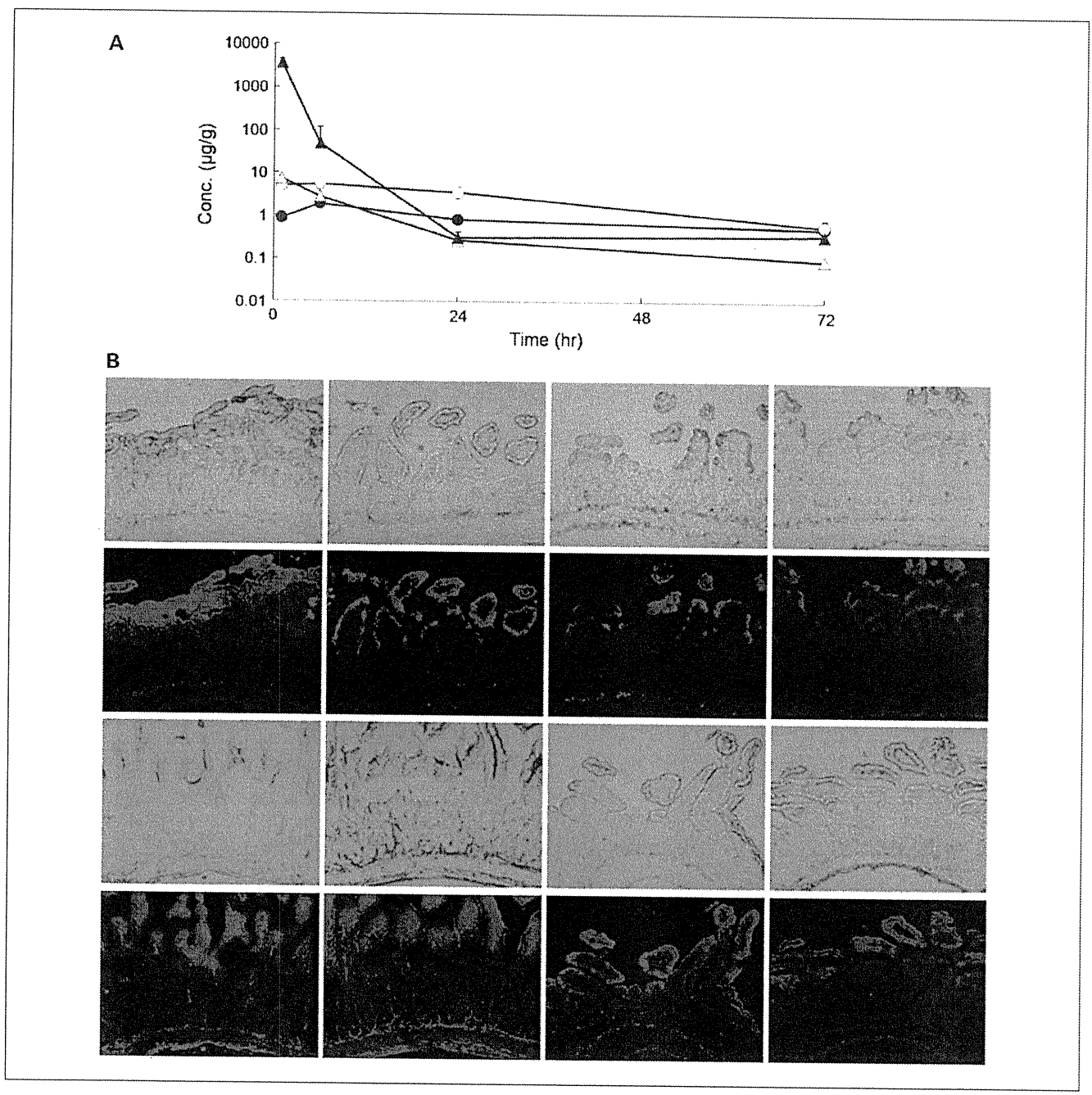

Fig. 4. Fecal concentrations of NK012, CPT-11, and free SN-38, and NK012 or CPT-11 distribution in the small intestine. *A*, distribution of NK012, CPT-11, and free SN-38 after i.v. administration of CPT-11 (30 mg/kg) or NK012 (20 mg/kg). ●, polymer-bound SN-38; ○, free SN-38 (polymer-unbound SN-38); Δ, SN-38 converted from CPT-11; ▲, CPT-11. *B*, small intestines were excised 1, 6, 24, and 72 h after i.v. administration of CPT-11 (30 mg/kg) or NK012 (20 mg/kg). Frozen sections were examined under a fluorescence microscope at a 358-nm excitation wavelength and a 461-nm emission wavelength. NK012 and CPT-11 were visualized as blue. The first or third columns are a bright-field image and the second or forth columns are a fluorescence image. Sections of small intestines were most well visualized in bright field. First, second, third, and fourth lines from the left side are images obtained 1, 6, 24, and 72 h after drug administration, respectively. CPT-11 was strongly distributed in the epithelium of the small intestine, whereas NK012 tended to be distributed weakly and uniformly in the mucosal interstitium.

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and kidney toxicities, there was no significant difference between NK012/CDDP and CPT-11/CDDP in the present treatment schedule (data not shown).

In conclusion, NK012/CDDP showed a significantly higher antitumor activity with no severe diarrhea toxicity than CPT-11/CDDP, one of the most active regimens against SCLC and NSCLC. The present data suggest the clinical evaluation of NK012/CDDP in patients with SCLC and NSCLC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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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 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

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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.
* $p < 0.05$.

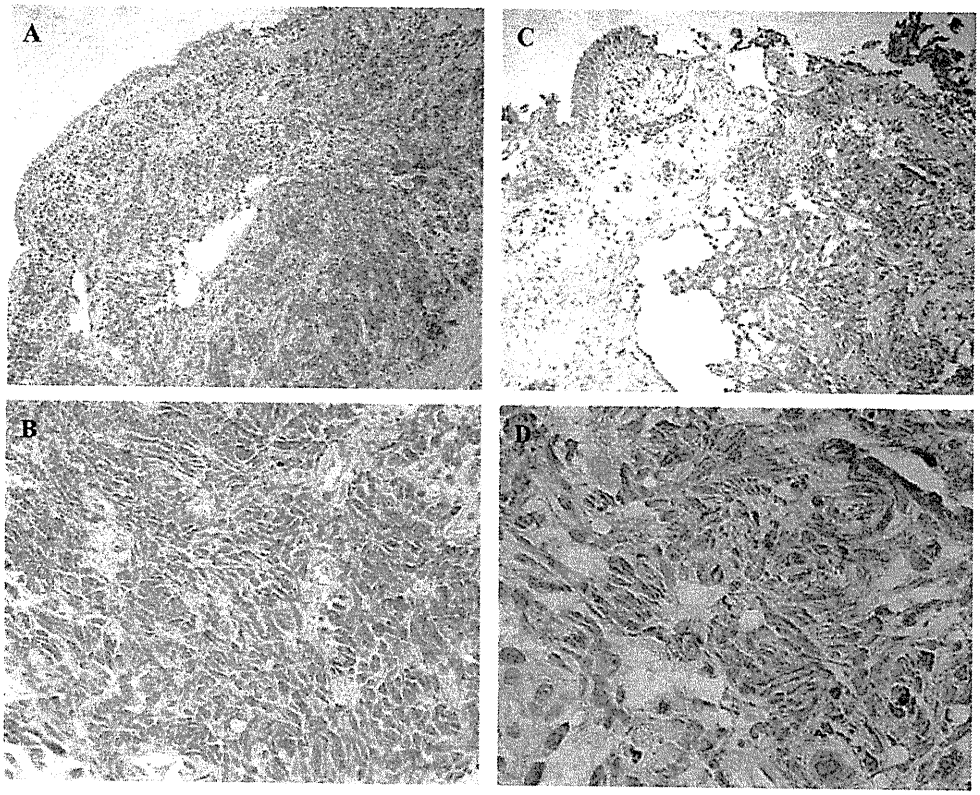

Fig. 1. Representative cases of positive immunostaining for BCRP (A, ×100; B, ×400) and MRP2 (C, ×100; D, ×400). BCRP and MRP2 in the apical membrane of the bronchial layer have been immunostained as a positive control.

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.
* $p < 0.05$.

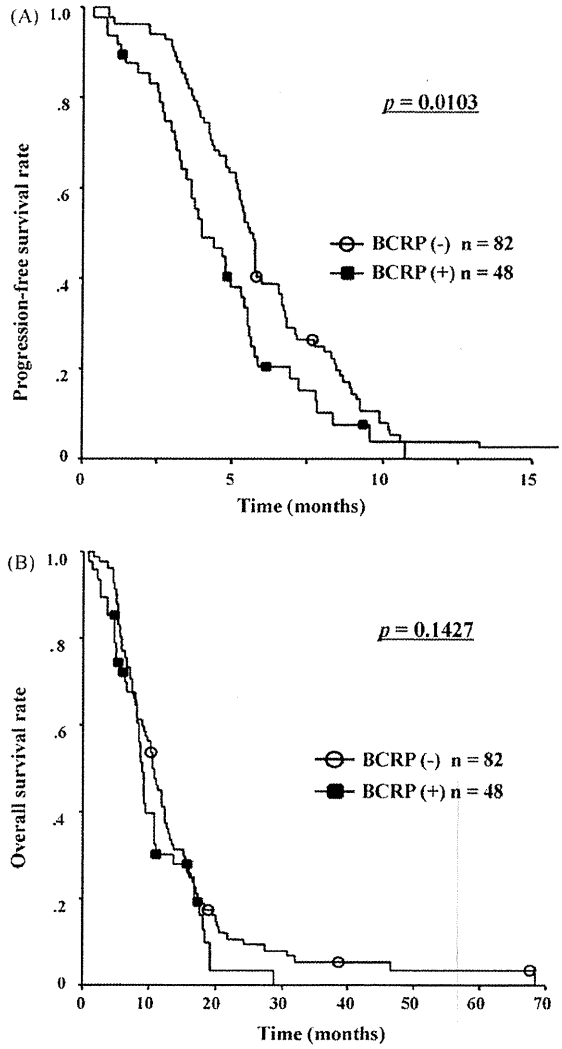

Fig. 2. Progression-free survival curves (A) and overall survival curves (B) for 130 SCLC patients, according to breast cancer resistance protein (BCRP) expression.

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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Differences in the Quality of Information on the Internet about Lung Cancer between the United States and Japan

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Introduction: Quality of information available over the Internet has been a cause for concern. Our goal was to evaluate the quality of information available on lung cancer in the United States and Japan and assess the differences between the two.

Methods: We conducted a prospective, observational Web review by searching the word "lung cancer" in Japanese and English, using Google Japan (Google-J), Google United States (Google-U), and Yahoo Japan (Yahoo-J). The first 50 Web sites displayed were evaluated from the ethical perspective and for the validity of the information. The administrator of each Web site was also investigated.

Results: Ethical policies were generally well described in the Web sites displayed by Google-U but less well so in the sites displayed by Google-J and Yahoo-J. The differences in the validity of the information available was more striking, in that 80% of the Web sites generated by Google-U described the most appropriate treatment methods, whereas less than 50% of the Web sites displayed by Google-J and Yahoo-J recommended the standard therapy, and more than 10% advertised alternative therapy. Nonprofit organizations and public institutions were the primary Web site administrators in the United States, whereas commercial or personal Web sites were more frequent in Japan.

Conclusion: Differences in the quality of information on lung cancer available over the Internet were apparent between Japan and the United States. The reasons for such differences might be tracked to the administrators of the Web sites. Nonprofit organizations and public institutions are the up-and-coming Web site administrators for relaying reliable medical information.

Key Words: Internet, Information quality, Lung cancer.

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The Internet has given rise to an information revolution of unprecedented magnitude. Whereas the Internet has great potential in marshaling the large volume of health information resources available, it is becoming increasingly difficult to discern which of the resources are reliable and accurate or appropriate for the users.[1–6] This issue has become a cause for great concern, especially in the field of oncology, and many studies have evaluated the pros and cons of obtaining information from the Internet.[2–6] Meanwhile, the medical community is being increasingly faced with patients asking us about the medical information available on the Internet. We can no longer neglect the public importance of the information available and have to use it effectively for patients to better understand their disease.

Although one of the main characteristics of the Internet is its worldwide accessibility, differences in language use around the world serve as a bottleneck for collecting information from the Internet. The estimated number of people using the Internet is about the same in the United States and Japan (70 and 67%,[7,8] respectively), and 80% of patients obtain health information via the Internet in the United States.[9] Until now, most studies that have evaluated the quality of the health care information available over the Internet are from the English-speaking community, and very few studies have been conducted in relation to information available in Japanese.[10,11] Furthermore, only a limited number of studies evaluating the differences in the quality of information available between two languages have been published,[12] and no such study comparing such information in the English and Japanese languages has been published.

Our goal was to imitate the search for medical information by the general population in Japan and United States and to evaluate the differences in the process between the two countries. We also investigated the administrators of the Web sites and attempted to identify any correlation existing between the Web site administrators and the quality of information available on the Internet. We focused on information available on lung cancer, which is the leading cause of cancer-related death in both the Unites States and Japan.[13,14] Because search engines are the leading tools to obtain any kind of information, whether general or medical, on the Internet,[15] we used Google and Yahoo, which are the two most commonly used search engines for Web search in both the United States and Japan.

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METHODS

Web Site Search

We conducted a prospective, observational Web review by performing keyword searches using Google in both Japanese and English, and Yahoo in Japanese. Japanese searches were conducted by author YG in Japan (Tokyo) on May 29, 2007, and the English search was conducted by author HS in the United States (New York) on May 25, 2007. We used "Hai-gan (both letters in Chinese characters)," "Hai (Chinese character)-gan (hiragana)," and "Hai (Chinese character)-gan (katakana)," for the Japanese search, and "lung cancer" and "lung carcinoma" for the English search. The search word that resulted in the largest number of search results was chosen for the subsequent study.

The first 50 Web sites displayed by Google and Yahoo in Japanese, and Google in English, excluding the advertisement area, were used for further evaluation. Web sites that were inaccessible, not designed to provide health information (i.e., news and advertisement of books), or displayed for the second (or more) time were excluded from the subsequent evaluation. Samples from the Yahoo in English were supplemented to compare the search utility on January 21, 2009.

Site Characteristics

Author YG evaluated the Web sites within a week of the original search. We evaluated the Web sites based on criteria known as the "JAMA" benchmark[16]: display of authorship (authors and contributors, their affiliations, and relevant credentials), attribution (references and sources for all content and all relevant copyright information), disclosure (Web site ownership, sponsorship, advertising, commercial funding arrangements or support, or potential conflicts of interest), and currency (dates on which the contents were posted and updated). We considered each criterion as fulfilled when it was fully displayed. For further evaluation, we focused on the description about the treatment of advanced non-small lung cancer. To our knowledge, there is no established tool-based instrument to evaluate the information available on cancer treatment. Therefore, we classified the information into three categories: acceptable (description of systematic reviews, such as guidelines from authorized facilities,[17–20] links to systematic reviews, or abstracts of systematic reviews), unacceptable (recommendation of alternative medicine or a generally unapproved treatment), and inevaluable (lack of adequate description). The administrators of the Web sites were classified into five categories: nonprofit organization (NPO) or public institution, medical institution, commercial (for specific treatments), personal (pages made by patients or their families), and others.

Analysis

Descriptive statistics were used to determine the numbers and percentages related to the characteristics of the Web sites. To compare the differences between two countries in view of user experience and search utility, Web sites displayed in Google-U was compared with that of Yahoo-J and Google-J, respectively. The χ^2 test or Fisher's exact test was used as appropriate.

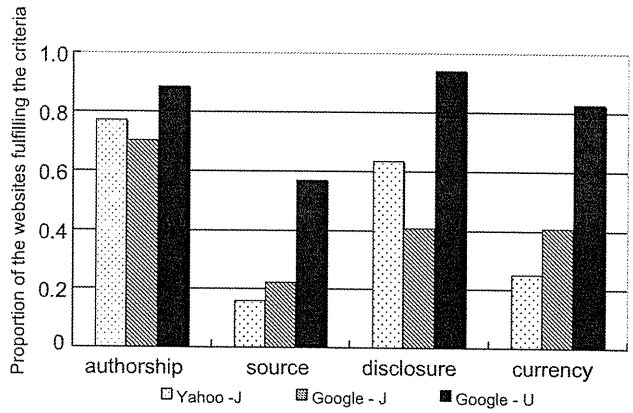

FIGURE 1. JAMA benchmark: Description of the JAMA benchmark[16] is listed by the search engines; display of authorship (authors and contributors, their affiliations, and relevant credentials); attribution (references and sources for all content, and all relevant copyright information); disclosure (Web site ownership, sponsorship, advertising, commercial funding arrangements or support, or potential conflicts of interest); and currency (dates on which the contents were posted and updated).

RESULTS

Differences by Notation

In Google Japan, search using the word "Hai-gan (both letters in Chinese characters)" resulted in a display of approximately 7.7 million Web sites, and in Google United States, search using the phrase "lung cancer" threw up approximately 52 million Web sites. These notations were, therefore, used for the subsequent evaluation. After excluding Web sites that were inaccessible, were not designed to provide health information, or ranked for the second (or more) time in each search, 44, 27, 39, and 35 Web sites displayed by Yahoo Japan (Yahoo-J), Google Japan (Google-J), Yahoo United States (Yahoo-U), and Google United States (Google-U), respectively, were evaluated for further study.

Web Site Characteristics

Figure 1 summarizes the quality of the Web sites that satisfied the criteria of the JAMA benchmark. Authorship was displayed in more than 70% of the Web sites displayed by the three searches: 31 in Google-U (88.6%), 34 in Yahoo-J (70.3%, $p = 0.243$), and 19 in Google-J (88.6%, $p = 0.106$). Attribution of the content was found in 20 (57.1%) of the Web sites in Google-U, and 7 (15.9%, $p < 0.001$) and 6 (22.2%, $p = 0.009$) of the Web sites in Yahoo-J and Google-J, respectively. Twenty-eight (63.6%, $p = 0.001$) Web sites in Yahoo-J, 11 (40.7%, $p < 0.001$) in Google-J, and 33 (94.2%) in Google-U made the disclosure. Display of currency was found in 29 (82.9%) sites in Google-U, but in less than 50% of the Web sites in the Japanese searches; 11 (25.0%, $p < 0.001$) in Yahoo-J and 11 (40.7%, $p = 0.001$) in Google-J.

Quality of Description of the Treatment

Evaluation of the treatment description for advanced non-small cell lung cancer is summarized in Figure 2. The

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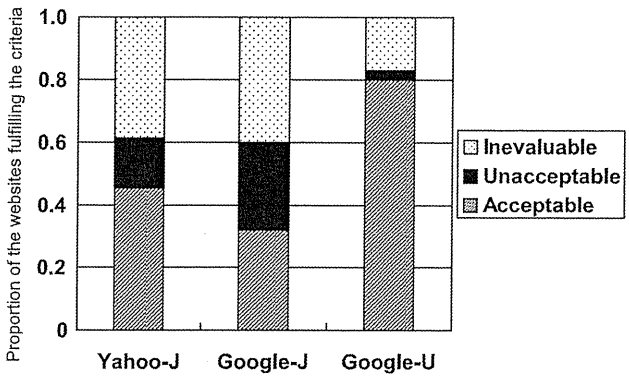

FIGURE 2. Evaluations of the treatment description in the Web sites: The treatment description is classified into three categories: acceptable (description of the systematic review such as guidelines from authorized facilities[17–20]; links to systematic reviews; abstracts of systematic reviews), unacceptable (recommendation of alternative medicine or a generally unapproved treatment), and invaluable (lack of description).

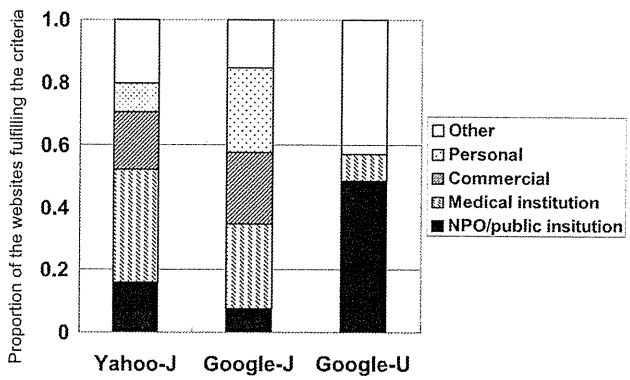

FIGURE 3. Administrators of the Web sites: Administrators were classified into five categories: NPO (nonprofit organization) or public institution, medical institution, commercial (for the specific treatments), personal (pages made by patients or their families), and others.

TABLE 1. Correlation of Sites Between the Top 50 Google and Yahoo, and the Rate of Reliable Sites in Each Engine

	United States	Japan
Correlation of titles in top 50 site of Google and Yahoo	11	10
Percentage of reliable sites in top 50 (%)		
Google	80.0	29.6
Yahoo	71.8[a]	45.5

Correlation of titles in both engines was almost the same in both countries. Proportions of reliable sites were comparable in countries but were not in search engines.

[a] Accessed and evaluated on January 21, 2009.

description was acceptable in 28 (80.0%) of the Web sites generated by Google-U, as these sites described chemotherapy as the standard treatment for advanced lung cancer. Only one site recommended alternative medicine. In Web sites ranked by Yahoo-J and Google-J, standard therapy was only described in 20 (45.5%, $p < 0.001$) and 10 (37.0%, $p < 0.001$) sites, respectively, whereas 7 (15.9%, $p = 0.070$) and 7 (25.9%, $p = 0.017$) sites, respectively, recommended alternative medicine. Table 1 summarizes the quality of the Web sites displayed in Yahoo and Google by both countries. Proportions of reliable sites were comparable in countries but were not in search engines.

Administrators of the Web sites

The administrators of the Web sites are shown in Figure 3. In Google-U, the administrators of 16 (45.7%) Web sites were NPO or public institution, whereas only 7 (15.9%, $p = 0.006$) and 2 (7.4%, $p = 0.001$), respectively, in Yahoo-J and Google-J were managed by them. Commercial site for specific treatments was not displayed in Google-U but was displayed in 8 (18.2%, $p = 0.007$) and 6 (22.2%, $p = 0.005$) Web sites in Yahoo-J and Google-J, respectively. Web sites administered personally by the patients themselves or their families were also not found among the Web site displayed in Google-U, whereas 4 (9.1%, $p = 0.125$) sites in Yahoo-J and 7 (25.9%, $p = 0.002$) sites in Google-J were personally managed.

Administrators and Quality of the Contents of the Web Sites

Table 2 shows the correlation between the Web site administrator and the quality of the contents of the sites. Ten sites generated by both Google-J and Yahoo-J were integrated. There was no site from NPO or public institution category, either Japanese or English, which provided misleading information. Most of the unacceptable sites were managed by commercial or personal sites, neither of which was found in the English-language sites.

DISCUSSION

By comparing the differences of quality of cancer information on the Internet between the different languages, we, for the first time, evaluated the correlation between the Web site administrator and the quality of the medical information in the Web sites. Furthermore, it is one of the few studies to evaluate the information on lung cancer available on the Internet.[15] We also showed that the Web sites displayed in the United States provide information of much higher quality than those displayed by Japanese Web sites, with regard to lung cancer treatment, and this may be related to the quality of the administrators of the displayed Web sites.

It is generally a difficult task to make people access reliable Web sites that would provide the precise information that they are looking for. Regulating access to only trustworthy Web sites that provide useful information is extremely difficult, because a global rule is a necessary step toward controlling the content of the worldwide Web sites. There are also no confirmed tools for weighting the information on the Internet in any field, including medicine. In this chaotic scenario, search engines such as Google and Yahoo have come up with a solution by developing an algorithm to rank the sites. Nowadays, their value is well established in the

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TABLE 2. Correlation Between the Quality of the Web site Administrators and the Quality of the Information

	NPO Public Institution	Med Institution	Commercial	Personal	Other	Total
Japanese						
Acceptable	6	10	0	1	5	22
Unacceptable	0	0	10	7	2	19
Inevaluable	2	10	1	1	6	20
Total	8	20	11	9	13	61
English						
Acceptable	15	3	0	0	10	28
Unacceptable	0	0	0	0	1	1
Inevaluable	2	0	0	0	4	6
Total	17	3	0	0	15	35

Ten sites generated by both Google-J and Yahoo-J were integrated. No site from the NPO or public institution category provided misleading information in either the Japanese or the English search. Commercial administrators recommending specific treatments and personal sites accounted entirely for the sites providing unacceptable information.

Internet, and people are generally using this tool for searching medical and other information. Even though there is a concern that the order in which the sites are placed by these tools is not entirely appropriate for the field of medicine,[3,21,22] the high frequency at which these are used has made it meaningless to say that they pose a problem in one-particular field. Therefore, what we must consider now is how to provide reliable information using these tools.

Why is misleading and nonreliable information provided on the Internet? One key characteristic of the Internet is the interaction between the provider and the consumer (in the medical field, patient). Web sites that are not accessed frequently will be ranked lower in the search engine system. Therefore, when discussing the results of Web sites ranked by the search engine, we should consider it from both the standpoint of the provider and the consumer. People access the Internet by requesting the information they want. Many cancer patients suffer from an incurable disease and look for a ray of hope in the Internet. This situation is most advantageous to the information senders. They can promote their treatment as the treatment that would bring about the miraculous cure that the patients are seeking. In this study, most of the sources recommending alternative or unapproved drugs were from commercial and personal sites. Information on medical subjects should be correct and be of assistance to the users to help them better understand their disease. People should be protected from disruptive information. Creating confusion in the minds of people by providing misleading information for profit to the administrator is a vexing situation.

One of the interesting findings in this study was that the correlation between the quality of the Web site administrator and the quality of the contents of the site was seen not only for sites providing misleading information but also for those providing reliable information. At present, there are two major administrators providing reliable information, namely, medical institutions and specialized organizations for information administered by patient advocate NPO or public institution. However, the type of information provided differed between the two types of administrators. In general, each medical institution provides reliable messages but not review articles, whereas the patient advocate group NPO and public institution provide a path to the review articles. This is not surprising because the aims of providing information are different between the two types of administrators. For each medical institution, the goal is to display the treatment that they are interested in, and describing the entire medical consensus is outside their reach. Therefore, sites specialized in providing information are the ones that can be most expected to provide general information. Differences in the number of reliable sites between the languages in this study may be because of the difference in the number of such organizations between the countries. The number of public institution sites may depend on the countries in which each language is spoken in, and the growth in the number of patient advocate NPO may depend on the social system or the differences in culture. However, it is noteworthy that patient advocate NPO can play a major role in providing reliable health information.

There were several limitations in this study. One is that we evaluated sites only from Yahoo Japan and Google Japan, and Google United States. We chose Google United States as the reference, because most previous studies on the Internet have been conducted in the United States, and Google is the most popular search engine in the United States.[23] In Japan, Yahoo ranks first as the most frequently used search engine, followed next by Google,[24] which is the reason we selected these two as the representative search engines for our search of Web sites in Japanese. Although this approach may limit evaluation of the overall Internet situation in the two countries, we believe that this was the closest way to reproduce the way people browse the Internet. Another concern is the number of sites generated by these tools. The total number of Web sites displayed by our search using the keywords differs between the two languages and maybe attributable to the differences in the quality of the administrators. Google-U generated approximately seven times as many Web sites as Google-J. This discrepancy could be because of the difference in the number of people using the two languages. However, we only evaluated the top 50 sites, which is far short of the total number of sites displayed but may already

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be too much for anyone seeking any type of information. Because the ranking system has prevailed, the quality of the highest ranked Web sites and not the total number of sites displayed is important to the user. Lastly, another important problem is whether people in the United States and Japan desire the same answers from the Internet. In general, search engines attempt to rank the Web sites sought by the users. If these differed between countries, the ranking would also reflect these differences. Differences in the social backgrounds of the populations in the two countries were confounding factors in this study. However, no studies evaluating the topic from this perspective have been conducted. These are topics of interest that need further investigation.

In this era of abundance of information, it is absolutely essential for people to make their choices based on the quality. As medical professionals, we have the responsibility of providing appropriate information to people who are unaware and anxious about their future. In the new era of the Internet technology, facilitating easy access to reliable information, and providing reliable information is important. This study may facilitate an understanding of the actual status of dispersal of information and pave the way for discussing methods to achieve better accessibility to high-quality health information.

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Cancer patients' reluctance to discuss psychological distress with their physicians was not associated with underrecognition of depression by physicians: A preliminary study

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ABSTRACT

Objective: To investigate the association between cancer patients' reluctance for emotional disclosure to their physician and underrecognition of depression by physicians.

Methods: Randomly selected ambulatory patients with lung cancer were evaluated by the Hospital Depression and Anxiety Scale (HADS), and those with scores over the validated cutoff value for adjustment disorder or major depressive disorder were included in this analysis. The data set included the responses to the 13-item questionnaire to assess four possible concerns of patients in relation to emotional disclosure to the treating physician ("no perceived need to disclose emotions," "fear of the negative impact of emotional disclosure," "negative attitude toward emotional disclosure," "hesitation to disturb the physician with emotional disclosure"). The attending physicians rated the severity of depression in each patient using 3-point Likert scales (0 [*absent*] to 2 [*clinical*]). Depression was considered to be underrecognized when the patients had a HADS score above the cutoff value, but in whom the depression rating by the attending physician was 0.

Results: The HADS score was over the cutoff value in the 60 patients. The mean age was 65.1 ± 10.0, and 82% had advanced cancer (Stage IIIb or IV or recurrence). Depression was underrecognized in 44 (73%) patients. None of the four factors related to reluctance for emotional disclosure was associated with the underrecognition of depression by the physicians. None of the demographic or cancer–related variables were associated with depression underrecognition by physicians.

Significance of results: The results did not support the assumption that patients' reluctance for emotional disclosure is associated with the underrecognition of depression by physicians.

KEYWORDS: Oncology, Communication, Psycho-Oncology, Depression, Quality of life

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INTRODUCTION

Cancer patients frequently experience psychological distress, especially depression (McDaniel et al., 1995). Because depression interferes with the quality

of life of the patients, induces a desire for death, increase the usage of health care and burdens on the family. Thus it is important to recognize depression and initiate intensive treatment (Block, 2000).

Appropriate assessment is the key and the first step to better management of depression. However, physicians often underestimate the severity of depressive symptoms in their patients (Passik et al., 1998; Fallowfield et al., 2001), and they are less likely recognize distress in more distressed patients (Merckaert et al., 2008). Emotional communication is an interactive phenomenon and is impacted by provider-, health care system-, and patient-related factors. Some review articles have cited cancer patients' hesitation to share their emotional distress and/or concerns with physicians as being possibly related to the underrecognition of depression in these patients by their physicians (Maguire, 1999). Furthermore, patients who were more anxious or depressed may be less likely to disclose their concerns to nurses (Heaven & Maguire, 1997). However, few studies have actually investigated the influence of such patient factors on underrecognition of depression by medical staffs, partly due to the lack of a suitable method of assessment of patients' attitude toward emotional disclosure.

We previously conceptualized four possible concerns on the part of the patients in relation to emotional disclosure: "hesitation to disturb the physician with emotional disclosure," "no perceived need to disclose emotions," "negative attitude toward emotional disclosure," and "fear of the negative impact of emotional disclosure" (Okuyama et al., 2008). The purpose of this study was to examine whether these concerns were actually associated with the recognition or underrecognition of depression by physicians.

METHODS

Subjects

This is a secondary analysis of data collected for a previously published study in which we conceptualized cancer patients' reluctance to disclose their emotional distress to their physicians (Okuyama et al., 2008). The study subjects were randomly sampled ambulatory patients with lung cancer attending the outpatient clinic of the Respiratory Medicine Division of the Tokai University Hospital, located in a suburban residential area, about 50 km from Tokyo, Japan. The eligibility criteria for patients in the original study were patients who were (a) 18 years of age or older, (b) aware of the cancer diagnosis, (c) well enough to complete the questionnaire and participate in a brief interview, and (d) not suffering from severe mental or cognitive disorders. Patients with a total HADS score above the validated cutoff for adjustment disorder or major depressive disorder (>10) (see Procedures section) were included in this analysis.

This study was approved by the Institutional Review Board and the Ethics Committee of Tokai University, Japan, and was conducted in accordance with the Helsinki Declaration. Written consent was obtained from each patient after full disclosure of the aims and procedures of the study.

Procedures

Patients were randomly sampled using a planned visiting list and a table of random numbers. After informed consent had been obtained, the patients were asked to complete the self-administered questionnaires described below at home and return them on the next visit day. In the case of inadequate answers, clarifications were sought over the telephone.

Reluctance for Emotional Disclosure Questionnaire

The Reluctance for Emotional Disclosure Questionnaire (REDQ) was developed for a series of studies to investigate cancer patient-related factors that are barriers to adequate psychological care (Okuyama et al., 2008). The scale assesses four aspects of patients' concerns in relation to emotional disclosure to the attending physician. "No perceived need to disclose emotions" includes four items, including: "No support is needed for my emotional distress, because it resolves spontaneously." "Fear of the negative impact of emotional disclosure" consists of two items, including: "My relation with my doctors will become poor if I discuss my emotional distress with them." "Negative attitude toward emotional disclosure" consists of four items, including: "In general, I do not like to speak about my emotions." "Hesitation to disturb the physician with emotional disclosure" consists of three items, including: "I don't want to bother my doctor by bringing up my emotional distress." Each item is rated on a 5-point Likert scale (1 [*not at all*] to 5 [*strongly agree*]). Each subscale score was obtained by calculating the mean score for the items included in the subscale. The validity and reliability of this assessment has been examined in a previous study (Okuyama et al., 2008). In that study, we found that patients with high distress levels were significantly more likely to endorse "Negative impact," older patients were more likely to report "Negative attitude," whereas male patients were more likely than females to report "Hesitation."