Table 5 Pharmacokinetic parameters of BNP7787 after administration of BNP7787 alone and BNP7787 in combination with paclitaxel and cisplatin

Parameter	Treatment	BNP7787 dose (g/m^2)					
		4.1 (n = 3/3)*	8.2 (n = 6/5)	12.3 (n = 4/4)	18.4 (n = 3/3)	27.6 (n = 3/3)	41.0 (n = 3/3)
$AUC_{0\text{-}t}$ (μg·h/mL)	BNP alone	499 ± 79**	1,332 ± 181	1,851 ± 485	2,748 ± 496	5,576 ± 733	8,551 ± 948
	BNP + CT	453 ± 73	1,180 ± 194	1,545 ± 320	2,552 ± 307	5,937 ± 2,799	6,577 ± 843
$AUC_{0\text{-inf.}}$ (μg h/mL)	BNP alone	501 ± 78	1,347 ± 194	1,870 ± 498	2,757 ± 500	5,780 ± 981	8,836 ± 1,267
	BNP + CT	455 ± 73	1,194 ± 209	1,563 ± 330	2,565 ± 316	5,974 ± 2,774	6,845 ± 1,124
C_{max} (μg/mL)	BNP alone	408 ± 16	946 ± 273	1,176 ± 299	1,864 ± 127	2,946 ± 271	4,233 ± 683
	BNP + CT	402 ± 29	702 ± 113	928 ± 93	1,627 ± 148	2,588 ± 672	3,250 ± 291
$T_{1/2}$ (h)	BNP alone	0.76 ± 0.11	0.92 ± 0.19	0.94 ± 0.13	0.75 ± 0.07	1.19 ± 0.43	1.25 ± 0.38
	BNP + CT	0.72 ± 0.09	0.92 ± 0.19	0.95 ± 0.17	0.78 ± 0.16	1.47 ± 0.75	1.27 ± 0.52
λz (h^{-1})	BNP alone	0.93 ± 0.13	0.78 ± 0.15	0.75 ± 0.11	0.93 ± 0.09	0.64 ± 0.22	0.59 ± 0.17
	BNP + CT	0.97 ± 0.13	0.78 ± 0.13	0.75 ± 0.16	0.91 ± 0.19	0.54 ± 0.22	0.61 ± 0.23
$MRT_{0\text{-}t}$ (h)	BNP alone	0.88 ± 0.18	1.18 ± 0.22	1.28 ± 0.08	1.11 ± 0.08	1.46 ± 0.28	1.52 ± 0.29
	BNP + CT	0.83 ± 0.13	1.22 ± 0.21	1.29 ± 0.09	1.21 ± 0.19	1.92 ± 0.93	1.50 ± 0.29
$MRT_{0\text{-inf.}}$ (h)	BNP alone	0.91 ± 0.17	1.24 ± 0.28	1.34 ± 0.11	1.13 ± 0.09	1.67 ± 0.52	1.72 ± 0.50
	BNP + CT	0.86 ± 0.13	1.29 ± 0.28	1.36 ± 0.11	1.24 ± 0.21	1.97 ± 0.89	1.75 ± 0.59
Vd_{ss} (L)	BNP alone	11.33 ± 2.36	11.79 ± 2.95	15.37 ± 3.34	12.44 ± 2.42	13.86 ± 1.67	12.19 ± 0.92
	BNP + CT	11.85 ± 2.43	13.53 ± 3.45	18.49 ± 3.94	14.27 ± 0.52	16.52 ± 3.52	16.11 ± 2.30
CL (L/h)	BNP alone	12.57 ± 1.84	9.59 ± 2.13	11.66 ± 3.22	11.20 ± 2.94	8.77 ± 2.56	7.40 ± 1.60
	BNP + CT	13.83 ± 2.02	10.69 ± 2.84	13.70 ± 3.29	11.75 ± 2.26	9.55 ± 4.65	9.71 ± 2.65

$AUC_{0\text{-}t}$ area under the plasma concentration–time curve up to last measurable point at 24 h from infusion, *$AUC_{0\text{-inf.}}$* area under the plasma concentration–time curve extrapolated to infinity, *C_{max}* maximum concentration, *λz* elimination rate constant, *$T_{1/2}$* half-life in terminal phase, *$MRT_{0\text{-inf.}}$* mean residence time extrapolated to infinity, *Vd_{ss}* volume of distribution at steady state, *CL* total body clearance, *CT* chemotherapy with cisplatin and paclitaxel

* Values are the no. of patients who received BNP7787 alone/no. of patients who had BNP preceding combination of paclitaxel and cisplatin

** Mean ± SD

mean Vd_{ss} for BNP7787 alone and in combination with chemotherapy were 8.45–17.78 and 9.06–21.72, respectively.

Clinical recommended dose

Following careful review of the phase I trial data from this and other studies, a BNP7787 dose of 18.4 g/m^2 was selected as the clinically recommended dose based on a balance of efficacy and safety observations. The recommended dose was not based on the maximum tolerated dose since no dose-limiting toxicities were observed with the administration of BNP7787 alone or in combination with chemotherapy at any of the dose levels tested.

The selection of 18.4 g/m^2 as the recommended phase II dose was based on the balance of toxicity, higher grades of neurotoxicity and nephrotoxicity observed with lower doses of BNP7787 (e.g., 4.1–12.3 g/m^2), and the increased frequency of local IV site discomfort observed at higher doses of BNP7787 (above 27.6 g/m^2).

We also note that the molar ratio of the doses of BNP7787 18.4 g/m^2 and paclitaxel 175 mg/m^2 and cisplatin 75 mg/m^2 are 275:1 and 226:1, respectively. These molar ratios of the drugs are consistent with dose molar ratios that have demonstrated neuroprotection and nephroprotection in preclinical studies.

Response

Twenty-one patients were assessed for objective tumor response. Although there was no complete response, nine patients attained a partial response, with an overall response rate of 43%. Stable disease was observed in eight patients (38%). One patient was non-evaluable because of atelectasis of the lung surrounding and obscuring the primary marker lesion. The remaining three patients (14%) had progressive disease. In 10 patients with prior chemotherapy, four (40%) achieved a partial response. Among 11 patients with no prior chemotherapy, five (46%) had a partial response.

Discussion

This trial demonstrated that administration of BNP7787 at doses of 4.1 g/m^2 up to 41.0 g/m^2 alone or in combination with paclitaxel and cisplatin was safe and feasible. Generally,

Table 6 Pharmacokinetic parameters of mesna following administration of BNP7787 alone and BNP7787 in combination with paclitaxel and cisplatin

Parameter	Treatment	BNP7787 dose (g/m^2)					
		4.1 (n = 3/3)*	8.2 (n = 6/5)	12.3 (n = 4/4)	18.4 (n = 3/3)	27.6 (n = 3/3)	41.0 (n = 3/3)
$AUC_{0\text{-}t}$ (μg·h/mL)	BNP alone	27.80 ± 9.67**	80.92 ± 25.01	86.49 ± 46.19	163.26 ± 71.90	398.21 ± 97.95	565.25 ± 183.38
	BNP + CT	34.75 ± 11.50	78.97 ± 28.84	85.41 ± 22.43	153.19 ± 32.24	363.39 ± 160.61	421.30 ± 153.04
$AUC_{0\text{-inf.}}$ (μg h/mL)	BNP alone	29.33 ± 10.14	81.96 ± 25.29	88.71 ± 45.58	164.97 ± 71.13	398.93 ± 98.18	566.32 ± 183.20
	BNP + CT	35.96 ± 12.14	80.47 ± 28.19	88.59 ± 22.42	155.99 ± 31.82	364.21 ± 161.12	421.81 ± 153.04
C_{max} (μg/mL)	BNP alone	10.20 ± 0.43	19.84 ± 2.37	23.21 ± 6.86	47.30 ± 13.64	63.22 ± 16.25	82.29 ± 2.48
	BNP + CT	14.55 ± 2.44	21.70 ± 2.19	24.40 ± 4.05	42.15 ± 1.91	52.76 ± 5.64	62.75 ± 4.79
T_{max} (h)	BNP alone	0.84 ± 0.16	1.00 ± 0.45	1.25 ± 0.28	1.17 ± 0.29	1.82 ± 0.59	1.69 ± 0.95
	BNP + CT	0.58 ± 0.14	0.90 ± 0.14	0.96 ± 0.12	1.18 ± 0.28	1.82 ± 0.60	1.40 ± 0.26
$T_{1/2}$ (h)	BNP alone	3.17 ± 2.87	4.59 ± 0.52	1.74 ± 0.95	1.59 ± 1.11	2.66 ± 0.04	2.70 ± 0.21
	BNP + CT	2.96 ± 2.88	2.96 ± 1.56	1.87 ± 1.10	1.65 ± 1.05	2.75 ± 0.10	2.52 ± 0.11
λz (h^{-1})	BNP alone	0.36 ± 0.26	0.15 ± 0.02	0.46 ± 0.17	0.57 ± 0.29	0.26 ± 0.00	0.26 ± 0.02
	BNP + CT	0.40 ± 0.25	0.31 ± 0.19	0.44 ± 0.17	0.53 ± 0.27	0.25 ± 0.01	0.27 ± 0.01
$MRT_{0\text{-}t}$ (h)	BNP alone	2.2 ± 1.3	3.1 ± 0.4	2.3 ± 0.8	2.1 ± 0.5	3.6 ± 0.7	3.8 ± 0.7
	BNP + CT	1.9 ± 0.8	2.6 ± 0.9	2.2 ± 0.5	2.2 ± 0.6	3.7 ± 0.8	3.7 ± 0.8
$MRT_{0\text{-inf.}}$ (h)	BNP alone	2.91 ± 1.75	3.48 ± 0.51	2.48 ± 0.71	2.15 ± 0.48	3.67 ± 0.73	3.86 ± 0.72
	BNP + CT	2.32 ± 1.11	2.91 ± 0.88	2.47 ± 0.46	2.35 ± 0.57	3.77 ± 0.78	3.73 ± 0.82

$AUC_{0\text{-}t}$ area under the plasma concentration–time curve up to last measurable point, *$AUC_{0\text{-inf.}}$* area under the plasma concentration–time curve extrapolated to infinity, *C_{max}* maximum concentration, *T_{max}* time to maximal concentration, *$T_{1/2}$* half-life in terminal phase, *λz* elimination rate constant, *$MRT_{0\text{-inf.}}$* mean residence time extrapolated to infinity, *CT* chemotherapy with paclitaxel and cisplatin

* Values are the no. of patients who received BNP alone/no. of patients who had BNP preceding combination of paclitaxel and cisplatin

** Mean ± SD

adverse effects attributable to BNP7787 were very mild and reversible. No grade 2 or worse toxicity was observed at all dose levels, except for grade 2 skin rash observed at dose level 2, which resulted in termination of study treatment in that patient (Table 3). The only frequent adverse effect was the transient local discomfort at the infusion site of grade 1 up to 18.4 g/m^2 of BNP7787. At dose level 5 and 6, which represented a BNP7787 dose of 27.6 and 41.0 g/m^2, all patients experienced transient local grade 1 discomfort at the iv site. This toxicity was to some degree, lessened by extending the infusion time from 30 to 45 min. Other toxicities encountered at these higher dose levels included thirst (3 of 6 patients), facial flush (4 of 6 patients), and nasal obstruction (2 of 6 patients; Table 3). These adverse effects usually disappeared promptly after the end of infusion. The starting dose of 4.1 g/m^2 was based on aggregate GLP safety data in rats and dogs and by the calculation of a BNP7787:cisplatin = 50:1 molar ratio that was required for partial cisplatin nephroprotection in rats [7]. Dose-limiting toxicities were not observed at any dose level, including the highest tested dose level of 41.0 g/m^2 as defined in the protocol. This dose level is far in excess for what is required for cytoprotection in non-clinical studies since the molar ratio of BNP7787:cisplatin at the BNP7787 41.0 g/m^2 dose level was 503:1. The dose of 41.0 g/m^2 of BNP7787 was well tolerated by NSCLC patients, and the overall safety profile in this study is consistent with prior phase I studies of paclitaxel followed by BNP7787 administered prior to cisplatin in patients with advanced solid malignancies [3, 19].

BNP7787 pharmacokinetics was similar to those reported in a previous human phase I study [3, 21]. After cessation of infusion, the BNP7787 curves showed single-exponential decay (Fig. 1). In accordance with the report by Verschraagen et al. [3, 21], with half-life times of 0.66–1.67 h and 0.62–2.33 h, the concentrations of the BNP7787 alone and in combination with cisplatin and paclitaxel declined in parallel, with identical slopes (Fig. 1a, b). Mean BNP7787 C_{max} and $AUC_{0\text{-inf}}$ increased linearly with dose in the dose ranges administered (Table 5). Verschraagen et al. [3, 21] also reported human pharmacokinetics of mesna following administration of IV BNP7787. Mesna formation following BNP7787 administration observed is consistent with extravascular non-enzymatic and enzymatic metabolism of BNP7787 [16]. Mesna is reported to be more reactive with hydrated cisplatin when compared with its disulfide BNP7787 [8, 9, 12, 22]. Mesna appeared in plasma after the start of BNP7787 infusion, which is consistent with extravascular metabolism and preservation of the law of mass action balance relationship of disulfide to

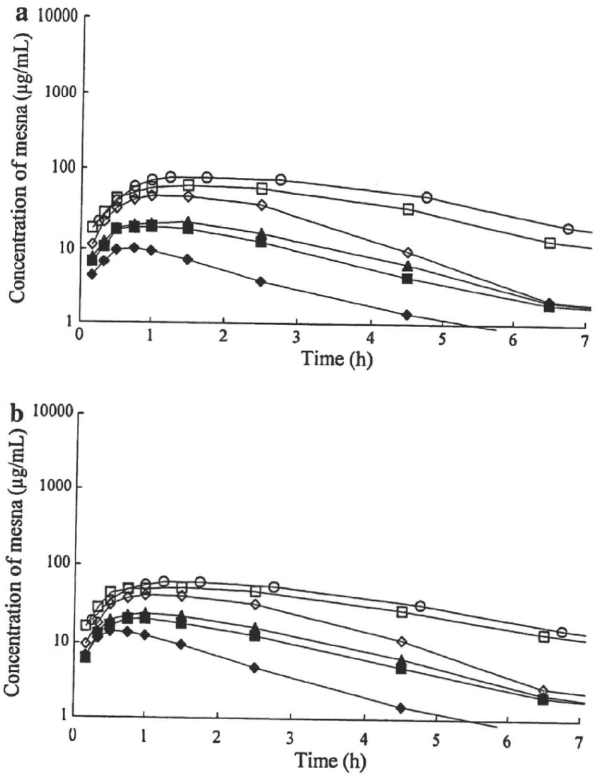

Fig. 2 Plasma concentration–time curves of the active metabolite, mesna in patients receiving BNP7787 treatment at dose of 4.1 g/m^2 (*filled diamond*), 8.2 g/m^2 (*filled square*), 12.3 g/m^2 (*filled triangle*), 18.4 g/m^2 (*open diamond*), 27.6 g/m^2 (*open square*) and 41.0 g/m^2 (*open circle*) in alone (**a**) and in combination with paclitaxel and cisplatin (**b**). Each point represents the mean of patients. Infusion time was 30 min except for three patients at the highest dose level of 41.0 g/m^2

thiol in plasma under physiological conditions [9]. The metabolite-to-BNP7787 ratio of C_{max} was about 2.2%, which is predicted by the free thiol hypothesis and prior studies [9]. Although plasma concentrations of BNP7787 began to decline at the end of the infusion, those of the metabolite increased initially and the maximal plasma levels were reached from 30 min to 1 h after the end of the infusion, with the half-life of from 0.88 to 6.45 h and from 0.90 to 6.28 h for BNP7787 alone and in combination with chemotherapy, respectively. The administration of paclitaxel and cisplatin in this study did not show any observable effect on BNP7787 pharmacokinetics when compared to the observed pharmacokinetics of single agent administration of BNP7787. This observation further supports prior studies demonstrating that BNP7787 is compatible and feasible with regard to co-administration of paclitaxel and cisplatin when all drugs are administered on this schedule and by the iv route (Table 5) [3, 7–9, 11, 20].

Of 21 patients, the overall response rate of 43% was observed during this phase I trial in patients with advanced NSCLC although no patients showed a complete response. The combination of paclitaxel and cisplatin in advanced NSCLC patients with no prior chemotherapy is historically reported to produce response rates between 21 and 41% [1, 6, 16–18]. A response rate of 46% in previously untreated patients observed here appears to be in the upper range of the reported trials in NSCLC with this regimen, and the co-administration of BNP7787 did not appear to interfere with the antitumor activity of paclitaxel and cisplatin in patients with advanced NSCLC. It is notable that four of 10 patients (40%) patients attained a PR in patients who had received prior chemotherapy, supporting further evaluation of BNP7787 in additional clinical studies. The number of patients in this study is too small to draw any valid conclusion about the ultimate clinical activity of this regimen, and it will be investigated in future studies.

In conclusion, this study confirmed that administration of a recommended BNP7787 dose level of 18.4 g/m^2 in combination with paclitaxel and cisplatin is safe to use to evaluate for potential efficacy in future studies in patients. The more common and non-dose-limiting BNP7787-related toxicities included local transient discomfort at the infusion site, facial flush, thirst, and nasal obstruction were very mild, and usually not greater than grade 2. A randomized multicenter phase III trial of paclitaxel, BNP7787 and cisplatin was subsequently initiated to determine the safety and efficacy of BNP7787 for the prevention of paclitaxel- and cisplatin-induced neurotoxicity, as well as other toxicities and also to evaluate the potential efficacy of BNP7787 in patients with advanced NSCLC.

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Conflict of interest statement Frederick Hausheer, MD, FACP, is a significant shareholder in BioNumerik Pharmaceuticals Inc. the originator of Tavocept, and is in a leadership and management position in this company. The funding source of this trial is ASKA Pharmaceuticals Co., which is in a Joint Venture relationship with BioNumerik Pharmaceuticals Inc. in Japan. Other authors indicated no potential conflicts of interest.

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Thymidylate synthase and dihydropyrimidine dehydrogenase expression levels are associated with response to S-1 plus carboplatin in advanced non-small cell lung cancer

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ABSTRACT

S-1 is an oral fluoropyrimidine derivative that is active against non-small cell lung cancer (NSCLC). Development of S-1 combination chemotherapy for advanced NSCLC is under way. Given the importance of designing therapeutic strategies based on specific tumor biology, we have evaluated the relation between immunohistochemical expression levels of thymidylate synthase (TS), orotate phosphoribosyltransferase (OPRT), or dihydropyrimidine dehydrogenase (DPD) and the response to treatment with S-1 plus carboplatin in patients with advanced NSCLC. Chemotherapy-naïve patients with advanced (stage IIIB or IV) NSCLC, an Eastern Cooperative Oncology Group performance status of 0 or 1, adequate organ function, and archival tumor tissue were assigned to receive S-1–carboplatin ($n = 22$). The predictive or prognostic relevance of the molecular markers was also examined by their evaluation in patients treated with paclitaxel plus carboplatin ($n = 25$). Expression levels of TS, OPRT, or DPD in tumor specimens did not differ significantly between patients treated with S-1–carboplatin and those treated with paclitaxel–carboplatin. A low expression level of TS or of DPD was associated with a better response and longer survival in patients treated with S-1–carboplatin but not in those treated with paclitaxel–carboplatin. Tumor expression levels of TS and DPD are predictive of response to S-1–carboplatin chemotherapy in patients with advanced NSCLC.

1. Introduction

Lung cancer is the most common cause of cancer-related death worldwide, with non-small cell lung cancer (NSCLC) accounting for ~75% of all lung cancer cases [1]. Platinum-based chemotherapy regimens are the standard first-line treatment for individuals with advanced NSCLC, but the efficacy of such regimens has reached a plateau [2]. Both experimental and clinical studies have revealed that many molecules contribute to the various biological behaviors of malignant tumors including NSCLC. New strategies based on a better understanding of tumor biology are thus needed to maximize the efficacy of current treatments. Indeed, certain molecular markers, such as excision-repair cross-complementation type 1 (ERCC1), ribonucleotide reductase subunit M1 (RRM1), and breast cancer 1 (BRCA1), have been associated with the sensitivity of NSCLC tumors to cisplatin-based regimens, although there is currently insufficient evidence to recommend their routine clinical use [3–5].

5-Fluorouracil (5-FU), a pyrimidine analog that is metabolized by pyrimidine metabolic pathways, has been used worldwide for chemotherapy in individuals with various solid organ malignancies. Encouraging clinical results have recently led to the development of a new generation of oral fluoropyrimidines, commonly referred to as dihydropyrimidine dehydrogenase (DPD)–inhibitory fluoropyrimidines (DIFs). S-1 is one anticancer agent developed on the basis of the DIF concept and contains the 5-FU prodrug tegafur, potassium oxonate, and 5-chloro-2,4-dihydroxypyridine (CDHP), an inhibitor of DPD. S-1 is active against a wide range of solid tumors including NSCLC, and the development of S-1 combination chemotherapy for advanced NSCLC is under way [6–10]. Phase I or II studies have shown that combination therapy with S-1 and platinum compounds (cisplatin or carboplatin) is feasible and well tolerated in patients with advanced NSCLC, with efficacy results similar to those obtained with other platinum doublets [7–9].

Several enzymes participate in the metabolic pathways of 5-FU or folate, including thymidylate synthase (TS), a target enzyme of 5-FU; DPD, which catalyzes the degradation of 5-FU; and orotate phosphoribosyltransferase (OPRT). Previous studies have demonstrated a correlation between the expression levels of TS, DPD, and

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OPRT in solid tumors and 5-FU sensitivity [11]. However, the clinical relevance of these enzymes has not been established for NSCLC patients treated with S-1 or S-1 combination chemotherapy. We have now investigated the predictive value of TS, DPD, or OPRT expression in individuals with NSCLC treated with S-1 plus carboplatin (CBDCA). These molecular markers were also examined by their evaluation in patients treated with paclitaxel plus carboplatin.

2. Patients and methods

2.1. Patient characteristics

The present retrospective study recruited consecutive patients with advanced NSCLC who received chemotherapy at Kinki University Hospital between June 2003 and October 2009. Patients met all of the following criteria: a histological diagnosis of NSCLC with at least one measurable lesion; a clinical stage of IIIB or IV; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; an age of 75 years or younger; adequate hematologic, hepatic, and renal function; treatment with CBDCA at an area under the curve (AUC) of 6 on day 1 and paclitaxel (PTX) at 200 mg/m^2 on day 1 or with CBDCA at an AUC of 5 on day 1 and S-1 at 80 mg/m^2 on days 1–14 every 3 weeks as first-line chemotherapy; and sufficient tissue available in paraffin blocks for assessment by immunohistochemistry. Tumor response was examined by computed tomography and was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECISTs). Many patients had already died before the initiation of immunohistochemical analysis, preventing us from obtaining informed consent. The institutional review board therefore approved our study protocol with the conditions that samples would be processed anonymously and analyzed for protein expression and that the study would be disclosed publicly, according to the Ethical Guidelines for Human Genome Research published by the Ministry of Education, Culture, Sports, Science, and Technology, the Ministry of Health, Labor, and Welfare, and the Ministry of Economy, Trade, and Industry of Japan. The present study also conforms to the provisions of the Declaration of Helsinki.

2.2. Immunohistochemistry and scoring of protein expression

Sections (thickness, 4 μm) were depleted of paraffin with xylene and then rehydrated, and endogenous peroxidase activity was quenched by incubation with 0.3% hydrogen peroxide in methanol. The antigen retrieval was carried out by microwaving in citrate buffer, pH 6.0 (TS, OPRT) or in 1 mM EDTA, pH 8.0 (DPD) for 10 min. After washing in phosphate buffered saline, the sections were then incubated with polyclonal antibodies (Taiho Pharmaceutical Co., Saitama, Japan) to either TS (dilution of 1:100), OPRT (dilution of 1:1000), or DPD (dilution of 1:1350) overnight at room temperature. Biotinylated goat anti-rabbit IgG was applied as a secondary antibody for 30 min, followed by streptavidin–biotinylated peroxidase complex for 30 min at room temperature. Peroxidase activity was visualized with diaminobenzidine tetrahydrochloride (DAB) solution (DAKO Co. Ltd., Santa Barbara, CA), and counter staining was performed with hematoxylin. The human colon cancer cell line DLD-1/FrUrd, human breast cancer cell line MDA-MB-435S, and human pancreatic cancer cell line MIAPaCa-2 were used as positive controls for the staining of TS, OPRT, and DPD, respectively. All of the immunostained sections were reviewed by two observers (N.H. and K.N.) without knowledge of the patients' characteristics. Sections with discrepant results were jointly re-evaluated until a consensus was reached. Cytoplasmic staining for TS, OPRT, and DPD was scored in a semiquantitative manner reflecting both the intensity of staining and the percentage of cells with staining at each intensity. Staining intensity was classified as 0 (no staining), +1 (weak staining), +2 (distinct staining), or +3 (strong staining). A value designated the HSCORE was obtained as $\Sigma(I \times PC)$, where I and PC represent staining intensity and the percentage of cells that stain at each intensity, respectively. The selection of clinically important cutoff scores for TS, OPRT, or DPD expression was based on receiver operating characteristic (ROC) curve analysis.

Table 1
Patient characteristics.

Characteristic	S-1 plus CBDCA*n* (%)	PTX plus CBDCA*n* (%)
Sex		
Male	15 (68)	17 (68)
Female	7 (32)	8 (32)
Age (years)[a]	63 (39–73)	65 (48–74)
Smoking history		
Never-smoker	3 (14)	7 (28)
Smoker	19 (86)	18 (72)
Tumor histology		
Adenocarcinoma	16 (73)	16 (64)
Squamous cell	1 (4)	5 (20)
Other	5 (23)	4 (16)
Disease stage		
IIIB	3 (14)	4 (16)
IV	19 (86)	21 (84)
Tumor response[b]		
ORR (CR+PR)	9 (41)	9 (36)
SD	6 (27)	10 (40)
PD	7 (32)	6 (24)

[a] Data are presented as median (range).
[b] ORR, overall response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

2.3. Statistical analysis

Expression levels of TS, OPRT, and DPD were compared between groups with the Mann–Whitney U test, and the relations between these variables were evaluated with Pearson's correlation test. Differences between the two treatment groups for demographic characteristics and the relation between treatment response and the expression of TS or DPD were evaluated with the two-sided Fisher's exact test. Overall survival and progression free survival were assessed from the first day of chemotherapy administration to the data of death from any cause and the date of objective disease progression, respectively. Patients without documented death at the time of the final analysis were evaluated at the date they were last known to be alive or of their last objective tumor assessment. The Kaplan–Meier method was used to estimate the probability of survival as a function of time, and differences in the survival of subgroups of patients were evaluated with the log-rank test. All P values were based on a two-tailed statistical analysis, and a P value of <0.05 was considered statistically significant. All statistical analysis was performed with GraphPad prism software (version 5.0; GraphPad Software, San Diego, CA).

3. Results

3.1. Patient characteristics

A total of 47 patients met the eligibility criteria (Table 1). Twenty-two patients were treated with S-1 plus CBDCA (S-1 arm) and 25 patients were treated with PTX plus CBDCA (PTX arm). Most (68%) patients were male in both groups. The median age of the patients was 63 years (range, 39–73) in the S-1 arm and 65 years (range, 48–74) in the PTX arm. Adenocarcinoma was the predominant histological type of NSCLC, accounting for 73% of patients

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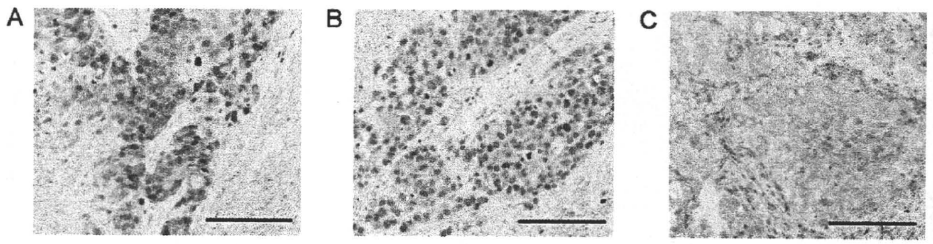

Fig. 1. Immunohistochemical staining of human NSCLC tissue. Representative sections of carcinomas with high levels of expression of TS (A), OPRT (B), or DPD (C) are shown. Scale bars, 125 μm.

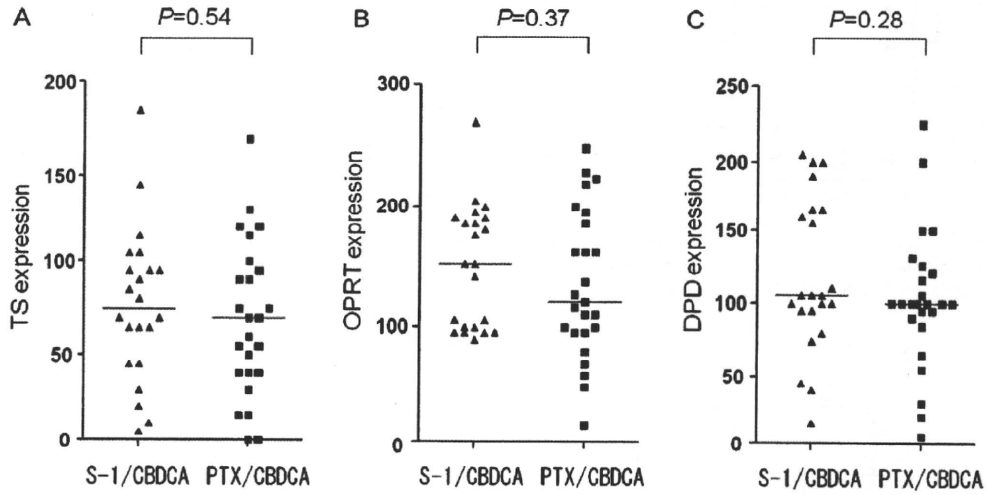

Fig. 2. Expression levels of TS (A), OPRT (B), and DPD (C) in NSCLC specimens of patients treated with S-1 plus CBDCA or with PTX plus CBDCA. Median values for expression level (HSCORE) are indicated by the horizontal lines. *P* values were determined with the Mann–Whitney *U* test.

in the S-1 arm and 64% in the PTX arm. The S-1 and PTX arms included 19 (86%) and 21 (84%) patients, respectively, with stage IV disease. There were no significant differences in sex distribution, age, smoking history, tumor histology, or disease stage between the S-1 and PTX arms. The median number of treatment cycles was 4 (range, 1–6) and 3 (range, 1–6) in the S-1 and PTX arms, respectively. The overall response rate (ORR = complete response [CR] + partial response [PR]) was 41% in the S-1 arm and 36% in the PTX arm (Table 1). The median follow-up time was 14.2 months, and the median overall survival was 15.5 months in the S-1 arm and 13.3 months in the PTX arm, with no significant difference in this parameter between the two arms ($P = 0.52$, log-rank test; data not shown).

3.2. Expression levels of TS, OPRT, and DPD in tumor specimens

We examined the expression levels of TS, OPRT, and DPD in tumor sections by immunohistochemistry (Fig. 1). In the S-1 arm, intratumoral TS, OPRT, and DPD expression levels (HSCOREs) varied from 5 to 185 (median, 75), from 90 to 270 (median, 150), and from 15 to 205 (median, 105), respectively (Fig. 2). In the PTX arm, these values ranged from 0 to 170 (median, 70), from 15 to 250 (median, 120), and from 5 to 225 (median, 100), respectively. No significant difference in TS ($P = 0.54$), OPRT ($P = 0.37$), or DPD ($P = 0.28$) expression levels was apparent between the two arms. The expression level of DPD was not correlated with that of TS ($R^2 = 0.0090$, data not shown), and the expression level of OPRT was not correlated with that of TS or DPD ($R^2 = 0.0074$ and 0.11, respectively; data not shown).

We next evaluated the relation between the expression of these enzymes and the tumor response to treatment. Tumors were categorized as either responding (CR or PR) or nonresponding (stable disease [SD] or progressive disease [PD]). In the S-1 arm, the TS expression level for the responding groups (range, 5–105) was significantly ($P = 0.006$) lower than that for the nonresponding groups (range, 65–185) (Fig. 3A). In contrast, the level of TS expression did not differ significantly ($P = 0.63$) between responders and nonresponders in the PTX arm (Fig. 3A). The expression levels of OPRT (Fig. 3B) and DPD (Fig. 3C) were not significantly associated with tumor response in the S-1 arm or the PTX arm, although the expression level of DPD tended to be lower in responders than in nonresponders of the S-1 arm ($P = 0.055$).

3.3. Predictive relevance of TS and DPD expression levels in NSCLC

We performed ROC curve analysis to establish the optimal cutoff values for the HSCORE of enzyme expression level for differentiation of responders from nonresponders. Values of 55, 97.5, and 162.5 for TS, OPRT, and DPD, respectively, were obtained for the S-1 arm (Fig. 4). In patients treated with S-1 plus CBDCA, response rates were 100% (6 out of 6) and 19% (3 out of 16) for tumors with low (<55) or high (≥55) levels of TS expression ($P = 0.001$), respectively (Table 2). In contrast, there was no significant ($P = 1.0$) difference in response rate between tumors with high or low levels of TS expression in the PTX arm. In the S-1 arm, the response rate for tumors with a high level (≥162.5) of DPD expression was significantly lower than that for those with a low level (<162.5) of DPD expression (0 versus 56%, $P = 0.046$) (Table 2), whereas no such difference was observed in the PTX arm ($P = 0.52$). In the S-1 arm, the expression level of DPD was not correlated with that of TS ($R^2 = 0.046$); however, the responder in low DPD levels ($n = 9$) included all the responder in low TS levels ($n = 6$) (Fig. 5). No signif-

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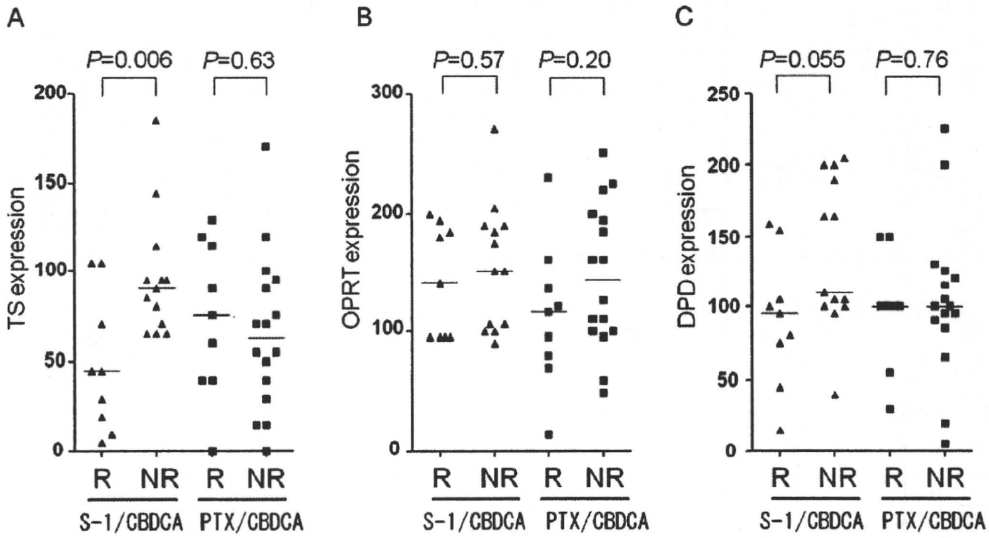

Fig. 3. Relation of expression levels of TS (A), OPRT (B), or DPD (C) in NSCLC specimens of patients treated with S-1 plus CBDCA or with PTX plus CBDCA to treatment response. NR and R represent nonresponders and responders, respectively, and median values for expression level (HSCORE) are indicated by the horizontal lines. *P* values were determined with the Mann–Whitney *U* test.

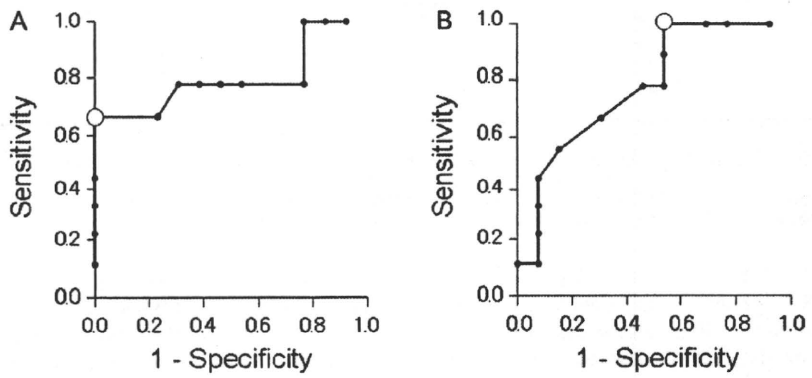

Fig. 4. Receiver operating characteristic (ROC) analysis based on intratumoral TS (A) and DPD (B) expression levels with response to S-1/CBDCA therapy. The optimal cut-off point (open circle) was 55 and 162.5 for TS and DPD, respectively, which yielded the maximum sensitivity plus specificity.

icant association between high or low OPRT expression level and response rate was apparent in either arm.

Finally, for patients treated with S-1 plus CBDCA, the progression-free survival in low TS group tended to be longer than that in high TS group, although the difference was not statistically significant ($P = 0.11$) (Fig. 6A). Patients with a low level of TS expression had a significantly ($P = 0.02$) longer overall survival than did those with a high level (Fig. 6B). In contrast, there was no significant difference in progression-free survival ($P = 0.62$) and overall survival ($P = 0.83$) between patients with a high or low level of TS expression in the PTX arm (Fig. 6C and D). Progression-free survival and overall survival for patients with a low level of DPD expression was significantly ($P = 0.013$ and 0.009, respectively; data not shown) longer than that for those with a high level in the S-1 arm, whereas no such difference ($P = 0.57$ and 0.27, respectively) was apparent in the PTX arm (data not shown). As shown in Table 3, 73% of patients with recurrence disease in the S-1 arm and 84% in the PTX arm received subsequent treatment. No bias for subsequent treatments between the two arms was observed.

4. Discussion

We have investigated the relation between intratumoral expression levels of TS, OPRT, or DPD and clinical outcome for NSCLC patients treated with S-1 plus CBDCA (S-1 arm) or with PTX plus CBDCA (PTX arm). The expression level of these proteins was assessed by immunohistochemical analysis in a semiquantitative manner by scoring the proportions of tumor cells with defined staining intensities relative to the total number of tumor cells. ROC curves are commonly used to determine biologically or clinically

Table 2
Tumor response to treatment according to TS or DPD expression level. All *P* values were determined with Fisher's exact test.

Relative expression level	S-1 plus CBDCA ($n = 22$)			PTX plus CBDCA ($n = 25$)		
	Responders*n* (%)	Nonresponders*n* (%)	*P*	Responders*n* (%)	Nonresponders*n* (%)	*P*
High TS	3 (19)	13 (81)	0.001	6 (38)	10 (63)	1.0
Low TS	6 (100)	0 (0)		3 (33)	6 (67)	
High DPD	0 (0)	6 (100)	0.046	0 (0)	2 (100)	0.52
Low DPD	9 (56)	7 (44)		9 (39)	14 (61)	

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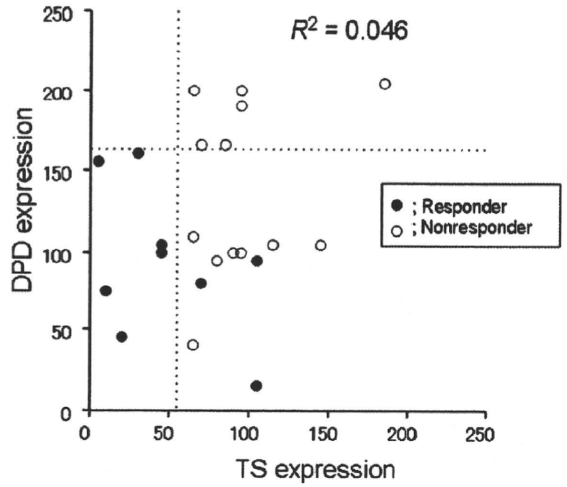

Fig. 5. Correlation between TS and DPD expression levels in patients treated with S-1 plus CBDCA. The dotted lines indicate the optimal cutoff values for the HSCORE of each expression level. The closed circles represent the responder, and the open circles represent the nonresponder.

relevant cutoff scores in such analysis [12,13], and we therefore used ROC curves to define the optimal cutoff values of TS or DPD expression level (HSCORE) for discrimination of responders from nonresponders in the S-1 arm. We found that low levels of TS and DPD expression were associated with a better treatment response and a longer survival time in NSCLC patients in the S-1 arm. To examine the predictive or prognostic relevance of the cutoff values for TS or DPD expression level determined in the S-1 arm, we applied these values to the results obtained for patients treated with PTX plus CBDCA, which is a standard first-line chemotherapy regimen for advanced NSCLC. Neither the expression of TS nor that of DPD showed a significant association with treatment response or survival in the PTX arm. These results thus indicate that the expression levels of TS and DPD are independent predictive markers, rather than prognostic markers, in patients with advanced NSCLC receiving S-1-based chemotherapy.

Table 3
Treatment after recurrence in each arm.

Variable	Treatment arm		*P*
	S-1 plus CBDCA *n* (%)	PTX plus CBDCA *n* (%)	
Any treatment	16 (73)	21 (84)	0.48
Radiotherapy	3 (14)	4 (16)	1.00
Any chemotherapy	11 (50)	17 (68)	0.25
Docetaxel	5 (23)	9 (36)	0.36
Gefitinib	3 (14)	8 (32)	0.18

TS is an essential enzyme that catalyzes the transfer of a methyl group from methylenetetrahydrofolate to dUMP in order to generate dTMP [14,15]. The subsequent phosphorylation of dTMP to dTTP provides a direct precursor for DNA synthesis. Several in vitro studies with tumor cell lines have implicated up-regulation of TS expression as a mechanism of resistance to 5-FU that develops after exposure to the drug [16–19]. Previous clinical studies have also shown that a low level of TS expression was associated with high sensitivity to 5-FU, to 5-FU plus cisplatin, or to 5-FU plus methotrexate in colorectal or gastric cancer [11]. A low level of TS expression in NSCLC tumors has also been associated with longer survival in patients treated with oral 5-FU–based agents after curative resection [20–22]. S-1 is an oral fluoropyrimidine derivative

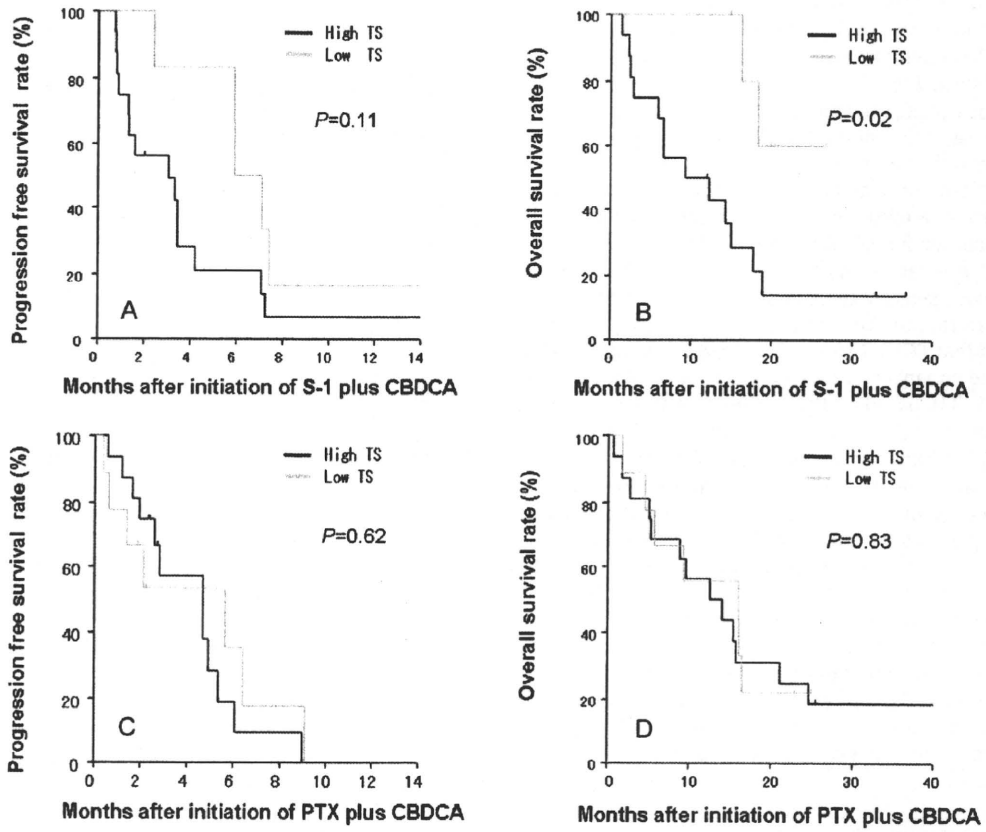

Fig. 6. Progression-free survival and overall survival according to expression level of TS in NSCLC tumors of patients treated with S-1 plus CBDCA (A and B) or with PTX plus CBDCA (C and D). *P* values were determined with the log-rank test.

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that contains the 5-FU prodrug tegafur, and it is therefore expected to have an antitumor effect in patients with tumors sensitive to 5-FU. Indeed, low levels of TS expression in gastric cancer have been linked to a favorable clinical outcome after S-1 treatment [23–25]. However, the relation between TS status and tumor response to S-1 or to S-1 combination therapy has not previously been examined for NSCLC. We have now shown that a low level of TS expression was significantly associated with response to treatment with S-1 plus CBDCA in patients with advanced NSCLC.

DPD is an initial and rate-limiting enzyme in the catabolism of 5-FU, with >80% of 5-FU being degraded to inactive metabolites by this enzyme in human tissues, and DPD activity therefore modulates the antitumor effects of 5-FU. In vitro studies have shown that overexpression of DPD in cancer cell lines confers resistance to 5-FU [16,26]. Several clinical studies have also shown that a high level of DPD expression in tumors was associated with poor survival in NSCLC patients treated with oral 5-FU-based agents after curative surgery [20,21,27,28], whereas a relation between DPD expression level and clinical outcome after 5-FU treatment has not been definitively demonstrated for colorectal or gastric cancer [11]. S-1 contains CDHP, an inhibitor of DPD, and an antitumor effect of S-1 is therefore expected even in tumors with a high level of DPD activity. Indeed, patients with gastric cancer expressing DPD at high levels were found to benefit from S-1 treatment [23–25]. No previous studies have evaluated the relation between DPD expression and S-1 sensitivity in NSCLC, however. We have now shown that a high level of DPD expression in NSCLC predicts resistance to S-1-based chemotherapy. DPD activity levels have been shown to be higher in NSCLC tissue than in other solid tumors including gastric, colorectal, and breast cancer [29]. The apparent discrepancy between the demonstrated clinical efficacy of S-1 in patients with gastric cancer expressing DPD at high levels [23–25] and our finding that no NSCLC patients with a high level of DPD expression responded to treatment with S-1 plus CBDCA may be attributable to the fact that DPD activity levels in NSCLC tissue are about twice those in gastric cancer [29]. In cancers with a high level of DPD expression, such as NSCLC, the amount of the free enzyme may be maintained in excess of that of the CDHP-bound enzyme.

Molecular targeting therapies have been developed as a new strategy for the treatment of advanced NSCLC, and somatic mutations in the epidermal growth factor receptor (*EGFR*) gene are the most robust biomarker for EGFR tyrosine kinase inhibitor (TKI) therapy in NSCLC. A recent study has reported that *EGFR* mutant tumors have a lower sensitivity to another oral 5-FU derivative, uracil-tegafur, than that of *EGFR* wild-type tumors [30]. We have previously shown that EGFR-TKI-induced downregulation of TS is responsible for the enhanced antitumor effect of combined treatment with S-1 [31–33]. Based on these results, further studies are warranted to investigate the relationship between the presence of *EGFR* mutation and TS/DPD expression levels in NSCLC.

In conclusion, we have shown that the tumor expression levels of TS and DPD were predictive of tumor response to S-1-based chemotherapy in patients with advanced NSCLC. S-1 in combination with platinum compounds (cisplatin or CBDCA) is currently under evaluation as a first-line treatment for advanced NSCLC in randomized phase III studies. It will be necessary to confirm that the expression levels of TS and DPD can predict clinical outcome in these clinical trials, given that our findings derive from a limited retrospective study of a relatively small number of patients. Further prospective studies of these biomarkers are also needed to address the issue of reproducibility in a large series of patients.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

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SHORT REPORT

Perirenal hematoma associated with bevacizumab treatment

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Summary We now describe the first example of a patient who developed perirenal hematoma during the course of bevacizumab-containing chemotherapy. A 59-years-old woman with metastatic rectal cancer treated with bevacizumab, who developed low back pain after 11 cycles of chemotherapy. CT-scan was consistent with perirenal hematoma and discontinuation of bevacizumab resulted in symptomatic improvement. Nontraumatic perirenal hematoma is a rare condition that can cause shock in severe cases. Given that several types of bleeding complication are known to be associated with bevacizumab treatment, we concluded that bevacizumab likely contributed to the perirenal hematoma in this case. Although the appropriate modification of bevacizumab treatment in the setting of perirenal hematoma is still unclear, physicians should be aware of this potential bevacizumab-associated bleeding complication.

Keywords Bevacizumab · Perirenal hematoma · VEGF · Rectal cancer

A 59-year-old woman was diagnosed with rectal cancer accompanied by multiple liver metastases in March 2009. A palliative rectectomy was performed to resolve partial rectal obstruction, but thereafter the patient refused to receive systemic chemotherapy. Four months later, she presented with symptoms of tumor progression and abdominal pain. Computed tomography (CT) revealed progression of multiple hepatic metastases, and she was placed on salvage therapy of FOLFOX and bevacizumab (5 mg/kg, intravenous, for 90 min biweekly) after she gave her full informed consent. After the third cycle of treatment, a partial response was confirmed by CT and treatment was continued with no severe adverse effects. After 11 cycles of FOLFOX with bevacizumab, however, the patient complained of low back pain, which was not associated with microscopic hematuria. Positron emission tomography–CT revealed progression of disease with recurrence in peritoneal metastasis with hydronephrosis. In addition, a mass around the left kidney, which was round and sharply marginated with homogeneously high attenuation, was observed (Fig. 1, with the mass indicated by the arrow). Although the mass was not evaluated surgically, its appearance was suggestive of a nontraumatic perirenal hematoma, and an urologist recommended adoption of a wait-and-see approach. The patient was immediately instructed to discontinue chemotherapy including bevacizumab. CT examination 2 weeks later revealed the size of the perirenal mass to be stable. The low back pain of the patient was relieved with complete bed rest.

Vascular endothelial growth factor (VEGF) is a proangiogenic molecule that has been implicated in several steps of normal and pathologic angiogenic processes. Bevacizumab, a humanized monoclonal antibody specific for VEGF, shows substantial activity against various types of solid tumor. Bleeding complications, including epistaxis, hemoptysis, hematemesis, gastrointestinal or vaginal bleeding, and brain hemorrhage, have been observed in patients treated with the combination of chemotherapy and bevacizumab [1]. Patients with colorectal cancer who receive bevacizumab plus chemotherapy generally show a higher incidence of serious hemorrhage (3 to 9%) than do those on chemotherapy alone [2–5]. With regard to a possible explanation for the high incidence of bleeding following bevacizumab treatment [6],

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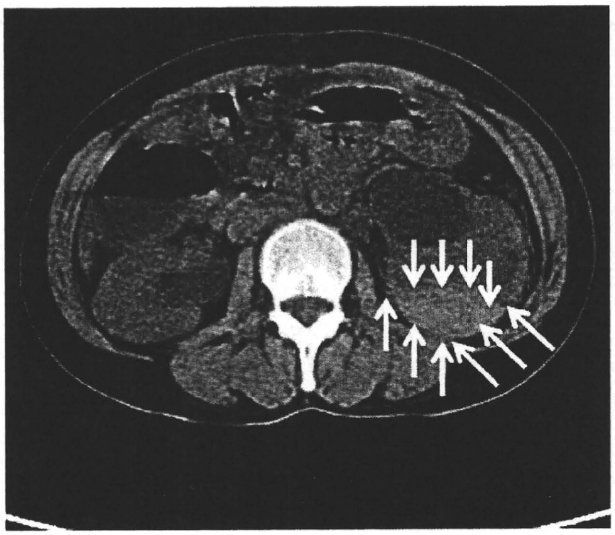

Fig. 1 A mass around the left kidney, which was round and sharply marginated with homogeneously high attenuation

VEGF not only stimulates endothelial cell proliferation during new tumor vessel formation but also promotes endothelial cell survival and helps maintain vascular integrity. Inhibition of VEGF signaling might therefore interfere with the regenerative capacity of endothelial cells and induce defects in the endothelial layer that expose the underlying matrix, leading to hemorrhage.

Nontraumatic perirenal hematoma is rare, whereas traumatic perirenal hematoma commonly results from renal injury or occurs as a severe complication after kidney-damaging surgery or other procedures. The most frequently identified cause of nontraumatic perirenal hematoma is renal neoplasms [7], with vascular disease, such as polyarteritis nodosa, being the second. In addition, antiplatelet therapy has been identified as an underlying cause of drug-induced perirenal hematoma [8]. Symptoms of perirenal hematoma include flank or abdominal pain, either gross or microscopic hematuria, and, in severe cases, signs of shock. CT is the valuable examination for diagnosis of perirenal hematoma [9].

The present patient complained of pain in the low back region and denied any relevant past history, including renal neoplasms, vascular disease, and antiplatelet therapy. CT findings were compatible with perirenal hematoma, which is visualized as fluid collection of high attenuation. To our knowledge, no reports associate FOLFOX with the development of perirenal hematoma or other type of hemorrhage with the exception of gastrointestinal bleeding caused by thrombocytopenia. Given that several types of bleeding complication are known to be associated with bevacizumab treatment, we concluded that bevacizumab likely contributed to the perirenal hematoma in this case.

Bevacizumab was discontinued and the patient was followed closely without surgical intervention. As a result, her symptoms resolved without a further increase in volume of the perirenal hematoma as evaluated by CT. To the best of our knowledge, this is the first reported case of the potential association of perirenal hematoma with bevacizumab therapy. Although the appropriate modification of bevacizumab treatment in the setting of perirenal hematoma is unclear, a conservative approach in this patient led to symptomatic improvement. Given the increasing number of patients receiving bevacizumab, physicians should be aware of this potential bevacizumab-associated complication.

Conflict of interest The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

High-dose dexamethasone plus antihistamine prevents colorectal cancer patients treated with modified FOLFOX6 from hypersensitivity reactions induced by oxaliplatin

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Abstract
Background Oxaliplatin is a third-generation platinum compound and a key agent for the management of colorectal cancer. Patients treated with oxaliplatin are at risk for hypersensitivity reactions. We designed a modified premedication regimen to prevent oxaliplatin-related hypersensitivity reactions and assessed if this approach is effective.
Methods A retrospective cohort study of patients with advanced colorectal cancer who received modified FOLFOX6 (mFOLFOX6) was performed. Patients received routine premedication with dexamethasone 8 mg and granisetron 3 mg for the first five cycles of mFOLFOX6. From the sixth cycle onward, cohort 1 received the same premedication, and cohort 2 received modified premedication (diphenhydramine 50 mg orally, followed by dexamethasone 20 mg, granisetron 3 mg, and famotidine 20 mg). We compared the incidence of hypersensitivity reactions, duration of treatment, and reasons for treatment withdrawal between the two cohorts.
Results A total of 181 patients were studied (cohort 1, 81; cohort 2, 100). Hypersensitivity reactions developed in 16 patients (20%) in cohort 1 and 7 (7.0%) in cohort 2 ($P = 0.0153$). The median number of cycles increased from 9 in cohort 1 to 12 in cohort 2. Apart from progressive disease, neurotoxicity was the reason for discontinuing treatment in 20% of the patients in cohort 1, as compared with 53% in cohort 2.
Conclusion Increased doses of dexamethasone and antihistamine significantly reduced oxaliplatin-related hypersensitivity reactions. This effective approach should be considered for all patients who receive FOLFOX, allowing treatment to be completed as planned.

Keywords Colorectal cancer · FOLFOX · Hypersensitivity reaction · Oxaliplatin · Premedication

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Introduction

Oxaliplatin, a third-generation platinum derivative, in combination with fluorouracil and leucovorin (FOLFOX) is among the most effective chemotherapies for metastatic colorectal cancer. The increasing use of oxaliplatin for chemotherapy has led to an increased incidence of oxaliplatin-related hypersensitivity reactions. The MOSAIC trial, in which more than 1,100 patients with colorectal cancer received 5-fluorouracil with oxaliplatin in an adjuvant setting, reported a 10.3% incidence of hypersensitivity reactions, which were one of the major reasons for discontinuing treatment [1].

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Hypersensitivity is defined as an unexpected reaction inconsistent with a drug's usual toxicity profile. Such reactions usually occur during or immediately after treatment. Once sensitized, patients have recurrent hypersensitivity reactions on subsequent exposure to oxaliplatin. Desensitization protocols have been designed to prevent hypersensitivity reactions. Such protocols have allowed successful rechallenge with oxaliplatin [2, 3]. However, clinical criteria for rechallenge with oxaliplatin remain a matter of debate. Reliable methods for predicting the risk of severe hypersensitivity reactions to oxaliplatin have not been established. The potential risks of rechallenge with oxaliplatin after severe anaphylaxis should be weighed against the expected benefits according to the specific clinical situation.

Hypersensitivity reactions to platinum salts (cisplatin, carboplatin) are classically type I (i.e., immediate) reactions [4], the incidence of which increases with multiple cycles of therapy [5]. The symptoms can resolve after treatment with antihistamines and steroids. More recent series have documented a considerably higher incidence of hypersensitivity reactions, ranging between 8% and 19% [6–10]. Besides these reports, studies assessing the preventative effect of premedication on oxaliplatin-related hypersensitivity are scant.

We have designed a modified premedication regimen, which includes a higher dose of dexamethasone (20 mg) plus an antihistamine. This dose of dexamethasone has been shown to be safe and effective for the prophylaxis of paclitaxel-associated hypersensitivity reactions [11]. Dexamethasone (20 mg) can be administered intravenously for desensitization against oxaliplatin hypersensitivity [3, 12]. These findings suggested that the prophylactic use of dexamethasone (20 mg) would reduce the incidence or severity of hypersensitivity reactions. We gave our modified regimen for premedication to patients with advanced colorectal cancer after they had received five cycles of a modified regimen of FOLFOX6 (mFOLFOX6) with standard premedication. We retrospectively compared the frequencies of hypersensitivity reactions between patients who received this modified premedication regimen with those who received standard premedication for the duration of FOLFOX treatment to determine whether our regimen was effective.

Patients and methods

Patient selection

This investigation was a retrospective cohort study of patients with advanced colorectal cancer who received modified FOLFOX6 (mFOLFOX6: oxaliplatin 85 mg/m^2 plus concurrent leucovorin 400 mg/m^2 as a 2-h intravenous infusion on day 1, followed by a bolus injection of 5-fluorouracil 400 mg/m^2 and by a 46-h continuous intravenous infusion of 5-fluorouracil 2,400 mg/m^2, repeated every 2 weeks) at Kinki University Hospital from September 2005 through September 2009. Eligible patients had to have adenocarcinoma of the colon or rectum; unresectable metastases; adequate bone marrow, liver, and kidney functions; a World Health Organization performance status of 0–2; and an age of $\geq$18 years. Patients who received five cycles of mFOLFOX6 without any allergic reactions were eligible. Patients with central nervous system metastases, only bone metastases, second malignancies, bowel obstruction, peripheral neuropathy of grade 3 or higher, symptomatic angina pectoris, or disease confined to previous radiation fields were excluded.

Chemotherapy and premedication

The patients were divided into two cohorts. In cohort 1, patients received routine premedication for the first five and subsequent cycles of mFOLFOX6 from September 2005 through September 2007. In cohort 2, treated between October 2007 and September 2009, patients similarly received routine premedication for the first five cycles. The premedication included routine antiemetic prophylaxis with dexamethasone 8 mg and granisetron 3 mg in 50 ml 0.9% saline, given intravenously 15 min before oxaliplatin. To reduce the risk of hypersensitivity reactions associated with continued treatment, from the sixth cycle onward all patients in cohort 2 received a modified premedication regimen, consisting of diphenhydramine 50 mg given orally 30 min before oxaliplatin, followed by dexamethasone 20 mg, granisetron 3 mg, and famotidine 20 mg in 50 ml saline, given intravenously 15 min before oxaliplatin.

Definition of allergic reactions

A hypersensitivity reaction to oxaliplatin was defined as the development of at least one of the following signs or symptoms after treatment with oxaliplatin: palmar erythema, pruritus, urticaria, diffuse erythroderma, tachycardia, angina, wheezing, facial or tongue edema, dyspnea, hypertension, hypotension, respiratory arrest, anaphylaxis, seizure, or death. Clinically significant respiratory compromise (wheezing associated with hypoxia or hypercarbia, and respiratory arrest), clinically significant cardiovascular compromise (angina, symptomatic hypotension or hypertension, and cardiovascular collapse), anaphylaxis, seizure, and death were all considered manifestations of a severe allergic reaction.

Study objectives and outcome measures

The primary objective of this study was to evaluate whether the modified premedication regimen reduced the incidence of hypersensitivity reactions. The primary outcome measure was the reduction in such reactions as compared with routine premedication. Secondary objectives were to evaluate the safety of the modified premedication regimen and to compare the duration of treatment with mFOLFOX6 and the reasons for treatment discontinuation between the two cohorts. Progressive disease was excluded from the analysis of reasons for treatment discontinuation.

Statistical analysis

A primary analysis was performed to compare cohorts 1 and 2. To assess the effect of premedication on hypersensitivity reactions to oxaliplatin in cohorts 1 and 2, we calculated risk ratios and 95% confidence intervals (95% CI). In addition, we calculated adjusted risk ratios with 95% CI for covariates (age, sex, diagnosis, prior treatment) by performing a Poisson regression analysis. To assess the effect of treatment exposure to the premedication on hypersensitivity reactions to oxaliplatin in cohorts 1 and 2, we compared the number of cycles between the cohorts with the use of the Wilcoxon test. All tests were two-sided with a significance level ≤ 0.05.

Results

Patient characteristics

The characteristics of the 181 eligible patients are listed in Table 1 (81 in cohort 1 and 100 in cohort 2). The patients' characteristics were well balanced between the cohorts, except for bevacizumab, because bevacizumab was approved in July 2007 in Japan. In 2007, bevacizumab was introduced to Japan; we therefore assessed the number of cycles administered for mFOLFOX6 alone in cohort 1 ($n = 81$) and for mFOLFOX6 alone ($n = 49$) and mFOLFOX6 plus bevacizumab ($n = 51$) in cohort 2. No patient in cohort 1 received bevacizumab, whereas nearly half the patients in cohort 2 received bevacizumab. No patient had a known history of allergy to a platinum salt. Five patients had a history of drug allergy.

Table 1 Patient characteristics

	Routine premedication (cohort 1)	Modified premedication (cohort 2)
No. of patients	81	100
Median age, years (range)	62 (29–82)	62 (34–84)
Sex		
Male/female	53/28	66/34
Diagnosis		
Colon	44	51
Rectum	37	49
Line of therapy		
First-line therapy	43	50
Second-line therapy	27	42
Third-line or subsequent therapy	11	8
mFOLFOX6 + bevacizumab	0	51
Median cumulative oxaliplatin dose for the first five cycles (mg/m^2)	414	419

FOLFOX6 chemotherapy with oxaliplatin plus fluorouracil and leucovorin

Incidence of hypersensitivity reactions to oxaliplatin

In cohort 1, hypersensitivity reactions developed in 16 (20%) of 81 patients who received routine premedication (Table 2). Six of these patients (7.4%) had manifestations of severe allergic reactions. In cohort 2, hypersensitivity reactions occurred in 7 (7.0%) of 100 patients who received modified premedication (Table 2). Three of these patients (3.0%) had manifestations of severe allergic reactions. The incidence of hypersensitivity reactions differed significantly between the cohorts (risk ratio, 0.3544; 95% CI, 0.1532–0.8196; $P = 0.0153$). Poisson regression analysis yielded a risk ratio of 0.3581 (95% CI, 0.1541–0.8324; $P = 0.0170$) (Table 2). None of the patients with a history of drug allergy had hypersensitivity reactions.

Treatment exposure

The 81 patients in cohort 1 received a total of 382 cycles of mFOLFOX6 (Table 2). The median number of cycles of mFOLFOX6 was 9 (9 as first-line therapy, 9 as second-line or subsequent therapy) (Table 3). The 100 patients in cohort 2 received a total of 781 cycles (Table 2). The median number of cycles of mFOLFOX6 was 12 overall (Table 2). The number of cycles differed significantly between the cohorts on the Wilcoxon test ($P < 0.0001$) (Table 2). In cohort 2, the median number of cycles of mFOLFOX6 without bevacizumab was 11 (10 as first-line therapy, 11 as second-line or subsequent therapy) (Table 3). The median number of cycles of mFOLFOX6 plus bevacizumab was 12 (12 as first-line therapy, 12 as second-line or subsequent therapy) (Table 3). The number of cycles in patients who additionally received bevacizumab did not differ significantly on the Wilcoxon test. The reasons for treatment

Table 2 Effect of premedication on incidence of hypersensitivity reactions to oxaliplatin

	Incidence of hypersensitivity reactions/ total patients (%)	Risk ratio (95% CI) (*P* value)	Adjusted risk ratio (95% CI) (*P* value)	Incidence of hypersensitivity reactions/total cycles (%)	Median cycles	*P* value
Routine premedication (cohort 1)	16/81 (20)	0.3544 (0.1532–0.8196) ($P = 0.0153$)	0.3581 (0.1541–0.8324) ($P = 0.0170$)	16/382 (4.2)	9	<0.0001
Modified premedication (cohort 2)	7/100 (7.0)			7/781 (0.90)	12	

CI confidence interval

Table 3 Effect of modified premedication on median number of treatment cycles

Cohort	Regimen	Line of therapy	Median cycles of mFOLFOX6 (range)	No. of patients
Routine premedication (cohort 1)	mFOLFOX6	First-line therapy	9 (6–17)	43
		Second-line or subsequent therapy	9 (6–22)	38
Modified premedication (cohort 2)	mFOLFOX6	First-line therapy	10 (6–28)	27
		Second-line or subsequent therapy	11 (6–29)	22
	mFOLFOX6 + bevacizumab	First-line therapy	12 (7–31)	23
		Second-line or subsequent therapy	12 (7–31)	28

discontinuation differed between the cohorts (Table 4). The main reason for treatment discontinuation in cohort 1 was hypersensitivity reactions (53%). Hypersensitivity was the second reason for discontinuing treatment in 11% of the patients in cohort 2. The main reason for treatment discontinuation in cohort 2 was neurotoxicity (53%). Neurotoxicity was the second reason for discontinuing treatment in 20% of the patients in cohort 1.

Table 4 Reasons for treatment discontinuation

Reasons for discontinuation	Routine premedication (cohort 1) ($n = 30$)		Modified premedication (cohort 2) ($n = 62$)	
	No. of patients	%	No. of patients	%
Neurotoxicity	6	20	33	53
Hypersensitivity reactions	16	53	7	11
Fatigue	0	0	3	4.8
Vomiting	0	0	2	3.2
Thrombocytopenia	0	0	1	1.6
Febrile neutropenia	2	6.7	4	6.5
Liver dysfunction	2	6.7	0	0
Thrombosis	0	0	1	1.6
Diarrhea	0	0	1	1.6
Others	4	13	10	16

Safety

Modified premedication did not increase the incidence of adverse effects related to the high dose of dexamethasone, such as exacerbation of diabetes, osteoporosis, and compression fracture. Diphenhydramine was associated with mild somnolence in two patients, but this symptom resolved promptly.

Discussion

The incidence of hypersensitivity reactions in cohort 1 was similar to that in previous studies. Allergic reactions usually develop after several infusions of oxaliplatin [13]. In cohort 2 of our study, the use of modified premedication decreased the incidence of hypersensitivity reactions to 7.0%. Modified premedication with increased doses of dexamethasone and antihistamines thus reduced the incidence of hypersensitivity reactions by 14 percentage points as compared with cohort 1, treated with routine premedication. Gowda et al. [9] evaluated the incidence of hypersensitivity reactions to oxaliplatin and reported 32 hypersensitivity reactions in 169 patients (incidence, 18.9%) who received oxaliplatin preceded by dexamethasone (10 mg) and ondansetron (Zofran, 8 mg). Brandi et al. [7] reported that hypersensitivity reactions occurred in 18.1% of patients who received oxaliplatin preceded by ondansetron. Other than these reports, studies assessing the

preventative effect of premedication on oxaliplatin-related hypersensitivity are scant.

In our study, all patients received mFOLFOX6. Kim et al. retrospectively investigated 247 patients given oxaliplatin-containing regimens and reported that the incidences of hypersensitivity reactions did not depend on the oxaliplatin-containing regimen employed [6]. The modified premedication regimen used in the present study might thus be useful for the management of hypersensitivity reactions to other oxaliplatin-containing regimens.

The patient characteristics were well balanced between the cohorts. The median number of cycles increased from 9 to 12 when modified premedication was used instead of routine premedication. This three-cycle increase in the median number of cycles administered to patients who received modified premedication is particularly important, because prolonged therapy might contribute to improved survival. In cohort 2, patients could receive mFOLFOX6 plus bevacizumab, newly approved in Japan. The addition of bevacizumab to oxaliplatin-based, first-line chemotherapy has been shown to significantly improve progression-free survival in patients with metastatic colorectal cancer [14, 15]. We therefore examined if increasing the number of treatment cycles was associated with the inclusion of bevacizumab. The median number of cycles in patients who additionally received bevacizumab was similar to that in patients treated with mFOLFOX6 without bevacizumab. We found no association between bevacizumab and the number of cycles administered to cohort 2. Bevacizumab thus apparently did not contribute to a longer duration of treatment. Kim et al. [6] reported that anti-vascular epithelial growth factor (anti-VEGF) monoclonal antibody bevacizumab was not associated with hypersensitivity reactions when given with combination chemotherapy regimens. Consistent with their results, we found no difference in the frequency of hypersensitivity reactions according to the presence or absence of bevacizumab.

The major reasons for discontinuing treatment with mFOLFOX6 were neurotoxicity and hypersensitivity reactions. Neurotoxicity was the most remarkable as well as the most common dose-limiting factor. Treatment withdrawal was based on the highest grade adverse effects occurring during the previous cycle. Sensory neuropathy was treatment limiting in patients who received FOLFOX4 (85 mg/m^2 oxaliplatin) because it generally occurred after 8–10 cycles [16]. Tournigand et al. [17] reported that oxaliplatin was associated with grade 3 neuropathy in 20% of patients who received FOLFOX6 (100 mg/m^2 oxaliplatin) and in 34% of patients after 12 cycles. In our study, neurotoxicity was the reason for discontinuing treatment in 20% of the patients in cohort 1, as compared with 53% of those in cohort 2. These reports supported our results that a decreased frequency of hypersensitivity reactions was associated with an increased rate of treatment discontinuation caused by neurotoxicity.

If treatment is discontinued because of neurotoxicity, oxaliplatin-based therapy may be able to be resumed after this adverse effect resolves. This strategy enables treatment for longer periods. When oxaliplatin is used in an adjuvant setting, in which the median number of courses of treatment ranges from 10 to 12, it is important to note that the use of modified premedication reduced the frequency of hypersensitivity reactions from 20% to 7.0%, allowing treatment to be completed as planned. Completion of adjuvant treatment by our strategy may reduce the relapse rate, thereby contributing to improved survival.

The exact mechanism responsible for platinum-related hypersensitivity reactions is unknown, but several mechanisms may be involved. Hypersensitivity reactions have been linked to the release of histamine and other vasoactive substances and ascribed to type I hypersensitivity IgE-mediated reactions [9, 18]. Hypersensitivity reactions usually develop after multiple infusions of oxaliplatin (7 on average) [19], clearly showing that repeated exposure to the drug is prerequisite to the induction of an allergic immune response.

The optimal strategy for resuming treatment after discontinuation caused by an episode of hypersensitivity remains controversial. Because resumption of treatment can be fatal, several preventive procedures have been proposed. Patient desensitization is of interest because of its consistent efficacy but has been studied in only a small number of subjects [19]. Moreover, desensitization is cumbersome to implement. The prick test, using a concentration of 1 mg/ml oxaliplatin, appears not to be very sensitive. Skin tests are useful for detecting IgE-mediated reactions, but their sensitivity is not high enough. When hypersensitivity reactions to oxaliplatin do occur, symptoms generally subside on discontinuation of treatment and administration of steroids and antihistamines. Mild sensitivity reactions to oxaliplatin can be controlled by treatment with antihistamines, steroids, or both. Interestingly, all the patients in cohort 1 of our study received premedication with dexamethasone 8 mg and granisetron 3 mg as a part of a "standard antiemetic" regimen before the infusion of oxaliplatin. In cohort 2, we confirmed that modified premedication with an increased dose of dexamethasone plus an antihistamine effectively decreased hypersensitivity reactions. Premedication was not associated with any side effects. In particular, adverse events potentially associated with a high dose of dexamethasone, such as exacerbation of diabetes, osteoporosis, and compression fractures, did not occur.

In conclusion, our study showed that modified premedication with an increased dose of dexamethasone plus an antihistamine from the sixth cycle of mFOLFOX6

greatly reduced the frequency of hypersensitivity reactions, an important dose-limiting toxic effect of oxaliplatin. A reduced incidence of hypersensitivity reactions to oxaliplatin enhances the effectiveness of mFOLFOX6 by allowing treatment to be prolonged. Our results were statistically significant, although the study was performed in a single institution. We therefore recommend our modified premedication regimen to reduce hypersensitivity reactions in clinical practice. Phase III prospective studies are highly warranted to confirm the effectiveness of modified premedication.

Conflict of interest No author has any conflict of interest.

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Short Communication

Safety of BLP25 Liposome Vaccine (L-BLP25) in Japanese Patients with Unresectable Stage III NSCLC after Primary Chemoradiotherapy: Preliminary Results from a Phase I/II Study

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Preliminary safety findings are presented from the open-label Phase I part of a combined Phase I/II study of BLP25 liposome vaccine (L-BLP25) in Japanese patients with unresectable Stage III non-small-cell lung cancer after primary chemoradiotherapy. Six patients received four or more once-weekly vaccinations with L-BLP25 1000 μg subcutaneously prior to a preliminary safety evaluation. Treatment continued with once-weekly vaccinations with L-BLP25 1000 μg subcutaneously until week 8, then maintenance vaccinations every 6 weeks until progressive disease. Cyclophosphamide (300 mg/m^2 i.v. single dose) was given 3 days before first vaccination. Median age was 63.5 years and performance status was 0–1. No serious adverse events occurred; none necessitated discontinuation. L-BLP25-related adverse events (Grade 1) were myalgia, arthralgia and nausea; cyclophosphamide-related adverse events comprised dysgeusia, anorexia and nausea. The first evaluation of L-BLP25 in Japanese patients shows that it is well tolerated, and the safety profile is consistent with that seen in previous studies of Caucasian patients.

Key words: immunotherapy – chemo-respiratory tract – lung medicine

INTRODUCTION

Lung cancer is the most common cause of cancer-related death worldwide (1) and at the national level in Japan (2). Non-small-cell lung cancer (NSCLC) accounts for the majority of lung cancers (~80%) (3,4), and survival rates for patients with Stage III/IV disease are poor (4). The standard of care for unresectable Stage III NSCLC is combined-modality therapy with chemotherapy and thoracic radiation therapy (TRT) (5). A Phase III study by the West Japan Lung Cancer Group showed that the combination of mitomycin, vindesine and cisplatin with concurrent TRT was associated with a median survival time of 16.6 months and a 5-year survival rate of 16% (6). There clearly remains an unmet need for novel treatment approaches to improve clinical outcomes in this patient population.

Therapeutic cancer vaccines contain tumor-associated antigens, which are supposed to stimulate the immune system to recognize the antigen expressed on cancer cells (7) and to respond with tumor cell destruction. One such vaccine in Phase III clinical development is the BLP25 liposome vaccine (L-BLP25, Merck KGaA), which targets mucin-1 (MUC1), a glycoprotein that is strongly expressed in many types of cancer (8,9). Cellular immune responses