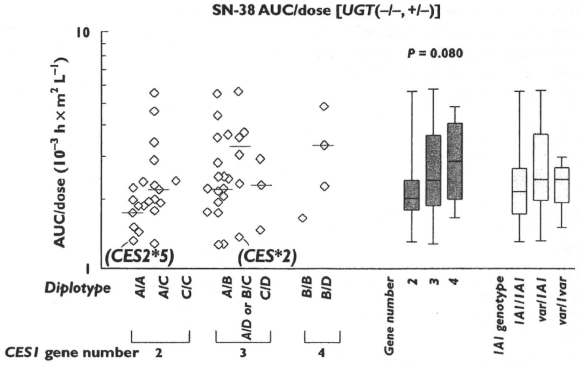

Figure 4

Association of *CES1* genotypes with SN-38 AUC/dose in *UGT*(–/– and +/–) patients treated with irinotecan monotherapy ($n = 51$). '*CES1* gene number' means the number of functional genes (*1A1*, *var1A1* and *1A2*). One patient with an outlying value who had *ABCB1*2* [2677G>T (A893S)] and **14* [2677G>T (A893S) and 1345G>A 230 (E448K)] was excluded from this analysis [10]. A slightly increasing trend in SN-38 AUC/(dose) was observed depending on functional *CES1* gene number. ($P = 0.080$, Jonckheere-Terpstra test). The patients with *CES2*5* [*CES2* 1A>T (M1L)] (*CES2*5*) and *CES2*2* [*CES2* 100C>T (R34W)] (*CES2*2*) [13, 14] are marked

CES1A2 [18] and had strong linkage with SNPs in the proximal promoter region (between –62 to –32) which resulted in additional Sp1 binding sites in the *1A2* promoter region [19]. However, our current study showed no significant effect of –816A>C on the AUC ratio. This can be explained by our finding that –816C and several linked SNPs were mostly located on the *CES1A3* psuedogene but not the functional *1A2* gene.

We newly detected three SNPs (–258C>T, –233C>A and –161A>G) in the 5′-flanking region and one SNP (–30 G>A) in the 5′-UTR of *CES1A1* (Table 3). The effect of –30 G>A on the AUC ratio was not significant (Figure 3c). The frequencies of three other SNPs in the 5′-flanking region were very low (0.003–0.014) which made statistical analysis difficult. These SNPs are not located in the putative transcriptional regulatory regions of *CES1A1*, the binding sites of transcription factors Sp1 and C/EBP [17]. The AUC ratios of the patients with these SNPs were within the 25th–75th percentiles except that slightly higher values were shown in the two -258T patients who received platinum-combination therapy (data not shown). Thus, clinical impact of these SNPs would be small.

With respect to the clinical importance of *CES1* genotyping for irinotecan therapy, the effects of *CES1* genotypes on the AUC level of the active metabolite SN-38 and incidence of grade 3/4 neutropenia should be considered. Since the patients homozygous for *UGT1A1*6 or *28* (*UGT+/+*: **6/*6*, **6/*28* and **28/*28*) showed higher SN-38 AUC/dose levels and severe neutropenia [7], we examined the effects of *CES1* genotypes and SNPs in the non-*UGT+/+* patients. Increasing trends of SN-38 AUC/dose (Figure 4) and incidence of grade 3/4 neutropenia were observed depending on the functional *CES1* gene number in patients with irinotecan monotherapy although statistical significance was not obtained. For the platinum-containing regimens, no significant effects of *CES1* genotypes were shown. Thus, although possible effects of the *CES1* genotypes on neutropenia could not be excluded in irinotecan monotherapy, this study was still insufficient to establish the clinical importance of *CES1* genotyping in irinotecan therapy. Since the sample size will be twice that of the present study to detect a statistically significant decrease of absolute neutrophil counts in the patients with four functional *CES1* genes, future clinical data obtained in a larger number of patients could clarify this point.

In conclusion, this study suggests that the total number of functional *CES1A* genes could influence the formation of the active metabolite of irinotecan in Japanese cancer patients.

Competing interests

HK has received lecture honorarium from Yakult Honsha, the manufacturer of irinotecan. HM has been paid by Yakult Honsha, the manufacturer of irinotecan, for speaking and research.

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A Phase I Study of Enzastaurin Combined with Pemetrexed in Advanced Non-small Cell Lung Cancer

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Introduction: Enzastaurin is an oral serine/threonine kinase inhibitor, which suppress signaling through protein kinase C-β and the phosphatidylinositol 3-kinase/AKT pathway. Preclinical studies suggested synergic antitumor activity of enzastaurin and pemetrexed. We conducted this phase I study to evaluate the safety, pharmacokinetics, and clinical activity of this combination in patients with previously treated advanced non-small cell lung cancer.

Methods: An oral daily dose of 500 mg enzastaurin was administered once daily (QD) or twice daily (BID) in combination with 500 mg/m^2 pemetrexed on day 1 in repeated 21-day cycles. Cycle 1 started with a 7-day enzastaurin lead-in treatment that preceded pemetrexed administration: a loading dose of 1125 mg enzastaurin on day 1 followed by a 500 mg total daily dose on days 2–7.

Results: Twelve patients were treated QD (n = 6) or BID (n = 6). One dose-limiting toxicity (grade 3 QTc prolongation) was reported in the QD cohort. Grade 3/4 hematological toxicities were slightly increased in the BID cohort compared with the QD cohort. After beginning the combination therapy, enzastaurin exposures decreased slightly but remained above the target plasma concentration of 1400 nmol/L. Compared with QD, there was a higher exposure with BID. The enzastaurin dosing regimen (QD or BID) had no effect on pemetrexed pharmacokinetics. Two patients had partial responses as defined by RECIST. Five patients received more than 10 cycles of treatment without disease progression.

Conclusions: Both schedules of enzastaurin in combination with pemetrexed were well tolerated and clinically active in patients with advanced non-small cell lung cancer.

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Lung cancer remains the leading cause of cancer mortality in the world. Platinum-based combination chemotherapy offers a survival benefit in patients with chemo-naïve advanced non-small cell lung cancer (NSCLC).[1,2] Nevertheless, the efficacy of platinum-based combination chemotherapy seems to have reached a therapeutic plateau, with a median survival of 8 to 12 months.[3,4] Several agents have been approved for second- and third-line therapy, including docetaxel,[5,6] pemetrexed[7], and erlotinib.[8] Nevertheless, they improve survival by only approximately 2.2 to 6.5 months compared with best supportive care.[5–8] A more effective therapy is needed.

Pemetrexed, a multitargeted antifolate, is currently approved as a first- and second-line therapy for locally advanced or metastatic NSCLC. The standard regimen of pemetrexed is 500 mg/m^2, administered intravenously (IV) on day 1 in repeated 21-day cycles, supplemented with folic acid and vitamin B_{12}.

Enzastaurin (LY317615), an oral serine/threonine kinase inhibitor, suppress signaling through protein kinase C (PKC)-β and the phosphatidylinositol 3-kinase/AKT pathway.[9–12] Enzastaurin is metabolized primarily by cytochrome P450 3A (CYP3A) to form a desmethylenepyrimidyl metabolite (LY326020) and a desmethyl metabolite (LY485912), two active metabolites with comparable potency against PKCβ. In vitro analysis has shown that the IC_{90} of enzastaurin for PKCβ is 70 nmol/L.[9] In light of the 95% plasma protein binding value of enzastaurin, the targeted mean steady state total concentration for clinical efficacy is estimated to be 1400 nmol/L. In a previous dose-escalation study (20–700 mg once daily [QD]) of patients with cancer, enzastaurin exposures reached a plateau above the targeted steady-state plasma concentration of 1400 nmol/L when administered at doses of 525 mg.[13] This dose was well tolerated, and enzastaurin at 500 mg QD demonstrated clinical activity as a single agent and in combination with cytotoxic agents.[14–17]

The combination of enzastaurin and pemetrexed has shown synergic antitumor activity in NSCLC cells.[18–20] Pre-

clinical studies revealed that enzastaurin suppressed thymidylate synthase (TS) expression through downregulation of E2F.[21] TS expression is one of the known sensitivity markers for pemetrexed, with pemetrexed treatment markedly increasing transcription of the *TS* gene. However, the combination of enzastaurin and pemetrexed significantly decrease TS activity and reduced glycogen synthase kinase-3beta/AKT phosphorylation and vascular endothelial growth factor secretion.[21] Therefore, evidence points to enzastaurin as a promising agent to increase the activity of pemetrexed.

In vitro analysis showed that twice daily (BID) dosing could maintain enzastaurin exposures above the targeted plasma concentration longer than QD dosing (data at Eli Lilly on file), which was confirmed in phase I studies.[15,17] We conducted this study to assess safety and tolerability of two dosing regimens of total daily 500 mg enzastaurin (QD or BID) in combination of pemetrexed.

The primary objective was to evaluate the safety of the combination of enzastaurin and pemetrexed in Japanese advanced NSCLC patients with prior systemic chemotherapy. The secondary objectives were to evaluate the toxicities of this combination and determine the pharmacokinetics of enzastaurin with or without pemetrexed. Antitumor activity of this combination was also assessed. We started with enzastaurin 500 mg QD in combination with pemetrexed to confirm safety in the QD cohorts. After this, we proceeded to 250 mg BID.

PATIENTS AND METHODS

Patients

Eligibility criteria included the following: histologically or cytologically documented NSCLC; clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy) or relapse after surgery; one or two prior systemic chemotherapy regimens, including at least one platinum-based regimen for NSCLC; presence of at least one measurable disease as defined by the Response Evaluation Criteria in Solid Tumors (RECIST); 20 to 75 years of age; Eastern Cooperative Oncology Group performance status of 0 to 2; adequate bone marrow reserve, hepatic, renal, and pulmonary function; predicted life expectancy of at least 12 weeks; recovery from toxicities of all previous therapies for NSCLC (had not received radiotherapy <28 days, chemotherapy <28 days, nitrosourea <42 days, hormone therapy <14 days, molecular targeted therapy <14 days, Uracil-Tegafur <14 days, or doxifluridine <14 days before enrollment).

Exclusion criteria were the following: interstitial pneumonia or pulmonary fibrosis detectable on radiologic evaluation; history of tube thoracostomy drainage for pleural effusion; inability to swallow capsules; myocardial infarction that occurred less than 6 months before entry; symptomatic angina pectoris, cardiac failure, arrhythmia not controlled by medication; prolonged QTc interval >450 milliseconds; symptomatic central nervous system metastasis; chronic use of nonsteroidal anti-inflammatory drugs; pregnancy; and serious comorbidity.

The study was approved by the institutional review boards of each participating institution and was conducted in accordance with the Declaration of Helsinki and good clinical practice and in compliance with all applicable laws and regulations. Written informed consent was given by each patient before study enrollment.

Study Design and Treatment Plan

This was an open-label, nonrandomized, multicenter study designed to assess safety and pharmacokinetics of enzastaurin administered QD (cohort 1) or BID (cohort 2) in combination with the standard dose of pemetrexed. During stage 1 of cycle 1, patients received a loading dose of enzastaurin (375 mg, 3 times, 1125 mg total dose) on day 1 followed by 500 mg total daily dose on days 2 through 7 of cycle 1 (Figure 1) to achieve the targeted steady-state concentration (1400 nmol/L).[9,13] During stage 2 of cycle 1, an oral daily dose of 500 mg enzastaurin was administered within half an hour after meals QD or BID. This was continued in cycle 2 and thereafter. Patients received standard pemetrexed as a 10-minute IV dose of 500 mg/m^2 on day 1 of stage 2 of cycle 1 and subsequently in a repeated 21-day cycle. On days of pemetrexed dosing, enzastaurin was administered after pemetrexed. We started with 500 mg QD (cohort 1), and after confirming safety, serially enrolled the BID cohorts and proceeded with 250 mg BID (cohort 2).

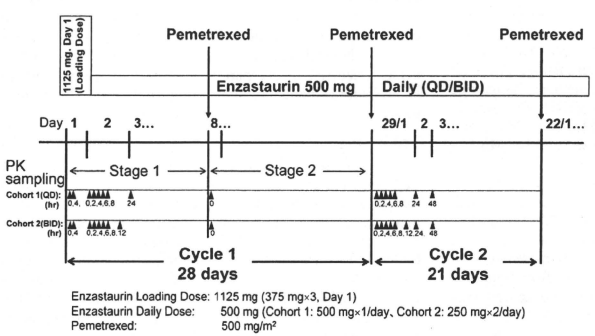

FIGURE 1. Study design and pharmacokinetic plan. An oral daily dose of 500 mg enzastaurin was given once daily (QD) in cohort 1 or twice daily (BID) in cohort 2 in combination with 500 mg/m^2 pemetrexed on day 1 in repeated 21-day cycles. Cycle 1 started with a 7-day enzastaurin lead-in treatment that preceded pemetrexed administration: a loading dose of 1125 mg enzastaurin on day 1 followed by 500 mg total daily dose on days 2–7. Pharmacokinetic (PK) sampling is indicated by chevrons. All patients received standard daily folate and vitamin B12 supplementation per standard treatment guidelines for pemetrexed infusion.

Each cohort was initially designed to enroll six patients. If three or more patients in each cohort experienced dose-limiting toxicities (DLTs) during cycle 1, the recruitment was to be ended. A DLT was defined as any of the following drug-related adverse events during cycle 1: (1) hematological toxicity, as determined by the Common Terminology Criteria for Adverse Events version 3.0: grade 4 neutropenia that persisted for 7 days or more, febrile neutropenia, grade 4 thrombocytopenia, or grade 3 thrombocytopenia with hemorrhage or requiring a blood transfusion; (2) grade 3/4 nonhematologic toxicities except for the following manageable events: nausea, vomiting, loss of appetite, fatigue, constipation, diarrhea, transient aspartate aminotransferase or alanine transaminase elevation, and transient electrolyte abnormality; (3) grade 3 corrected QT (QTc) prolongation >500 milliseconds or an increase ≥60 milliseconds over baseline QT measured at entry.

Safety Assessments

Physical examination results, vital signs (blood pressure, pulse rate, and body temperature), and performance status were evaluated at baseline, on day 1, and weekly during treatment. Complete blood count, serum chemistry, and urinalysis were performed at baseline and weekly during treatment. Twelve-lead electrocardiograms were recorded at baseline, 4–6 hours after the first dosing of enzastaurin on days 1 to 3 during stage 1, and at one point determined by the investigator between days 1 and 8 of each cycle. QTc values were obtained using Bazett's method of correction.[22]

Pharmacokinetic Measurements and Analyses

In cohort 1, blood samples for enzastaurin pharmacokinetics were collected on the following days (Figure 1): day 1 (before dosing and 4 hours after dosing), day 2 (before dosing and 2, 4, 6, 8, and 24 hours after dosing), and day 8 (before dosing) of cycle 1, and day 1 (before dosing and 2, 4, 6, 8, and 24 hours after dosing) of cycle 2. In cohort 2, blood samples were collected at the same points as cohort 1 except that the 24-hour collection time points after the first dose in cycles 1 and 2 was changed to 12 hours. Blood samples for pemetrexed pharmacokinetics were collected on day 1 (10 minutes, 2, 4, 6, 8, and 24 hours after dosing) and day 3 (before dosing) of cycle 2.

Pemetrexed plasma concentrations were measured using two validated high-pressure liquid chromatography with tandem mass spectrometry (LC/MS/MS) methods, high range and low range (SFBC Taylor, Princeton, NJ). Enzastaurin (LY317615) and its two active metabolites (LY326020 and LY485912) were also detected using a validated LC/MS/MS method (Advion BioSciences, Inc., Ithaca, NY).

Pharmacokinetic (PK) parameters for enzastaurin, its metabolites LY326020, LY485912, and pemetrexed were analyzed using noncompartmental methods (WinNonlin Enterprise Version 5.0.1; Pharsight Corporation, Mountain View, CA). PK parameters calculated for pemetrexed were area under the concentration versus time curve from time zero extrapolated to infinity (AUC_{0-inf}), maximum observed concentration (C_{max}), apparent clearance, apparent volume of distribution, and terminal half-life ($t_{[1/2]}$). PK parameters calculated were AUC_{0-24} for QD enzastaurin or AUC_{0-12} for BID enzastaurin, LY326020, LY485912, total analytes (enzastaurin + LY326020 + LY485912), C_{max}, and the observed time to reach peak drug concentration (t_{max}). PK parameters between cycle 1 and cycle 2 were compared to evaluate the effect of the loading dose to reach steady state in a short time. AUC_{0-24} for QD enzastaurin or AUC_{0-12} for BID enzastaurin, C_{max}, t_{max}, and metabolic ratio (metabolite AUC/parent AUC) were also calculated for LY326020 and LY485912.

Assessment of Tumor Response

Tumor measurement by radiologic imaging was done at baseline and every 42 days during treatment. Poststudy evaluation was conducted 30 ± 7 days after the last administration of enzastaurin. Tumor response was evaluated using the RECIST guideline.[23]

Statistical Analyses

All patients who received at least one enzastaurin dose were evaluated for safety, and those who received both enzastaurin and pemetrexed at least once were evaluated for efficacy. All analyses were descriptive, with no formal statistical test performed on the data from this study.

RESULTS

Patient Characteristics

Twelve patients were enrolled into the study at two cancer center hospitals in Japan from November 2007 to March 2008. All the 12 patients received at least one study treatment: six patients each were enrolled in the QD (cohort 1) and BID (cohort 2) groups. A single patient (cohort 1) discontinued the study before pemetrexed administration because of an adverse event. Baseline characteristics for the 12 patients are summarized in Table 1.

Dose Administration

In the study, a total of 91 cycles of therapy were completed, with a median number of cycles per patient of 4 (range, 1–19). Five patients (two in QD and three in BID) received more than 10 cycles of therapy (range, 13–19).

The reasons for discontinuation during the first four cycles were disease progression ($n = 5$) and adverse events ($n = 2$). Nine dosing delays of pemetrexed during the four cycles occurred in six patients because of adverse events (four in three patients), scheduling conflict (three in three patients), and others (two in one patient).

Toxicities

All 12 patients were evaluated for toxicities during the first four cycles. Table 2 lists all grade 3/4 drug-related toxicities; all drug-related toxicities with at least a 20% incidence in the overall population, regardless of grade, are also shown. One patient (BID) experienced grade 4 hematological toxicities: neutropenia, anemia, and thrombocytopenia. Grade 3 hematological toxicities occurred in four patients. Grade 4 nonhematological toxicities were not observed. All grade 3/4 toxicities were reversible and manageable, except for toxicities whose recovery could not be

TABLE 1. Patient Characteristics

	500 mg QD (n = 6)	250 mg BID (n = 6)	Total (n = 12)
Age, yr			
Median	63.5	59.0	61.5
Range	49–74	49–71	49–74
Gender, n (%)			
Male	5	3	8
Female	1	3	4
ECOG PS, n (%)			
0	4	4	8
1	2	2	4
Histology, n (%)			
Adenocarcinoma	5	3	8
Squamous cell carcinoma	1	2	3
Undifferentiated NSCLC	0	1	1
Disease stage, n (%)			
IIIB	1	3	4
IV	4	3	7
Relapse after surgery	1	0	1
Prior therapy, n (%)			
Chemotherapy[a]			
1 regimen	3	3	6
2 regimens	3	3	6
Surgery	1	0	1
Radiotherapy	3	4	7

[a] Cisplatin-gemcitabine, carboplatin-paclitaxel, cisplatin-S1, cisplatin-vinorelbine, docetaxel, gefitinib, and others.

ECOG, eastern cooperative oncology group; NSCLC, non-small cell lung cancer.

confirmed because of disease progression. During the first four cycles, two patients discontinued the study because of drug-related toxicities: grade 3 QTc prolongation (QD) and grade 1 increased serum creatinine (BID). Four dosing delays of pemetrexed occurred in three patients (one in QD and two in BID) because of adverse events: neutropenia, thrombocytopenia, anemia, increased alanine transaminase, hyponatremia, and increased blood creatinine. Grade 3/4 hematological toxicities were slightly increased in BID dosing compared with QD dosing.

One DLT was reported: grade 3 QTc prolongation was observed 1 day after the enzastaurin loading dose in the QD cohort. This male patient with a history of coronary spastic angina experienced asymptomatic QTc prolongation ≥60 milliseconds over baseline (baseline: 430 milliseconds, post dose: 510 milliseconds). Enzastaurin was halted and his QTc normalized (420 milliseconds) in 6 days without the need of any medication. The patient was withdrawn from the study because of the event. There were no treatment-related deaths.

Treatment Response

Other than the single patient who discontinued before administration of pemetrexed because of DLT, all patients were assessed for response. Based on the results at the end of the study, two patients (one in QD and one in BID) (18%) achieved partial response (PR) and five patients (two in QD and three in BID) (45%) had stable disease (SD). Five patients (45%) received more than 10 cycles of treatment without disease progression.

TABLE 2. All Grade 3/4 Toxicities and Toxicities with at Least 20% Incidence

	500 mg QD (n = 6)		250 mg BID (n = 6)		Overall (n = 6)	
Toxicity, n	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4
Nonhematological toxicity						
Chromaturia	5	0/0	6	0/0	11	0/0
Anorexia	5	0/0	5	0/0	10	0/0
Rash	5	0/0	5	0/0	10	0/0
Increased ALT	4	1/0	5	1/0	9	2/0
Increased AST	4	1/0	5	1/0	9	2/0
Fatigue	3	0/0	4	0/0	7	0/0
Nausea	4	0/0	3	0/0	7	0/0
Constipation	3	0/0	1	0/0	4	0/0
Discolored faeces	1	0/0	3	0/0	4	0/0
Hyponatremia	1	0/0	3	2/0	4	2/0
Increased LDH	1	0/0	3	0/0	4	0/0
Diarrhea	1	0/0	2	0/0	3	0/0
Fever	2	0/0	1	0/0	3	0/0
Hematuria	2	0/0	1	0/0	3	0/0
Hypoalbuminemia	1	0/0	2	0/0	3	0/0
Increased ALP	2	0/0	1	0/0	3	0/0
Arthralgia	2	1/0	0	0/0	2	1/0
QT prolongation	1	1/0	1	0/0	2	1/0
Hematological toxicity						
Anemia	5	0/0	5	0/1	10	0/1
Leukocytopenia	4	1/0	4	2/0	8	3/0
Lymphocytopenia	3	1/0	4	0/0	7	1/0
Neutropenia	3	1/0	3	2/1	6	3/1
Thrombocytopenia	2	0/0	2	1/1	4	1/1

Common Toxicity Criteria for Adverse Events version 3.0.

ALT, alanine transaminase; AST, aspartateaminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

Pharmacokinetics

PK parameters after 500 mg QD or 250 mg BID dosing of enzastaurin are shown in Table 3. The C_{max} for total analyte was reached within about 2 hours after BID dosing and about 5 hours after QD dosing. Total analytes in both BID and QD dosing declined in a monophasic manner after reaching maximum concentrations (Figure 2). Total analyte exposure (enzastaurin and its metabolites $AUC_{\tau,ss}$) of enzastaurin was approximately 21% and 3% lower, respectively, for 500 mg QD and 250 mg BID when administered with pemetrexed (cycle 2) compared with administration of enzastaurin alone (stage 1 in cycle 1). Maximum concentrations ($C_{max,ss}$) of total analyte were 14% and 2% lower, respectively, for 500 mg QD and 250 mg BID when administered with pemetrexed (cycle 2) compared with administration of enzastaurin alone (stage 1 in cycle 1) (Figure 2 and Table 3). Compared with QD dosing, there was a higher mean exposure for BID dosing. Total analyte concentrations reached

TABLE 3. Pharmacokinetic Parameters of Enzastaurin Total Analytes

	Geometric Mean (CV%)			
	500 mg QD		250 mg BID	
Parameter	Cycle 1, Day 2 One Day After Loading Dose[a]	Cycle 2, Day 1 (Steady State) + Pemetrexed	Cycle 1, Day 2 One Day After Loading Dose[a]	Cycle 2, Day 1 (Steady State) + Pemetrexed
N	6	4	6	5
C_{max} (nmol/L)	4870 (36.0)	4200 (50.1)	4420 (44.5)	4340 (31.3)
t_{max}[b] (h)	4.99 (0.00–6.00)	5.09 (2.02–8.02)	1.97 (1.78–3.95)	2.23 (1.93–4.22)
$AUC_{\tau,ss}$ (nmol/L·h)	86200 (36.1)	68500 (53.5)	42400 (49.3)	41100 (39.5)
$C_{av,ss}$ (nmol/L)	NC	2850 (53.5)	NC	3420 (39.5)

[a] Non-steady-statevalues, $AUC_{\tau,ss}$ = AUC(0–24 h) (QD) or AUC(0–12 h) (BID).

[b] Values are in median (range).

BID, twice daily; QD, once daily; *N*, numberof subjects used in the pharmacokinetic analysis; C_{max}, maximum plasma concentration; t_{max}, time to reach maximumconcentration; $AUC_{\tau,ss}$, area under the concentration versus timecurve during one dosing interval at steady state (QD = 24 h and BID = 12 h); $C_{av,ss}$, average drug concentration under steady-state conditions during multipledosing; CV, coefficient of variation; NC, not calculated.

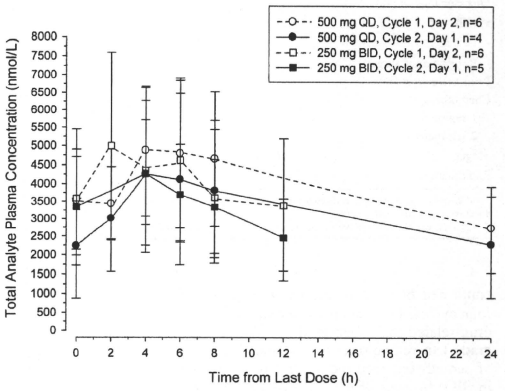

FIGURE 2. Plasma concentration of enzastaurin total analytes. Arithmetic mean (SD) total analytes (enzastaurin and its metabolites) plasma concentration time profiles after enzastaurin administered 500 mg once daily (QD) or 250 mg twice daily (BID) without concurrent pemetrexed (QD, open circle; BID, open square) or with concurrent pemetrexed (QD, black circle; BID, black square).

steady-state by day 8 of cycle 1, with mean steady-state concentrations (CV %) of 2850 nmol/L (53.5%) and 3420 nmol/L (39.5%) after QD and BID dosing, respectively (Figure 3). Total analyte exposure in cycle 1 was relatively higher than that in cycle 2, suggesting that the loading dose regimen of enzastaurin was instrumental in achieving a steady-state level of total analytes on day 2.

PK parameters of pemetrexed for QD and BID dosing are summarized in Table 4. Pemetrexed declined in a triphasic manner with an elimination half-life of 3.22 hours, which was consistent with previous observations of pemetrexed single dosing.[24] Interpatient variability in CL and Vss was less than 40%, implying constant systemic exposure to pemetrexed. Pemetrexed pharmacokinetics were not altered by enzastaurin dosing regimen, either QD or BID.

DISCUSSION

PKC overexpression and increased activity have been detected in a variety of tumors including several hematological malignancies, colon cancer, renal cell cancer, hepatocellular cancer, prostate cancer, and NSCLC.[25–30] Enzastaurin, a potent PKC inhibitor has been shown to have antiangiogenic and antitumor effects in NSCLC.[13,14,20,31] The synergic antitumor activity of enzastaurin and pemetrexed combination was shown in NSCLC cell lines,[18–20] and previous combination studies of enzastaurin with cytotoxic agents showed neither increased toxicity nor PK drug-drug interactions.[15,16,31,32] Therefore, in this study, we decided to assess safety of the recommended clinical doses of enzastaurin and pemetrexed (enzastaurin 500 mg/d and pemetrexed 500 mg/m^2).

The combination regimen was well tolerated for both QD and BID dosing. The observed range of grade 3/4 toxicities in this study was consistent with those seen in the monotherapy studies of enzastaurin and pemetrexed. All grade 3/4 toxicities, including DLT, were reversible and manageable. One patient with a history of ischemic heart disease developed grade 3 QTc prolongation that was considered a DLT. However, this event was asymptomatic and

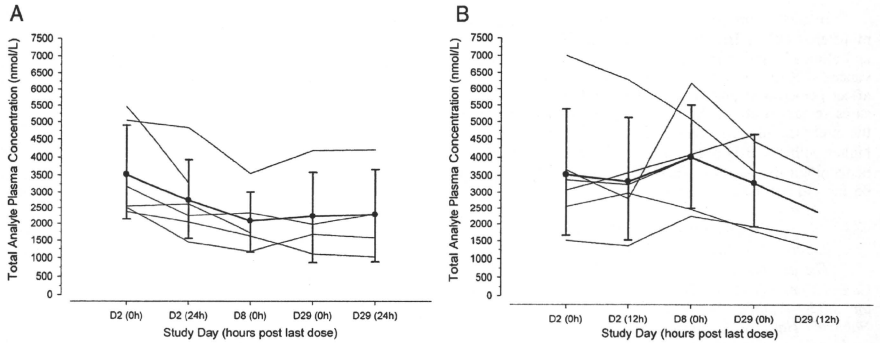

FIGURE 3. Pharmacokinetic result of enzastaurin: trough concentration of total analytes. Arithmetic mean (solid circles), SD, and individual trough concentrations (lines) of enzastaurin total analytes (enzastaurin and its metabolites) after enzastaurin administered 500 mg once daily (*A*) or 250 mg twice daily (*B*) without or with concurrent pemetrexed.

TABLE 4. Pemetrexed Pharmacokinetic Parameters After Enzastaurin Dose

	Geometric Mean (CV%)		
Parameter	**+Enzastaurin (Cycle 2) 500 mg QD**	**+Enzastaurin (Cycle 2) 250 mg BID**	**+Enzastaurin (Cycle 2) 250 mg BID and 500 mg QD**
N	4	5	9
C_{max} (μg/mL)	143 (20.4)	127 (10.6)	134 (15.8)
t_{max}[a] (h)	0.15 (0.15–0.18)	0.15 (0.15–0.17)	0.15 (0.15–0.18)
AUC(0–∞) (μg·h/mL)	265 (38.1)	236 (36.3)	248 (35.2)
CL (L/h/m^2)	1.88 (38.3)	2.12 (36.3)	2.01 (35.2)
V_{ss} (L/m^2)	5.70 (17.3)	6.12 (9.60)	5.93 (13.1)
$t_{1/2}$[b] (h)	3.13 (2.67–3.60)	3.29 (2.42–4.85)	3.22 (2.42–4.85)

[a] Values are in median (range).
[b] Values are in geometric mean (range).
AUC(0–∞), area under the plasma concentration time curve; CL, systemic clearance; C_{max}, maximum plasma concentration; CV, coefficient of variation; *N*, number ofpatients; $t_{1/2}$, elimination half-life; t_{max}, time of maximal plasma concentration; V_{ss}, volume ofdistribution at steady state.

transient. In the enzastaurin preclinical toxicology study in dogs, prolonged QT and QTc values were observed after 5 weeks of dosing at a high daily dose of enzastaurin. In the enzastaurin phase I study in recurrent glioma patients, one grade 3 QTc prolongation was also reported.[33]

The PK results of this study indicated no significant PK interaction between enzastaurin and pemetrexed, which was consistent with a previously published phase I study report.[32] One possible reason for the absence of any effect on pharmacokinetics was the different pathways used for elimination. Pemetrexed is renally eliminated, whereas a phase I study using [^{14}C] enzastaurin indicated that enzastaurin undergoes extensive hepatic metabolism with minimal renal elimination (Eli Lilly and Company, Internal Clinical Study Report, October 2006). Based on these results, it is not likely that enzastaurin and its metabolites inhibit the renal elimination of pemetrexed. In fact, a previous combination study of enzastaurin with pemetrexed reported by Hanauske et al.[17] showed that pemetrexed pharmacokinetics (systemic clearance and half-life) did not seem to be altered by enzastaurin. In addition, it is not likely that pemetrexed inhibits the metabolism of enzastaurin by CYP3A4 because results from in vitro studies with human liver microsomes predicted that pemetrexed would not cause clinically significant inhibition of metabolic clearance of drugs metabolized by CYP3A, CYP2D6, CYP2C9, and CYP1A2.[34] In this study, the comparatively high concentrations observed in cycle 1 resulted from the large loading dose on day 1 of cycle 1. In addition, we confirmed two more findings that were reported by Hanauske et al.[17] First, the maximum concentrations ($C_{max,ss}$) of total analyte in both QD and BID regimens were slightly decreased in the presence of pemetrexed (14% QD and 2% BID in this study; 17% QD and 8% BID in the Hanauske phase Ib study). Second, the average steady-state plasma concentration ($C_{av,ss}$) of enzastaurin total analyte was slightly higher in the BID versus QD regimen (20% higher in this study and 11% higher in the Hanauske phase Ib study).

In light of the fact that two patients achieved PR, five patients achieved SD, and five patients remained on therapy for more than 9 months (13 cycles), the results from this study suggest that the combination of enzastaurin and pemetrexed might be beneficial in previously treated patients with advanced NSCLC. The histology of both the patients who achieved PR was nonsquamous cell carcinoma. One of three patients with squamous cell carcinoma remained on therapy for 14 cycles with SD, whereas PD was observed during cycle 1 for the other two patients. Further research is warranted to determine whether enzastaurin might improve the effect of pemetrexed that works preferentially in nonsquamous cell carcinoma.

In conclusion, combination therapy for enzastaurin administered QD or BID with pemetrexed was well tolerated and clinically active in patients with previously treated advanced NSCLC. Both dosing regimens of enzastaurin did not affect pemetrexed pharmacokinetics, and enzastaurin exposures remained above the targeted plasma concentration in the presence of pemetrexed. Enzastaurin exposures were higher with the BID regimen, with slightly more grade 3/4 hematological toxicities. These were manageable and BID dosing did not indicate any major tolerability issues.

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Clinical Studies

A phase II trial of dose-dense chemotherapy, followed by surgical resection and/or thoracic radiotherapy, in locally advanced thymoma: report of a Japan Clinical Oncology Group trial (JCOG 9606)

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BACKGROUND: This study aimed to evaluate the safety and efficacy of dose-dense weekly chemotherapy, followed by resection and/or thoracic radiotherapy.

METHODS: Patients with histologically documented thymoma with unresectable stage III disease received 9 weeks of chemotherapy: cisplatin 25 mg m^{-2} on weeks 1–9; vincristine 1 mg m^{-2} on weeks 1, 2, 4, 6 and 8; and doxorubicin 40 mg m^{-2} and etoposide 80 mg m^{-2} on days 1–3 of weeks 1, 3, 5, 7 and 9. Patients went on to surgery and post-operative radiotherapy of 48 Gy; those with unresectable disease received 60 Gy radiotherapy.

RESULTS: A total of 23 patients were entered. The main toxicities of the chemotherapy regimen were neutropenia and anaemia, and 57% of patients completed the planned 9 weeks of therapy. There were no toxic deaths. Of the 21 eligible patients, 13 (62%) achieved a partial response (95% confidence interval: 38–82%). Thirteen patients underwent a thoracotomy and nine (39%) underwent complete resection. Progression-free survival at 2 and 5 years was 80 and 43%, respectively. Overall survival at 5 and 8 years was 85 and 69%, respectively. Survival did not seem to be affected by resection.

CONCLUSION: In thymoma patients, weekly dose-dense chemotherapy has activity similar to that of conventional regimens. Although some patients could achieve complete resection, the role of surgery remains unclear.

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Thymoma is one of the most common tumours to originate in the mediastinum (Giaccone, 2005; Girard *et al*, 2009). Although its clinical behaviour tends to be indolent, it eventually disseminates into the pleural space or sometimes leads to distant metastases. Masaoka's classification has been widely used for clinical staging (Masaoka *et al*, 1981; Girard *et al*, 2009).

The majority of thymomas are discovered at a limited stage, Masaoka's stage I or II, and surgical resection is the treatment of choice for such cases (Giaccone, 2005; Girard *et al*, 2009). Even when the tumour invades neighbouring organs, namely, stage III disease, surgical resection with post-operative radiotherapy is the preferred treatment when the tumour can be completely resected (Curran *et al*, 1988; Urgesi *et al*, 1990; Ogawa *et al*, 2002; Strobel *et al*, 2004).

However, for stage III, unresectable tumours, a combination of chemotherapy and radiotherapy with or without surgical resection is frequently used, but optimal management remains controversial (Ciernik *et al*, 1994; Loehrer *et al*, 1997; Kim *et al*, 2004; Mangi *et al*, 2005; Lucchi *et al*, 2006). There are very few prospective trials with limited numbers of cases, some including stage IV cases (Loehrer *et al*, 1997; Kim *et al*, 2004; Girard *et al*, 2009).

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On the other hand, thymomas are generally reported to be chemotherapy-sensitive tumours, with a response rate of 50–70% to combination chemotherapy (Loehrer *et al*, 1994, 1997, 2001; Giaccone *et al*, 1996; Berruti *et al*, 1999; Kim *et al*, 2004; Lucchi *et al*, 2006; Yokoi *et al*, 2007). Active agents include cisplatin (CDDP), vincristine (VCR), doxorubicin (ADM), etoposide (ETP), cyclophosphamide (CPM) and ifosfamide (IFX).

Dose-dense chemotherapy with the CODE combination (CDDP–VCR–ADM–ETP), combined with granulocyte colony-stimulating factor (G-CSF), has been shown to be safe when administered to patients with advanced lung cancer (Murray *et al*, 1991; Fukuoka *et al*, 1997). Theoretically, it might be suitable for chemo-sensitive tumours such as small-cell lung cancers and thymomas, especially in cases with limited tumour burden (Goldie and Coldman, 1983; Levin and Hryniuk, 1987; Murray, 1987). Because of the pilot data in Japan that had suggested that administration of 12 weeks of CODE chemotherapy was barely feasible, subsequent Japanese trials used a modified schedule that was shortened to 9 weeks (Fukuoka *et al*, 1997; Furuse *et al*, 1998).

In 1996, we, the Japan Clinical Oncology Group (JCOG), initiated two clinical trials for advanced thymoma: one aimed to evaluate the safety and efficacy of the CODE regimen in stage IV, disseminated thymoma (JCOG 9605), and the other aimed to evaluate the safety and efficacy of CODE combination chemotherapy, followed by surgical resection and post-operative radiotherapy, in initially unresectable stage III thymoma (JCOG 9606). The primary end point in each study was progression-free survival (PFS). The results of JCOG 9606 are reported herein.

PATIENTS AND METHODS

Eligibility criteria

Patients with previously untreated, histologically documented thymomas with Masaoka's stage III disease that was judged to be unresectable by the surgeons, radiologists and medical oncologists at each institute were eligible for entry. Thymoma had to be confirmed histologically, and thymic tumours with other histology, such as thymic carcinoma, carcinoid or lymphoma, were excluded. Each patient was required to fulfil the following criteria: 15–70 years of age; Eastern Cooperative Oncology Group (ECOG) performance status, 0–2; and adequate organ function, that is, leukocyte count $\geqslant 4000/\mu l$, platelet count $\geqslant 10^5/\mu l$, haemoglobin $\geqslant 10.0$ g per 100 ml, serum creatinine <1.5 mg per 100 ml, creatinine clearance $\geqslant 60$ ml min^{-1}, serum bilirubin <1.5 mg per 100 ml, serum alanine aminotransferase and aspartate aminotransferase less than double the upper limit of the institutional normal range, $PaO_2 \geqslant 70$ mm Hg and predicted post-operative forced expiratory volume in 1 s to be 50% or more of the age-, sex- and height-predicted vital capacity. The exclusion criteria included patients with uncontrolled heart disease, uncontrolled diabetes or hypertension, pulmonary fibrosis or active pneumonitis as evident on chest X-ray, infections necessitating systemic use of antibiotics, disease necessitating emergency radiotherapy, such as superior vena cava obstruction syndrome, active concomitant malignancy, as well as pregnant or lactating women. Also excluded were those with grave complications of thymoma, such as pure red cell aplasia or hypogammaglobulinaemia; myasthenia gravis was allowed and these patients were not excluded *per se*.

Patient eligibility was confirmed by the JCOG Data Center before patient registration. This study protocol was confirmed by the JCOG protocol committee, and then approved by the institutional review boards at each participating centre. Written informed consent was obtained from all patients.

Treatment plan

Chemotherapy Patients received the 9-week CODE combination chemotherapy described below. Each chemotherapeutic agent was administered intravenously.

Week 1: CDDP 25 mg m^{-2} on day 1 with antiemetics and ample hydration; VCR 1 mg m^{-2} on day 1; ADM 40 mg m^{-2} on day 1; and ETP 80 mg m^{-2} on days 1–3.
Weeks 2, 4, 6 and 8: CDDP 25 mg m^{-2} on day 1 with antiemetics and ample hydration and VCR 1 mg m^{-2} on day 1.
Weeks 3, 5, 7 and 9: CDDP 25 mg m^{-2} on day 1 with antiemetics and ample hydration, ADM 40 mg m^{-2} on day 1 and ETP 80 mg m^{-2} on days 1–3.

Each week, G-CSF (filgrastim 50 μg m^{-2} per day or lenograstim 2 μg kg^{-1} per day) was administered by subcutaneous injection, except on days when chemotherapy was administered or when the leukocyte count was $\geqslant 10\,000/\mu l$. Corticosteroid was used only as part of the antiemetic regimen, and the specific drug and dosage were not regulated by the protocol.

Dose and schedule modifications were carried out as previously reported (Kunitoh *et al*, 2009).

Surgery and radiotherapy

When the tumour was clinically judged to be resectable by the surgeons, radiologists and medical oncologists in each institution, surgical resection of the tumour and a total thymectomy were performed within 6 months (preferably within 3 months) after completion of chemotherapy. For completely resected tumours, post-operative thoracic radiotherapy up to 48 Gy/24 fractions was administered to the surgical margin and the mediastinum. For incompletely resected or unresected tumours, thoracic radiotherapy of up to 60 Gy/30 fractions was administered to the mediastinum and the residual tumour with 1.5-cm margins. The radiation dose per fraction, 2 Gy, and the total doses were determined by the study group in view of previous reports (Girard *et al*, 2009). The actual treatment delivery method was determined at each institution.

Thoracic radiotherapy was started with a linear accelerator ($\geqslant$4 MeV) within 6 months of surgery or, for those who did not undergo surgery, on completion of chemotherapy.

Patient evaluation and follow-up Before enrolment into the study, each patient underwent a complete medical history and physical examination (including neurological examination for signs of myasthenia gravis), blood cell count determinations, serum biochemistry testing, arterial blood gas analysis, pulmonary function test, electrocardiogram, chest X-ray, computed tomography (CT) scan of the chest, CT scan or ultrasound of the upper abdomen, whole-brain CT or magnetic resonance imaging and an isotope bone scan. Blood cell counts were determined, serum biochemistry testing was carried out and chest X-rays were taken weekly during each course of chemotherapy.

The toxicity of the chemotherapy was evaluated according to the Japan Clinical Oncology Group Toxicity Criteria (Tobinai *et al*, 1993), modified from the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 1. Tumour responses were assessed radiographically according to the standard, two-dimensional WHO criteria (Miller *et al*, 1981) and classified into complete response (CR), partial response (PR), no change (NC), progressive disease (PD) and non-evaluable. Response confirmation at 4 weeks or longer intervals was not required in the protocol. After completion of the protocol therapy, the patients were followed up with periodic re-evaluation, including chest CT every 6 months for the first 2 years and yearly thereafter.

Central review Radiographic reviews for eligibility of the enrolled patients and their clinical responses were carried out at the time of the study group meetings. The study coordinator (HK) and a few selected investigators reviewed the radiographic films. The clinical data presented below were all confirmed by this central review. Reviews of pathological specimens were not carried out, because the logistics of the study group were insufficient at the time of study activation in 1997.

End points and statistical considerations

Because of the rarity of the tumour and the accrual to the US trials (Loehrer *et al*, 1994, 1997), we presumed that we would be capable of accruing 30 patients in the target accrual period of 4 years. The sample size was, therefore, not based on statistical calculations. The expected 5-year PFS rate was 60%, which would give a 95% confidence interval of 40–77% with 30 cases.

Hence, the initial study design envisioned enrolment of 30 fully eligible cases over 4 years, with a follow-up period of 5 years.

The secondary end points included toxicity and safety, objective tumour response to chemotherapy, pattern of relapse, overall survival (OS) and complete resection rate.

The PFS and OS were calculated from the date of enrolment and estimated by the Kaplan–Meier method. Progression-free survival was censored at the date verifiable to be progression free, and OS was censored at the date of last follow-up. During the accrual period, an interim analysis for futility was planned after half of the patients were registered and followed up for at least 6 months. All analyses were performed using SAS software version 8.2/9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics

A total of 23 patients from eight institutions were enrolled from July 1997 to April 2005, when the study was terminated because of slow accrual. Two patients were ineligible because of wrong histology; one had thymic carcinoma, and the other had lymphoma. These mistakes occurred because of technical problems in the patient registry. As the ineligible cases did receive the protocol therapy, all 23 patients were analysed for characteristics and toxicity. In all, 21 eligible patients were analysed for clinical response, survival (PFS and OS) and surgical results. The patients' characteristics are shown in Table 1. Diagnostic procedure was CT-guided needle biopsy in most of the cases.

Reasons for surgical unresectability (one patient could have more than one reason) included invasion into the following: the pulmonary artery trunk in 10 cases, superior vena cava in 8, aorta in 6, extensive pericardium or myocardium in 4, and sternum in 1.

Chemotherapy delivery and toxicity

Thirteen patients (57%) received the planned 9 weeks of chemotherapy. The other 10 patients included 2 who received 8 weeks, 5 who received 7 weeks, 2 who received 6 weeks and 1 who received 1 week of therapy. Reasons for ceasing chemotherapy were patient refusal (six cases), attending doctors' decision for earlier local therapy (two cases), disease progression (one case) and ineligibility (one case). The median duration of chemotherapy for the 13 patients who underwent the planned 9 cycles was 9 weeks (range: 9–12 weeks). Among the nine patients who received 6–8 cycles, six received chemotherapy without delay and the remaining three received chemotherapy with a delay of 1–4 weeks.

Table 2 summarises the major toxicities of the chemotherapy. They were mainly haematological, and although about half of the patients experienced grade 4 neutropenia, it was generally transient and complicated by infection in only 1 case. Substantial anaemia was frequently observed, consistent with other reports of dose-dense CODE therapy (Fukuoka *et al*, 1997; Furuse *et al*, 1998). Overall, the toxicities were well tolerated. There were no deaths related to toxicity.

Table 1 Patients' characteristics

Item	Number
Sex (male/female)	17/6
Age, years (median/range)	56 (28–70)
ECOG performance status	
PS0/PS1/PS2	9/14/0
Smoking history	
No	13
Yes (median pack-years)	10 (28)
Myasthenia gravis (no/yes)	21/2
Histology: thymoma and eligible	21
Lymphocyte predominance	10
Mixed cell	4
Epithelioid cell	6
Spindle cell	1
Histology: not thymoma (ineligible)	2
Carcinoma	1
Lymphoma	1

Abbreviations: ECOG = Eastern Cooperative Oncology Group; PS = performance status.

Table 2 Toxicity of the chemotherapy ($N = 23$)

Toxicity	Grades 1/2	Grade 3	Grade 4	%Grade 3/4
Leukopenia	4/5	8	5	57
Neutropenia	1/6	3	11	61
Anaemia	0/3	19	ND	83
Thrombocytopenia	6/4	4	2	26
ALT	10/1	1	0	4
Creatinine	2/0	0	0	0
PaO_2	5/6	0	0	0
Emesis	10/8	3	ND	13
Diarrhea	3/3	1	0	4
Stomatitis	5/2	0	0	0
Constipation	2/1	0	0	0
Neuropathy	7/2	0	ND	0
Infection	5/2	3	0	13

Abbreviations: ALT = alanine aminotransaminase; ND = not defined (the Japan Clinical Oncology Group toxicity criteria did not define grade 4 in these toxicities).

Clinical response to induction therapy

The clinical responses of the 21 eligible patients to the chemotherapy were judged radiologically and confirmed by central review. The responses were as follows: CR, 0; PR, 13; NC, 7; and PD, 1. The overall response rate was 62% (95% confidence interval: 38–82%).

Surgical and pathological results

Of the 21 eligible patients, a thoracotomy was performed in 13 (62%). Thoracotomy was performed 26–73 days (median: 47 days) after completion of chemotherapy. The results of the surgery were as follows: probe thoracotomy, two cases; gross residual tumour (R2 resection), one case; microscopically residual tumour on pathological review (R1 resection), one case; and complete surgical and pathological resection (R0 resection), nine cases (43% of all eligible cases). A combined resection of the adjunct organs included pericardium in eight, lung parenchyma in eight, pleura

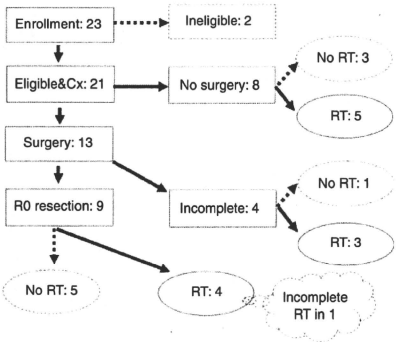

Figure 1 Study schema of the Japan Clinical Oncology Group (JCOG) 9606 trial, with the number of patients who actually received each of the protocol therapies. Cx, chemotherapy; RT, radiotherapy.

in seven, superior vena cava in two, brachiocephalic vein in two and others in five cases. Pathological CR (pCR), with no residual viable tumour cells in resected specimens, was achieved in three patients (14% of the 21 eligible patients).

The major post-operative morbidities included one case of pulmonary infarction, which subsequently recovered.

Boost radiotherapy

Post-operative radiotherapy was administered to 7 of the 13 patients who underwent thoracotomy: four of the nine patients with R0 resection received radiotherapy of 48, 48, 48 and 8 Gy, respectively; one of the two patients with incomplete resection received radiotherapy of 50 Gy; and each of the two patients with probe thoracotomy received radiotherapy of 60 Gy. Reasons for not carrying out radiotherapy included surgery-related complication or incomplete recovery (three cases), disease progression (two cases) and patient refusal (one case). Of the eight patients without thoracotomy, five received radiotherapy, with a dose of 60 Gy for each case. The other three patients refused radiotherapy.

The study schema with the actual numbers of patients receiving the protocol therapy is shown in Figure 1.

Other and late complications

Thirteen patients received thoracic radiotherapy. The toxicities were generally mild and manageable. There were four patients with grade 2 oesophagitis, one patient with a grade 3 skin reaction and another with a grade 2 skin reaction. All other adverse events were grade 0 or 1.

One patient was reported to have pure red cell aplasia, which occurred while receiving post-operative radiotherapy. Radiotherapy was terminated, and the patient recovered with immunosuppressant therapy.

Progression-free and overall survival

Survival data were last updated in May 2009, 4 years after accrual of the last patient. Figure 2 shows the PFS and OS curves for the 21 eligible cases. The median PFS was 4.5 years (95% confidence interval: 2.3 not calculable years), and the PFS at 2, 5 and 8 years was 80, 43 (95% confidence interval: 21–63%) and 32%,

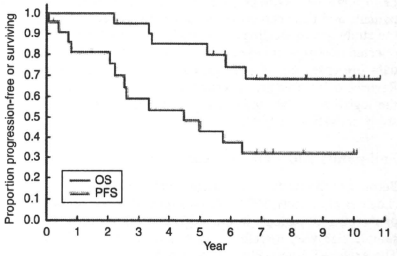

Figure 2 Progression-free survival (PFS) and overall survival (OS) of the 21 eligible patients.

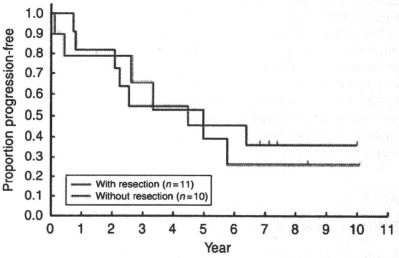

Figure 3 Progression-free survival of the 21 eligible patients, according to the surgery undergone. Resection was performed in 11 patients (complete resection in nine), and 10 patients did not undergo resection (including two with probe thoracotomy). There was no significant difference (log rank $P = 0.75$).

respectively. The median OS was not reached, and the OS at 2, 5 and 8 years was 100, 85 (95% confidence interval: 61–95%) and 69%, respectively.

Of the 21 eligible patients, 11 underwent surgical resection (nine complete resection and two incomplete resection), whereas 10 did not (including two who underwent a probe thoracotomy). The PFS and OS were quite similar for those with or without surgical resection. The 5- and 8-year PFS rates for those who underwent resection were 46 and 36% for those with surgical resection and 39 and 26% for those without, respectively (Figure 3). The 5- and 8-year OS rates were 91 and 73% for those with surgical resection and 79 and 63% for those without, respectively (Figure 4).

For the nine patients who underwent R0 resection, the outcomes were marginally better, with 5- and 8-year PFS rates of 56 and 44%, respectively, and 5- and 8-year OS rates of 89 and 78%, respectively. The case with R1 resection had relapse at 2.3 years, and the case with R2 resection had relapse at 0.7 year.

All three patients who achieved pCR were alive and disease free at 6.3–7.4 years of follow-up.

Pattern of relapse

So far, 13 of the 21 eligible patients have had tumour relapse. All of the 13 relapsed patients initially demonstrated regrowth of the primary and/or pleural or pericardial dissemination: primary only

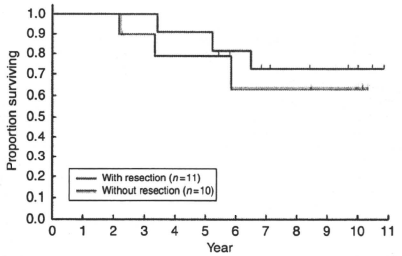

Figure 4 Overall survival of the 21 eligible patients, according to the surgery undergone. Resection was performed in 11 patients (complete resection in nine), and 10 patients did not undergo resection (including two with probe thoracotomy). There was no significant difference (log rank $P = 0.59$).

in five; pleura or pericardium only in six, and both in two2. None had initial relapse involving distant organs. There was no report of needle biopsy-track recurrence.

DISCUSSION

The optimal management of unresectable stage III thymoma remains unclear. There are some reports of combined modality approaches including chemotherapy and surgery, but many reports included stage IV disease and/or thymic carcinoma histology (Berruti *et al*, 1999; Kim *et al*, 2004; Lucchi *et al*, 2006; Yokoi *et al*, 2007). Reports of multicentre prospective trials are very few (Table 3).

In the current trial, we prospectively accrued patients with unresectable stage III thymoma, and excluded thymic carcinoma; it is now evident that thymoma and thymic carcinoma differ in clinical presentation and in prognosis, and trials on them should be reported separately (Eng *et al*, 2004; Giaccone, 2005).

We previously reported the results of another trial, JCOG 9605 (Kunitoh *et al*, 2009), in which we treated patients with stage IV thymoma with CODE chemotherapy. The results were similar to conventional chemotherapy, and we concluded that intensive chemotherapy does not seem to be promising enough in disseminated thymoma. However, dose-dense chemotherapy might still have a role in patients with limited tumour burden, in combination with definitive local therapy.

Although our results showed that CODE chemotherapy in combination with local therapy could be safely administered to thymoma patients, the efficacy was not remarkable. Compliance to chemotherapy was poorer; only 57% of patients completed the planned 9-week schedule, as compared with the 87% rate in the JCOG 9605 study for stage IV disease (Kunitoh *et al*, 2009). Doctors' and patients' decisions were the main reasons for ceasing chemotherapy and early local therapy. Therefore, although chemotherapy itself was well tolerated, toxicities such as malaise or fatigue, which the old JCOG toxicity criteria did not define, might have compromised the completion of chemotherapy before surgery.

Moreover, although the sample size was smaller than expected because of poor accrual, the 5-year PFS rate was 43% (95% confidence interval: 21–63%), which fell short of the expected 60%. Although the OS rate was favourable, it would be difficult to make a valid conclusion because of the small sample size (Table 3).

Table 3 Reports of prospective trials of combined modality therapy for locally advanced thymoma

Treatment	Stage	Patients[a]	ORR	5-yr OS
PAC, R (Loehrer *et al*, 1997)	III	23	70%	52.5%[b]
PAC, S, R (Kim *et al*, 2004)	III/IV	22	77%	95%[c]
CODE, S, R (current study)	III	21	62%	85%[d]

Abbreviations: CODE = combination chemotherapy with cisplatin/vincristine/doxorubicin/etoposide; ORR: overall response rate; PAC = combination chemotherapy with cisplatin/doxorubicin/cyclophosphamide; R = thoracic radiotherapy; S = surgical resection; 5-yr OS = overall survival rate at 5 years. [a]Number of assessable patients. [b]Including patients with thymic carcinoma. [c]7-year OS rate was 79%. [d]8-year OS rate was 69%.

In this study, we did show that about half of the patients with an initially unresectable thymoma were able to undergo complete resection after induction CODE chemotherapy. However, both PFS and OS were surprisingly similar for patients with and without complete resection.

Those who underwent complete resection got numerically better PFS and OS rates, but the difference with unresected cases was marginal, especially considering the selection bias. Only those who received pathological CR enjoyed clearly favourable outcomes. Low compliance to radiotherapy in patients with surgery could partly account for the unexpected results.

Complete resection has been reported to be associated with good prognosis in patients with stage III thymoma (Regnard *et al*, 1996; Girard *et al*, 2009). On the other hand, the role of 'debulking' surgery, in patients in whom complete resection is not feasible, remains unclear. Although some have suggested it to be beneficial (Liu *et al*, 2006), others reported that it could not affect the outcome as compared with biopsy only, followed by radiotherapy (Ciernik *et al*, 1994).

Taken together with our results, we believe that the role of surgery in locally advanced thymoma, as compared with definitive radiotherapy, still remains to be established, especially in combination with systemic chemotherapy. More studies are warranted.

One major limitation of the study is that we did not perform a central review of the histology, and thus could not provide WHO classifications of histology (Okumura *et al*, 2002; Travis *et al*, 2004). This makes comparisons with results from other reports difficult. Central pathology review and, preferably, tissue collection would be very important in future trials.

Now JCOG is discussing our next study on thymoma. As intensification of the current chemotherapy does not seem to be promising enough, our next approach would be trials with new agents, cytotoxic (such as amrubicin or irinotecan) or target based. More translational research of the tumour would be necessary, as well as international cooperation, given the rarity of the disease.

In conclusion, we found that weekly dose-dense chemotherapy could be administered safely to patients with thymoma, even when combined with local therapy in localised disease. However, the efficacy seemed to be no better than that of conventional chemotherapy. More research on the optimal systemic therapy, as well as on the role of surgery in locally advanced disease, seems to be necessary.

ACKNOWLEDGEMENTS

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Appendix 1

Study participants

The following institutions and investigators participated in the trial:

National Cancer Center Hospital East (Yutaka Nishiwaki, Kaoru Kubota, Nagahiro Saijo), National Cancer Center Hospital (Tomohide Tamura, Noboru Yamamoto, Hideo Kunitoh), Kanagawa Cancer Center (Kazumasa Noda, Fumihiro Oshita), Yokohama Municipal Citizen's Hospital (Koshiro Watanabe, Hiroaki Okamoto), Niigata Cancer Center Hospital (Akira Yokoyama, Yuko Tsukada), Kinki University Hospital (Kazuhiko Nakagawa, Isamu Okamoto), Osaka City General Hospital (Koji Takeda, Haruko Daga), and Kobe City Medical Center General Hospital (Nobuyuki Katakami, Hisashi Nishimura).

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ORIGINAL ARTICLE

Additive effects of drug transporter genetic polymorphisms on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients

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Abstract
Purpose Effects of genetic polymorphisms/variations of *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* in addition to "*UGT1A1*28* or **6*" on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients were investigated.
Methods Associations between transporter haplotypes/variations along with *UGT1A1*28* or **6* and SN-38 area under the time–concentration curve (AUC) or neutropenia were examined in irinotecan monotherapy (55 patients) and irinotecan–cisplatin-combination therapy (62 patients).
Results Higher SN-38 AUC values were observed in *ABCB1* 2677G>T (A893S) (*2 group) for both regimens. Associations of grade 3/4 neutropenia were observed with *ABCC2* −1774delG (**1A*), *ABCG2* 421C>A (Q141K) and IVS12 + 49G>T (**IIB*) and *SLCO1B1* 521T>C (V174A) (**15* · *17*) in the irinotecan monotherapy, while they were

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evident only in homozygotes of *ABCB1*2*, *ABCG2#IIB*, *SLCO1B1*15 · 17* in the cisplatin-combination therapy. With combinations of haplotypes/variations of two or more genes, neutropenia incidence increased, but their prediction power for grade 3/4 neutropenia is still unsatisfactory.

Conclusions Certain transporter genotypes additively increased irinotecan-induced neutropenia, but their clinical importance should be further elucidated.

Keywords Irinotecan · Transporter · Genetic polymorphism · Haplotype

Introduction

Irinotecan, an anticancer prodrug, is widely used for treating a broad range of carcinomas including colorectal and lung cancers. However, unexpected severe diarrhea and neutropenia are important clinical side effects from irinotecan treatment. The active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases [1], and is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (UGTs), such as UGT1A1, UGT1A7, and UGT1A9, to form an inactive metabolite, SN-38 glucuronide (SN-38G) [2–4]. Irinotecan is also inactivated by CYP3A4 to produce 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin (NPC) [5]. Irinotecan and its metabolites are excreted into the bile and urine via the action of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp/ABCB1), multiple resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2) [6]. Transport of SN-38 from the plasma into the liver is mediated by the organic anion transporting polypeptide C (OATP-C/SLCO1B1) [7]. Most of the previous pharmacogenetic studies on irinotecan have focused on *UGT1A1* polymorphisms and have shown clinical relevance of *UGT1A1*28*, a repeat polymorphism in the TATA box [−54_−39A(TA)$_6$TAA>A(TA)$_7$TAA or −40_−39ins TA], to severe toxicities [8–10]. Based on these findings, in 2005, the Food and Drug Administration (FDA) of the United States approved an amendment for the label of Camptosar (irinotecan HCl) (NDA 20-571/S-024/S-027/S-028) and the clinical use of a genetic diagnostic kit for the *28* allele. In parallel with this advance in the USA, clinical relevance to severe neutropenia of *UGT1A1*6* [211G>A (G71R)], another low-activity allele detected specifically in East-Asians, as well as *28* was demonstrated in several studies on Asian patients [11–14]. Accordingly, in June 2008, the Ministry of Health, Labor and Welfare of Japan approved changes to irinotecan labels (Campto and Topotecin) by adding a caution for the risk of severe toxicities in patients either homozygous or compound heterozygous for *UGT1A1*28* and *6 (*28/*28*, *6/*6*, *28/*6*) and the clinical use of a diagnostic kit for *UGT1A1*28* and *6*. Severe toxicities, however, are found in patients without *6/*6*, *28/*28*, and *28/*6*; therefore, other factors responsible for irinotecan toxicities should be identified.

Several clinical studies have suggested polymorphisms of the drug transporter genes, such as *ABCB1*, *ABCC2*, *ABCG2*, and *SLCO1B1*, might affect irinotecan pharmacokinetics (PK)/pharmacodynamics (PD) in Caucasian and Asian patients. However, the results obtained from different ethnic populations with various irinotencan regimens are still controversial, and the genetic markers examined also differ [13, 15–26]. We previously identified a number of haplotypes/variations of transporter genes, including *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* in Japanese [12, 26–29], but their clinical significance, either alone or in combination, in irinotecan therapy has not yet been examined.

This study aimed to identify the genetic polymorphisms/variations of *ABCB1*, *ABCC2*, *ABCG2*, and *SLCO1B1* which can affect irinotecan PK/PD in Japanese cancer patients. We carefully stratified the patients considering the irinotecan regimen (irinotecan monotherapy or combination therapy with cisplatin) and *UGT1A1* genotype (*UGT1A1 *6* or *28*), and examined additive effects of transporter haplotypes/variations on the area under the time–concentration curves (AUC) of the toxic metabolite SN-38 and on the risk of severe neutropenia.

Patients and methods

Patients

The patients used in this study were the same as those described in a previous paper [12], where details on the eligibility criteria for irinotecan therapy, patient profiles, and irinotecan regimens were described. In this study, 55 patients with irinotecan monotherapy (100 mg/m^2 weekly or 150 mg/m^2 biweekly) and 62 patients with combination therapy of irinotecan (60 mg/m^2 weekly or 70 mg/m^2 biweekly) and cisplatin (60 or 80 mg/m^2, respectively) were included. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants.

Analyses on genetic polymorphisms and PK/PD

Patients' data on genetic variations and haplotypes of *UGT1A1*, *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* were

previously obtained [12, 26–29]. Regarding *ABCG2*, combination haplotypes were newly defined using the previously reported haplotypes from three linkage disequilibrium (LD) blocks [28]. Patients' PK data on the area under the concentration–time curve (AUC) and toxicities were previously obtained [12].

Association analyses

Associations of transporter genotypes with AUC/dose values for irinotecan, SN-38 and SN-38G, absolute neutrophil count (ANC) nadir, and incidence of grade 3 diarrhea or grade 3/4 neutropenia were investigated. For SN-38 AUC/dose and neutropenia, the patients were stratified by the presence of *UGT1A1*6* or **28* (*UGT+*). Statistical significance (two-sided, $P < 0.1$) was determined by the Mann–Whitney (MW) test or Jonckheere–Terpstra (JT) test for AUC/dose, and by Fisher's exact test and chi-square test (for trend) for incidence of grade 3 and 4 toxicities, using Prism version 4.0 (GraphPad Prism Software Inc., San Diego, CA, USA) and StatXact version 6.0 (Cytel Inc., Cambridge, MA). Multiplicity adjustment was not applied to bivariate analysis, and contributions of the candidate genetic markers to SN-38 AUC/dose values and ANC nadir were further determined by multiple regression analysis after logarithmic transformation of the AUC/dose values and ANC nadir counts. The variables examined were age, sex, body surface area, history of smoking or drinking, performance status, serum biochemistry (GOT, ALP, creatinine) at baseline, the ANC at baseline (for neutropenia), and genetic markers including *UGT1A1*6* or **28* (*UGT+*) and the transporter haplotypes. The variables in the final models were selected by the forward and backward stepwise procedure at a significance level of 0.20 using JMP version 7.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

Definition of major transporter haplotypes and their selected markers

For screening transporter gene polymorphisms affecting irinotecan PK/PD, major haplotypes and their tagging single nucleotide polymorphisms (SNPs) from *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* were selected (Table 1) according to their frequencies (more than 5%) and/or from preliminary results obtained from all patients treated with irinotecan.

For *ABCB1* block 1[26], the haplotype group *BJL*, which consists of **1B* (having −1789G>A), **1J* (having −1789G>A and −371A>G) and **1L* (having −1789G>A and −145C>G), was selected because an association of the marker SNP −1789G>A with lower expression levels of P-gp has been reported [30]. *ABCB1* block 2 **2* was originally defined as haplotypes containing three SNPs, 1236C>T, 2677G>T (A893S) and 3435C>T [31]. Since the **9* haplotype with 1236C>T, 2677G>T (A893S) without 3435C>T [16] showed the same trend for PK/PD as **2* (data not shown), the current study classified the

Table 1 List of major transporter haplotypes and their markers analyzed for Japanese cancer patients

Gene	Haplotype	Tagging SNP	Abbreviation used in this paper	Haplotype frequency	
				Monotherapy ($N = 110$)[a]	With cisplatin ($N = 124$)[a]
ABCB1	*BJL*[b] (block 1)	−1789G>A		0.182	0.210
	**2* group[c] (block 2)	2677G>T(A893S)	*B*	0.382	0.379
	**10* group[d] (block 2)	2677G>A(A893T)		0.182	0.169
	**1b* (block 3)	IVS27-182G>T		0.200	0.169
ABCC2	**1A*	−1774delG	*C*	0.373	0.371
	**1C/G*	3972C>T(I1324I)		0.218	0.266
ABCG2	*#IIB* [**1a–*2–*1b*][e]	421C>A(Q141K), IVS12 + 49G>T	*G*	0.200	0.274
	#IIIC [**1b–*3–*1c*][e]	34G>A(V12M), IVS9-30A>T		0.164	0.097
SLCO1B1	**1b*	388A>G(N130D)		0.373	0.573
	**15 · 17*	521T>C(V174A)	*S*	0.191	0.153

[a] Number of chromosome

[b] *BJL* consists of **1B* (having −1789G>A), **1J* (having −1789G>A and −371A>G) and **1L* (having −1789G>A and −145C>G) previously defined [26]

[c] **2* Group includes **2*, **9*, **12* and **14* haplotypes previously defined [26]

[d] **10* Group includes **10* and **13* haplotypes previously defined [26]

[e] Combination of *ABCG2* haplotypes of three blocks [block (−1)–block 1–block 2] previously defined [28]

haplotypes with 2677G>T (A893S), **2*, **9*, **12* and **14* [26], as the **2* group (**2* in this paper). Similarly, the **10* group was classified as haplotypes with 2677G>A (A893T), i.e., **10* and **13*, since no differences in PK/PD parameters were observed between these haplotypes. The **4*, **6*, and **8* haplotypes in block 2 [16, 26] showed no significant effect in the current analysis (data not shown). The *ABCB1* block 3 **1b* haplotype containing IVS27-182G>T was selected because our previous study showed it was associated with an increased renal clearance of SN-38 [16].

Based on reports showing possible functional alterations of −1774delG [32] and 3972C>T (I1324I) [18, 24], *ABCC2* haplotypes containing those variations were classified as **1A* and "**1C* and **1G* (**1C/G*)", respectively, according to our previous definition: **1A*, −1774delG; **1C*, −24C>T and 3972C>T; **1G*, 3972C>T [27]. *ABCC2*2* [1246G>A (V417I)] and **1H* [2934G>A (S978S)] [27] showed no statistically significant effects (data not shown).

The *ABCG2* combinatorial haplotypes were newly defined as combinations of haplotypes across the three blocks [block (−1)–block 1–block 2] previously reported [28]. Major combinations in 177 patients were the wild type $^{\#}$*1A* (frequency = 0.291), $^{\#}$*IIB* [containing 421C>A (Q141K) and IVS12 + 49G>T] (0.251) and $^{\#}$*IIIC* [containing 34G>A (V12M) and IVS9-30A>T] (0.107). Note that $^{\#}$*IIB* and $^{\#}$*IIIC* are subgroups of block 1 **2* [421C>A (Q141K)] and block 1**3* [34G>A (V12M)], respectively [28].

The *SLCO1B1* haplotypes used were the major haplotypes **1b* [containing 388A>G (N130D) without 521T>C (V174A)] [33] and **15* · *17* [containing 521T>C (V174A)], the functional relevance of which has been reported [34].

Association of transporter genotypes with AUC values

Since we previously found that some PK parameters, including AUC/dose, Cmax/dose and $t_{1/2}$ for irinotecan and/or its metabolites, as well as incidence of grade 3/4 toxicities were affected by irinotecan regimen [12], the following analyses were conducted using the two groups of patients; i.e., those treated with irinotecan monotherapy (100–150 mg/m^2 for initial dosage) or by combination therapy with cisplatin (60–70 mg/m^2 for initial dose of irinotecan). Since SN-38 AUC levels were largely dependent on the *UGT1A1* genotype "**6* or **28*" [12], the associations of transporter genotypes with SN-38 AUC values were analyzed within the groups stratified by the marker *UGT1A1* "**6* or **28*" (*UGT+*); i.e., *UGT−/−*, *UGT+/−* and *UGT+/+*. Since the SN-38 AUC/dose level of one patient with haplotypes *ABCB1*2* [2677G>T (A893S)] and **14* [2677G>T (A893S) and 1345G>A (E448K)] showed an outlying value (indicated as "*a*" in Fig. 1), this patient was excluded from the statistical analysis. In this study, we preliminarily found that effect of each transporter genotype on irinotecan PK/PD was generally small. However, it was hypothesized that multiple transporter genotypes might act additively as described below. Accordingly, we adopted a statistical significance level of $P = 0.1$ (two-sided) to pick up candidate polymorphisms for further evaluation of their combined effects.

Figure 1 shows the association of transporter genotypes with SN-38 AUC values in the irinotecan monotherapy. In all patients (ALL), higher values of the SN-38 AUC/dose were observed in the *ABCB1*2/*2* [1.64-fold of *−/−*, $P = 0.095$ (MW test)] (Fig. 1b) and *ABCG2$^{\#}$IIB* [1.24-fold of *−/−*, $P = 0.078$ (MW test)] genotypes (Fig. 1e) and lower values were observed in the *ABCB1*1b* (block 3) [0.78-fold of *−/−*, $P = 0.008$ (MW test)] (Fig. 1c) genotype. In *UGT−/−* patients, an increase in SN-38 AUC/dose was observed in the *ABCB1 BJL* [1.22-fold of *−/−*, $P = 0.073$ (MW test)] (Fig. 1a) and *ABCG2$^{\#}$IIB* [1.21-fold of *−/−*, $P = 0.082$, (MW test)] genotypes (Fig. 1e). In *UGT* (*+/−* and *+/+*) patients, an increase in SN-38 AUC/dose in *SLCO1B1*15* · *17* (*S*) [1.59-fold of *−/−*, $P = 0.036$ (MW test)] was also observed (Fig. 1f). Multiple regression analysis for the SN-38 AUC/dose (logarithm-transformed values) in the irinotecan monotherapy revealed significant associations of *ABCB1*2/*2* (coefficient = 0.212 ± 0.075, $P = 0.007$), along with *UGT+/−* (0.113 ± 0.054, $P = 0.040$) and *UGT+/+* (0.225 ± 0.088, $P = 0.014$) in the final model [$R^2 = 0.226$, Intercept = 0.281 (log 10^{-3}h m^2/L), $N = 53$].

Regarding other compounds, *ABCB1*2/*2* also showed higher irinotecan AUC/dose (1.27-fold) [66.2 (48.2–82.4) [median (25th–75th percentiles)] for **2/*2* vs. 52.2 (40.6–61.9) for *−/−* and **2/−*; $P = 0.063$ (MW test)] and SN-38G AUC/dose (1.62-fold) [18.0 (14.6–27.7) for **2/*2* vs. 11.1 (7.7–14.2) for *−/−* and **2/−*; $P = 0.002$ (MW test)]. Conversely, lower irinotecan AUC/dose for *ABCB1*10/*10* (0.79-fold) [54.8 (44.4–65.7) for *−/−* vs. 43.3 (40.6–54.1) for **10/*10*; $P = 0.062$ (JT test)] was detected.

For the combination therapy with cisplatin, an increase of the SN-38 AUC/dose for *ABCB1*2/*2* (1.43-fold) in *non-UGT+/+* patients (*UGT−/−* and *UGT+/−*) ($N = 55$) [3.57 (2.72–4.19) for **2/*2* vs. 2.51 (1.99–3.28) for *−/−* and **2/−*; $P = 0.032$ (MW test)], and a decrease for *ABCB1*1b* (0.80-fold) in *UGT−/−* patients ($N = 35$) [2.03 (1.72–2.33) for **1b/−* and **1b/*1b* vs. 2.55 (2.02–3.31) for *−/−*; $P = 0.026$ (MW test)] were observed. Multivariate analysis, however, showed no significant contributions of these transporter haplotypes to the SN-38 AUC/dose values.