of the ToGA study, we performed subgroup analyses using data from patients who were enrolled from institutions in Japan.

Preplanned sample size for Japanese patients

In the ToGA study, the HR for OS was expected to be 0.77, the expected number of events was 460, and the target sample size was set at 584 patients [6]. Before starting the ToGA study, we set the sample size of Japanese patients to allow us to evaluate similarities between the overall ToGA results and our subgroup analysis in an exploratory manner. Assuming a 70% probability that the HR for OS in the Japanese subgroup would be less than 0.88 (the midpoint between 0.77 and 1.00), the expected number of events was 70. To reach this expected number of events within the study period, the minimum sample size was determined to be 89 patients to allow us to conduct four analyses: preplanned (unadjusted and adjusted), post hoc, and exploratory analyses of the HR.

Unadjusted analyses

We calculated the unadjusted OS and progression-free survival (PFS) of the Japanese sub-group using the same methods as those used for the overall ToGA study [6]. Objective response rate of the Japanese sub-group was analysed with a χ^2 test in patients with measurable disease ($n = 45$ in the trastuzumab plus XP arm and 41 in the XP arm).

Preplanned analyses

Prior to carrying out the Japanese subgroup analysis, we predicted an imbalance in the baseline patient characteristics. Therefore, we planned to calculate an adjusted HR and 95% CI in the Japanese subgroup using a multivariate Cox regression analysis with 15 factors: extent of disease, primary tumor site, measurability of disease, Eastern Cooperative Oncology Group Performance Status (ECOG PS), chemotherapy regimen (stratification factors), sex, age, number of lesions, number of metastatic sites, type of gastric cancer, visceral metastasis, prior gastrectomy, prior chemotherapy, HER2 status, and region of origin (other prespecified covariates). All factors were prespecified in the ToGA study protocol. Each covariate was also evaluated using a univariate Cox regression analysis.

Post hoc analyses

During the preplanned multivariate Cox regression analysis, we excluded patients for whom HER2 status was reported as IHC 3+/FISH unknown (no result). In addition, estimates of effects were extremely unstable for covariates that contained a category which included only one patient. Therefore, to target all of the enrolled patients and ensure the stability of the model, a post hoc analysis was conducted using a multivariate Cox analysis. Among covariates, HER2 status was divided into two categories: high expression (IHC 2+ and FISH-positive or IHC 3+) and low expression (IHC0 and FISH-positive or IHC 1+ and FISH-positive). Covariates that contained a category with only one patient (extent of disease and previous chemotherapy) were excluded from the model to ensure its stability.

Exploratory analyses to evaluate deviation of patient prognosis

To identify factors that affect prognosis specifically in the Japanese subgroup, and to confirm the robustness of our preplanned and post hoc analyses, an exploratory multivariate Cox regression analysis on the HR for OS with various combinations of covariates was carried out. We created a series of models that included the treatment group as a base covariate with 3–6 other covariates, and selected the top four models ranked by value following a chi-square test. The procedure was repeated for the models with three, four, five, and six covariates, and a total of 16 models were selected. From the well-fitting model that was obtained, we compared the HR for OS with the results of preplanned and post hoc analyses. To ensure that HER2 status was not a confounding variable, we carried out a multivariate Cox regression analysis with HER2 expression (high or low) as the stratification factor, and determined the HR for OS in which selected covariates were included in the model.

Furthermore, scoring of the prognosis of each patient in both study arms using the Cox regression model and estimation of the risk for each patient were carried out with the selected covariates. The risk was shown by the estimated value of logarithm HR for each patient. To eliminate the influence of treatment on the mortality risk, we set the treatment group as the stratification factor and produced a histogram plot according to the distribution of patient risk to evaluate potential bias between the treatment arms.

Safety

Adverse events and serious adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0 and the International Conference on Harmonization guidelines, respectively.

Results

Patients

Between September 2005 and December 2008, 594 patients were enrolled in the primary ToGA study at 122

Table 1 HER2 testing results in the Japanese population of ToGA

FISH result	IHC score				
	IHC 0	IHC 1+	IHC 2+	IHC 3+	Total
FISH-positive, *n*	14	19	36	37	106
FISH-negative, *n*	155	57	14	1	227
NE, *n*	48	12	8	8	83
Total, *n*	217	88	58	46	409

FISH fluorescence in situ hybridization, *HER2* human epidermal growth factor receptor 2, *IHC* immunohistochemistry, *NE* not evaluable

centers in 24 countries, of whom 584 were included in the primary analysis. Four hundred twenty-one tumor samples were provided for HER2 testing from 16 centers in Japan. Twelve samples were not evaluated due to a lack of tumor tissue in the sample ($n = 7$), shipment failure ($n = 4$), or disease progression before shipment ($n = 1$). Of the 409 samples successfully screened, 115 (28.1%) were scored as HER2-positive (IHC 3+ or FISH-positive; Table 1) and 102 patients were registered into the study. After excluding one patient who did not receive the study drug, 101 Japanese patients (trastuzumab plus chemotherapy, $n = 51$; chemotherapy alone, $n = 50$) were included in this subgroup analysis. All patients received capecitabine as the chemotherapy partner of cisplatin.

Table 2 shows the baseline characteristics of the Japanese patients included in this subgroup analysis ($n = 101$) and the non-Japanese patients ($n = 483$). There is similarity in the baseline characteristics of patients from other countries between the study arms. On the other hand, number of metastatic sites, histologic type, and prior gastrectomy were imbalanced by approximately 10% between the study arms in the Japanese subgroup, and were considered to be prognostic factors. Median follow-up times were 18.6 months [interquartile range (IQR) 11–25] in the trastuzumab plus XP arm and 17.1 months (IQR 1–49) in the XP arm. The median number of cycles of trastuzumab therapy was eight (range 1–24). Forty-one patients in the trastuzumab plus XP arm (80.4%) and 41 patients in the XP arm (82.0%) received second-line treatment (at least one chemotherapy treatment after disease progression despite the study treatments).

Efficacy

Unadjusted analyses

Twenty-eight patients (54.9%) in the trastuzumab plus XP arm and 27 patients (54.0%) in the XP arm had died by the data cutoff point. As shown in Table 3, unadjusted median OS was 15.9 months (95% CI 12–25 months) in the trastuzumab plus XP arm and 17.7 months (95% CI 12–24 months) in the XP arm (HR 1.00, 95% CI 0.59–1.69). The number of PFS events (defined as disease progression or death) was 43 (84.3%) in the trastuzumab plus XP arm and 40 (80.0%) in the XP arm. Unadjusted median PFS was 6.2 months (95% CI 5–7 months) in the trastuzumab plus XP arm and 5.6 months (95% CI 5–7 months) in the XP arm (HR 0.92, 95% CI 0.60–1.43). The objective response rate was 64.4% (95% CI 48.8–78.1%) in the trastuzumab plus XP arm and 58.5% (95% CI 42.1–73.7%) in the XP arm.

Preplanned analyses

In the multivariate analysis, the HR for OS, adjusted by the 15 prespecified covariates above, was 0.68 (95% CI 0.36–1.27, $P = 0.2251$, Table 4). The adjusted HR for PFS was 0.66 (95% CI 0.40–1.09%), which was slightly improved compared with the results for the overall population. Among the covariates in the preplanned analysis, the univariate analysis showed that prior gastrectomy was the covariate most strongly associated with longer OS (HR 0.39, 95% CI 0.16–0.91). There were more patients with prior gastrectomy in the XP arm (26%) than in the trastuzumab arm (16%). After adjusting for gastrectomy only, the HR for OS between the treatment arms was 0.85 (95% CI 0.49–1.45).

Post hoc analyses

For the post hoc exploratory multivariate Cox regression analysis, the adjusted HRs for OS and PFS were 0.82 (95% CI 0.45–1.50) and 0.81 (95% CI 0.50–1.30), respectively (Fig. 1).

Exploratory analyses to evaluate deviation of patient prognosis

We evaluated the HR for OS with different combinations of covariates in the model. In the well-fitting models with high chi-square values, the HRs using three, four, five, and six covariates ranged between 0.79 (95% CI 0.49–1.38) and 0.89 (95% CI 0.52–1.54), 0.77 (95% CI 0.44–1.33) and 0.88 (95% CI 0.51–1.53), 0.68 (95% CI 0.39–1.20) and 0.80 (95% CI 0.45–1.42), and 0.68 (95% CI 0.38–1.20) and 0.76 (95% CI 0.44–1.33), respectively. In choosing the well-fitting models, the covariates sex, HER2 status, type of gastric cancer, prior gastrectomy, prior chemotherapy, and number of lesions tended to be chosen. The sets of covariates were similar to those used as prespecified covariates (15 factors). A similar analysis was carried out

Table 2 Baseline patient characteristics of the Japanese population and the non-Japanese population of ToGA

Characteristic	Japanese		Non-Japanese	
	Trastuzumab plus XP ($n = 51$)	XP/FP ($n = 50$)	Trastuzumab plus XP ($n = 243$)	XP/FP ($n = 240$)
Sex				
Male, *n*	40 (78.4%)	40 (80.0%)	186 (76.5%)	178 (74.2%)
Median age, years (range)	63.0 (29–76)	63.5 (45–81)	60.0 (23–83)	59.0 (21–82)
Extent of disease				
Locally advanced, *n*	0 (0.0%)	1 (2.0%)	10 (4.1%)	9 (3.8%)
Metastatic, *n*	51 (100.0%)	49 (98.0%)	233 (95.9%)	231 (96.3%)
Primary tumor site				
Stomach, *n*	49 (96.1%)	44 (88.0%)	187 (77.0%)	198 (82.5%)
Gastroesophageal junction, *n*	2 (3.9%)	6 (12.0%)	56 (23.0%)	42 (17.5%)
Measurability of disease				
Measurable, *n*	45 (88.2%)	41 (82.0%)	224 (92.2%)	216 (90.0%)
Nonmeasurable, *n*	6 (11.8%)	9 (18.0%)	19 (7.8%)	24 (10%)
ECOG performance status				
0–1, *n*	51 (100.0%)	50 (100.0%)	213 (87.7%)	213 (88.7%)
2, *n*	0 (0.0%)	0 (0.0%)	30 (12.3%)	27 (11.3%)
Chemotherapy regimen				
XP, *n*	51 (100%)	50 (100%)	205 (84.4%)	205 (85.4%)
FP, *n*	0 (0.0%)	0 (0.0%)	38 (15.6%)	35 (14.6%)
Number of lesions			($n = 242$)	
1–4, *n*	16 (31.4%)	18 (36.0%)	112 (46.3%)	98 (40.8%)
>4, *n*	35 (68.6%)	32 (64.0%)	130 (53.7%)	142 (59.2%)
Median value (range)	6 (1–15)	6 (1–15)	5 (1–20)	5 (1–16)
Number of metastatic sites			($n = 242$)	
1–2, *n*	28 (54.9%)	32 (64.0%)	124 (51.2%)	114 (47.5%)
>2, *n*	23 (45.1%)	18 (36.0%)	118 (48.8%)	126 (52.5%)
Median value (range)	2 (1–5)	2 (1–5)	2 (1–7)	3 (1–8)
Type of gastric cancer (central review)[a]			($n = 242$)	($n = 237$)
Intestinal type, *n*	37 (72.5%)	42 (84.0%)	188 (77.7%)	171 (72.2%)
Diffuse type, *n*	5 (9.8%)	4 (8.0%)	21 (8.7%)	21 (8.9%)
Mixed type, *n*	9 (17.6%)	4 (8.0%)	33 (13.6%)	45 (19.0%)
Visceral metastasis (liver or lung)				
Yes, *n*	35 (68.6%)	33 (66.0%)	134 (55.1%)	139 (57.9%)
No, *n*	16 (31.4%)	17 (34.0%)	109 (44.9%)	101 (42.1%)
History of treatment for gastric cancer				
Prior gastrectomy, *n*	8 (15.7%)	13 (26.0%)	62 (25.5%)	49 (20.4%)
Prior chemotherapy, *n*	1 (2.0%)	0 (0.0%)	26 (10.7%)	12 (5.0%)
HER2 status				
IHC 0/FISH-positive, *n*	3 (5.9%)	9 (18.0%)	20 (8.2%)	29 (12.2%)
IHC 1+/FISH-positive, *n*	10 (19.6%)	7 (14.0%)	28 (11.5%)	25 (10.4%)
IHC 2+/FISH-positive, *n*	18 (35.3%)	13 (26.0%)	62 (25.5%)	66 (27.5%)
IHC 3+/FISH-positive, *n*	16 (31.4%)	17 (34.0%)	115 (47.3%)	108 (45.0%)
IHC 3+/FISH-negative, *n*	1 (2.0%)	0 (0.0%)	8 (3.3%)	6 (2.5%)
IHC unknown/FISH-positive, *n*	0 (0.0%)	0 (0.0%)	5 (2.1%)	2 (0.8%)
IHC 3+/FISH unknown, *n*	3 (5.9%)	4 (8.0%)	5 (2.1%)	4 (1.7%)
Region of origin				
Japanese, *n*	51 (100%)	50 (100%)	0 (0.0%)	0 (0.0%)
Non-Japanese, *n*	0 (0.0%)	0 (0.0%)	243 (100%)	240 (100%)

ECOG Eastern Cooperative Oncology Group, *FISH* fluorescence in situ hybridization, *HER2* human epidermal growth factor receptor 2, *IHC* immunohistochemistry, *XP* capecitabine plus cisplatin

[a] Type of gastric cancer was described by the Lauren Classification

using HER2 expression (high or low) as the stratification factor. The HR was approximately 0.7, and the HRs using three, four, five, and six covariates were between 0.67 (95% CI 0.38–1.18) and 0.79 (95% CI 0.46–1.39), 0.70 (95% CI 0.40–1.24) and 0.82 (95% CI 0.47–1.42), 0.68 (95% CI 0.39–1.22) and 0.76 (95% CI 0.43–1.34), and 0.67 (95% CI 0.37–1.22) and 0.78 (95% CI 0.44–1.36), respectively. Influential covariates chosen in the well-fitting models included sex, prior gastrectomy, and number of lesions. Table 5 shows the covariate combinations that resulted in a good fit based on these analyses. Figure 2 shows the distribution of patient risk with these three models. The risk distribution is broad in each arm; however, the XP arm shows a somewhat greater distribution toward the left, indicating that this arm included a greater number of patients with better prognosis.

Table 3 Overall survival in the Japanese population of ToGA (unadjusted Cox regression analysis)

	Trastuzumab plus XP ($n = 51$)	XP ($n = 50$)
Number of events (%)	28 (54.9)	27 (54)
Median OS, months (95% CI)	15.9 (12–25)	17.7 (12–24)
Survival rate (%)		
6 months	92	92
12 months	68	64
18 months	48	49
24 months	41	35
Hazard ratio (95% CI)	1.00 (0.59–1.69)	

CI confidence interval, *OS* overall survival, *XP* capecitabine plus cisplatin

Safety

Table 6 shows the adverse events in the Japanese population of ToGA, and indicates that all patients experienced at least one adverse event in each arm. Grade 3/4 adverse events occurred in 43 patients (84%) in the trastuzumab

Table 4 Preplanned multivariate Cox regression analysis of overall survival by extent of disease, primary tumor site, measurability of disease, ECOG status, chemotherapy regimen, and other prespecified covariates: sex, age, number of lesions, number of metastatic sites, type of gastric cancer, visceral metastasis, prior gastrectomy, prior chemotherapy, HER2 status, and region of origin

	Hazard ratio (95% CI)		*P* value
Trastuzumab plus XP versus XP	0.68	(0.36–1.27)	0.2251
Sex (male vs. female)	0.16	(0.07–0.41)	<0.0001
Age (<60 vs. ≥60)	1.07	(0.54–2.13)	0.8382
Extent of disease (locally advanced vs. metastatic)	0.00	(0.00–.)	0.9902
Primary tumor site (stomach vs. gastroesophageal junction)	0.68	(0.25–1.87)	0.4559
Measurability of disease (measurable vs. nonmeasurable)	0.95	(0.29–3.05)	0.9268
ECOG performance status	–	–	–
Chemotherapy regimen	–	–	–
Number of lesions (1–4 vs. >4)	0.49	(0.22–1.09)	0.0818
Number of metastatic sites (1–2 vs. >2)	0.79	(0.41–1.50)	0.4695
Type of gastric cancer			
Diffuse type versus intestinal type	3.24	(1.08–9.70)	0.0356
Mixed type versus intestinal type	0.91	(0.30–2.71)	0.8644
Visceral metastasis (yes vs. no)	1.15	(0.48–2.74)	0.7510
Prior gastrectomy (yes vs. no)	0.22	(0.06–0.75)	0.0159
Prior chemotherapy (yes vs. no)	27.72	(1.11–694.38)	0.0432
HER2 status			
IHC 0/FISH-positive versus IHC 3+/FISH-positive	5.31	(1.29–21.86)	0.0208
IHC 1+/FISH-positive versus IHC 3+/FISH-positive	4.87	(1.73–13.70)	0.0027
IHC 2+/FISH-positive versus IHC 3+/FISH-positive	1.53	(0.73–3.18)	0.2578
IHC 3+/FISH-negative versus IHC 3+/FISH-positive	25.66	(1.72–382.49)	0.0186
Region of origin	–	–	–

Among 15 prespecified factors, chemotherapy regimen, performance status, and region of origin were not calculated in this table because all Japanese patients received capecitabine as the chemotherapy partner of cisplatin, had Karnofsky performance status of 0–1, and were from Asia (Japan)

CI confidence interval, *ECOG* Eastern Cooperative Oncology Group, *FISH* fluorescence in situ hybridization, *HER2* human epidermal growth factor receptor 2, *IHC* immunohistochemistry, *XP* capecitabine plus cisplatin

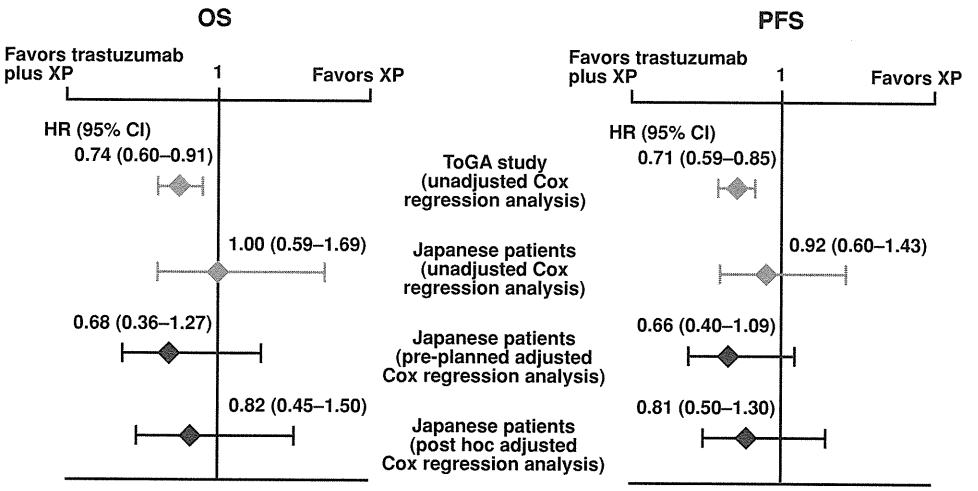

Fig. 1 Unadjusted and adjusted hazard ratios for overall and progression-free survival. *CI* confidence interval, *HR* hazard ratio, *OS* overall survival, *PFS* progression-free survival, *XP* capecitabine plus cisplatin

Table 5 Covariates included in the model

Number of covariates	Covariates included in the model
4	HER2 expression (low/high), sex (male/female), prior gastrectomy (yes/no), number of lesions (1–4/>4)
5	HER2 expression (low/high), sex (male/female), prior gastrectomy (yes/no), number of lesions (1–4/>4), type of gastric cancer (diffuse/intestinal)
6	HER2 expression (low/high), sex (male/female), prior gastrectomy (yes/no), number of lesions (1–4/>4), type of gastric cancer (diffuse/intestinal), number of metastatic sites (1–2/>2)

HER2 human epidermal growth factor receptor 2

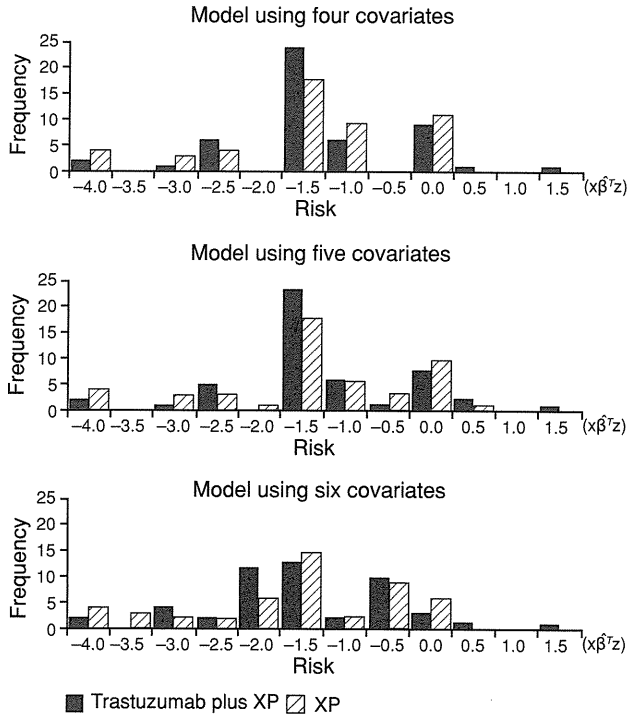

Fig. 2 Distribution of estimated values by linear predictor. *XP* capecitabine plus cisplatin. The ordinate is the number of patients and the abscissa is the risk score (estimated hazard number for each patient). The risk of mortality increases as the plot moves to the right

plus XP arm and 36 patients (72%) in the XP arm. Treatment was discontinued due to adverse events for one patient (2%) in the trastuzumab plus XP arm and four patients (8%) in the XP arm. Deaths due to adverse events occurred in two patients in the trastuzumab plus XP arm: one due to cardiac failure and unstable angina and the other due to gastrointestinal perforation. The case of cardiac failure and unstable angina was attributed to an adverse event likely related to trastuzumab.

Discussion

In the original ToGA study, patients with HER2-positive advanced gastric or GEJ cancer who received the combination treatment of trastuzumab plus XP/FP had significantly longer OS and PFS than patients who received XP/FP alone [6]. No differences in OS or PFS were detected between the two treatment arms in this subgroup analysis of Japanese patients when unadjusted data were analyzed. However, in preplanned and post hoc analyses, the HRs were 0.68 and 0.82 for OS and 0.66 and 0.82 for PFS, respectively, after adjusting for baseline characteristics. These values were similar to the overall ToGA study results. Taken together, these results strongly suggest that

Table 6 Adverse events in ≥10% of Japanese patients in ToGA

	Trastuzumab plus XP ($n = 51$)		XP ($n = 50$)	
	All grade n (%)	Grade ≥3 n (%)	All grade n (%)	Grade ≥3 n (%)
Total	51 (100)	43 (84)	50 (100)	36 (72)
Gastrointestinal disorders				
Nausea	44 (86)	7 (14)	44 (88)	7 (14)
Vomiting	33 (65)	1 (2)	28 (56)	2 (4)
Constipation	24 (47)	1 (2)	24 (48)	–
Diarrhoea	23 (45)	4 (8)	24 (48)	2 (4)
Stomatitis	29 (57)	–	16 (32)	1 (2)
Blood and lymphatic system disorders				
Neutropenia	30 (59)	18 (35)	34 (68)	20 (40)
Thrombocytopenia	11 (22)	1 (2)	8 (16)	3 (6)
Anemia	15 (29)	13 (25)	11 (22)	8 (16)
Febrile neutropenia	5 (10)	5 (10)	3 (6)	3 (6)
Skin and subcutaneous tissue disorders				
Palmar–plantar erythrodysaesthesia syndrome	21 (41)	–	23 (46)	1 (2)
Alopecia	12 (24)	–	9 (18)	–
Skin hyperpigmentation	6 (12)	–	5 (10)	–
Rash	10 (20)	–	5 (10)	–
Pigmentation disorder	10 (20)	–	7 (14)	–
Nail disorder	5 (10)	–	5 (10)	–
Metabolism and nutrition disorders				
Anorexia	43 (84)	12 (24)	46 (92)	10 (20)
Dehydration	3 (6)	1 (2)	6 (12)	1 (2)
General disorders and administration site conditions				
Fatigue	31 (61)	4 (8)	26 (52)	4 (8)
Pyrexia	19 (37)	1 (2)	12 (24)	–
Chill	7 (14)	–	0 (0)	–
Edema	19 (37)	–	23 (46)	–
Nervous system disorders				
Peripheral neuropathy	16 (31)	1 (2)	10 (20)	–
Dysgeusia	13 (25)	–	8 (16)	–
Peripheral sensory neuropathy	2 (4)	–	11 (22)	–
Dizziness	5 (10)	1 (2)	5 (10)	–
Respiratory, thoracic, and mediastinal disorders				
Hiccups	21 (41)	–	16 (32)	–
Epistaxis	5 (10)	–	3 (6)	–
Renal and urinary disorders				
Renal impairment	32 (63)	2 (4)	27 (54)	–
Vascular disorders				
Hypertension	4 (8)	1 (2)	3 (6)	–
Investigations				
Weight decreased	27 (53)	2 (4)	13 (26)	1 (2)
Weight increased	10 (20)	1 (2)	9 (18)	–
Psychiatric disorders				
Insomnia	11 (22)	–	8 (16)	–
Infections and infestations				
Nasopharyngitis	18 (35)	–	6 (12)	–
Musculoskeletal and connective tissue disorders				
Back pain	5 (10)	–	1 (2)	–

XP capecitabine plus cisplatin

the same benefit of adding trastuzumab to chemotherapy was obtained in the Japanese patient subgroup as in the overall population.

In our subgroup analysis, the change in HR pre- and post-adjustment may have been due to an uneven distribution of prognostic factors between the two treatment arms. The XP arm included more patients with factors generally considered to be associated with a good prognosis (history of gastrectomy [14, 15], intestinal type cancer [16–19], and metastasis in fewer than two organs [19]). In the overall ToGA study and in the Japanese subgroup, gastric resection was shown to be the most influential factor affecting prognosis, as assessed by univariate Cox regression analyses (HRs of gastrectomy were 0.54 and 0.39, respectively). In the Japanese subgroup, the number of patients who had undergone gastric resection in the XP arm ($n = 13$, 26.0%) was approximately 10% higher than that of the trastuzumab plus XP arm ($n = 8$, 15.7%).

When multiple factors influence prognosis, different combinations of factors could affect the HR between two treatment groups. Therefore, to confirm that the HR is robust, it is necessary to analyze different combinations of factors. In this regard, we found that the HRs for OS were approximately 0.7 for all combinations of factors, thus supporting the robustness of our results.

Median OS in the XP/FP alone arm was 11.1 months (95% CI 10–13) in the overall ToGA population [6], but was approximately 6.5 months longer in the Japanese subgroup (XP arm: 17.7 months). These findings are consistent with results of recent trials reporting longer survival for patients with gastric cancer in Japan than for patients in Europe and the USA. One possible reason for this difference is that more Japanese patients receive second-line or later treatment after the failure of first-line treatment [11–13]. In the ToGA study, more than 80% of Japanese patients in both treatment arms underwent second-line or further treatment, which was considerably higher than the overall rates of second-line treatment in the overall ToGA population (42% of patients in the trastuzumab plus XP/FP arm and 45% in the XP/FP arm) [6]. In the present study of Japanese patients, the OS of patients who received XP only was similar to that reported in other recent Japanese trials [2, 7, 8]. Furthermore, after adjusting for imbalances between the baseline characteristics of treatment arms, we detected an additive effect of trastuzumab among Japanese patients, similar to that of the overall population. By further exploratory analyses, we confirmed that the HRs in favor of trastuzumab were consistently observed after adjusting for prognostic factors. These findings strongly suggest that the benefits of trastuzumab were of the same magnitude in Japanese patients as in the whole study population, although the absolute length of survival was longer in the Japanese subgroup. In conclusion, trastuzumab in combination with XP can be considered a new standard therapy for Japanese patients with HER2-positive advanced gastric or GEJ cancer.

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Phase I study of dasatinib (BMS-354825) in Japanese patients with solid tumors

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Dasatinib is a potent oral inhibitor of tyrosine kinases including the SRC family kinases, which are activated in tumors, and implicated in invasion and bone metastasis. This phase I dose-escalation study assessed safety, tolerability, maximum tolerated dose (MTD), antitumor activity, pharmacokinetics and pharmacodynamics in Japanese patients with refractory, advanced solid tumors. Dasatinib was administered once daily at 100, 150 and 200 mg/day. Sixteen patients were treated with dasatinib in the following doses: 100 mg (nine patients), 150 mg (three patients) and 200 mg (four patients). The most frequent adverse events (AE; ≥50%) were anorexia, fatigue, pleural effusion, anemia, constipation, diarrhea, vomiting and increased aspartate aminotransferase (AST). The most frequent AE of grade ≥3 (≥10%) were anemia, decreased lymphocyte count, fatigue and increased blood magnesium. Dose-limiting toxicities were observed in two patients: grade 2 pleural effusion and bronchial wall thickening at the 100-mg level and grade 3 dyspnea at the 200-mg level. In addition, grade 2 pleural effusion was observed in all four patients treated with 200 mg. Therefore, 150 mg was determined to be the MTD. The pharmacokinetic parameters were comparable among the dose levels. As a pharmacodynamic study, markers of bone metabolism were assessed. Bone resorption markers, NTx and TRACP-5b, showed a decrease of 46.3% and 22.2%, respectively. No objective responses were observed, but three patients had stable disease that lasted for over 6 months. In this study population, the safety profile of dasatinib was generally acceptable and 150 mg of dasatinib administered once daily was determined to be the MTD. (*Cancer Sci* 2011; 102: 2058–2064)

SRC family kinases (SFK) such as SRC, YES, LCK and FYN are non-receptor tyrosine kinases that have important roles in cell proliferation, motility, adhesion and survival.[1,2] SRC family kinases regulate signals from membrane-associated growth factor receptors such as epidermal growth factor receptor (EGFR), insulin-like growth factor-1 receptor (IGF-1R) and vascular endothelial growth factor receptor (VEGFR).[3] SRC was the first proto-oncogene identified and is upregulated in various tumors[1] through epigenetic processes. SRC and other SFK are associated with epidermal-to-mesenchymal transformation (EMT), VEGF overexpression, a propensity for metastases and shorter survival. SRC is also essential for osteoclast function,[4,5] and SRC overexpression accelerates bone metastases.[6]

Dasatinib (BMS) is a multi-target tyrosine kinase inhibitor that inhibits LCK, SRC, YES, BCR-ABL, KIT and platelet-derived growth factor receptor (PDGFR) *in vitro* with IC_{50} of 0.40, 0.50, 0.50, <1.0, 5.0 and 28, respectively.[7] Dasatinib showed antitumor efficacy for several types of solid cancer both *in vitro* and *in vivo*.[8,9] With better inhibitory activity for abl kinase than imatinib, dasatinib has proven clinical efficacy in patients with imatinib-resistant chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL),[10] and has been approved in many countries including Japan.

In the present study, we conducted a phase I study of dasatinib in Japanese patients with advanced solid tumors that were refractory to standard therapies or for which no effective standard therapy existed. The primary objective of the present study was to establish the maximum tolerated dose (MTD) of dasatinib with once daily administration.

Materials and Methods

Patients and eligibility criteria. This was a multi-center, open-label, phase I, dose-escalation study to assess the safety, tolerability, pharmacokinetics, pharmacodynamics and preliminary efficacy of dasatinib in patients with refractory solid tumors. The study was conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice, and in accordance with the ethical principles that are in the current Declaration of Helsinki, and was approved by the institutional review board at each of the participating institutions. All patients provided written informed consent.

Eligible patients were aged 20 years or older with an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2, and had a solid cancer, verified cytologically or histologically, that was refractory to conventional therapy, or for which there was no established therapy. Patients were eligible if they had adequate bone marrow function (neutrophil count $\geq 2000/mm^3$, platelets $\geq 125\,000/mm^3$, hemoglobin ≥9.0 g/dL), liver function (total bilirubin, aspartate aminotransferase [AST], alanine aminotransferase [ALT] ≤ two times the upper limit of normal), renal function (creatinine ≤1.5 times the upper limit of normal) and serum potassium, magnesium and corrected calcium within the range of grade 0–1 of the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v3.0. Patients should not have received chemotherapy, immunotherapy or radiotherapy within 4 weeks before the start of therapy (6 weeks if nitrosourea or mitomycin C and 2 weeks if endocrine therapy).

Patients were excluded if they had symptomatic brain metastasis, pleural effusion, prolonged QT syndrome or QTc prolongation of more than 450 ms, grade II or III atrioventricular block, heart rate of <50 b.p.m., uncontrolled hypertension, history of a significant bleeding disorder, vasculitis, gastrointestinal bleeding within 6 months, recent ischemic heart disease or drug allergy. Patients who were pregnant or breastfeeding or those who were of childbearing potential but unwilling or unable to use adequate contraception were also excluded. Prohibited medications included those known to increase the risk of Torsades

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de Pointes, irreversible inhibitors of platelet function and drugs that increase intra-gastric pH.

Drug administration. Dasatinib was administered orally once daily after breakfast. The initial dose was 100 mg, increased by increments of 50 mg up to a maximum dose of 250 mg. Treatment for 4 weeks (28 days) was defined as one treatment course. Initially, three patients were treated at a given dose level. If no dose-limiting toxicity (DLT) was observed, the dose would be escalated to the next higher level. If a DLT was observed in one of the three patients treated during the first course at that particular dose level, three additional patients would be enrolled and treated at the same dose level. If no further DLT was observed during the first course in these additional patients, the dose could be escalated to the next higher dose level. When two or more (≥33%) DLT were observed at the same dose level, there would be no further dose escalation.

Study treatment continued until progressive disease, death, withdrawal of consent or unacceptable toxicity was observed. All patients were followed for a minimum of 4 weeks after the last dose of study therapy.

Evaluation of toxicity. Toxicity was graded according to the CTCAE v3.0. Physical examination and laboratory assessment were performed regularly. Electrocardiograms were performed before the start of therapy and at 1 and 4 h after administration on days 1, 14 and 28 of the first cycle, and on days 14 and 28 of subsequent cycles.

The DLT was defined as grade 4 neutropenia for five consecutive days or longer, febrile neutropenia, grade 4 thrombocytopenia or bleeding requiring platelet transfusion, grade 3–4 nausea, vomiting or diarrhea despite adequate prophylaxis and therapy, other non-hematological toxicities of grade 3 or more except anorexia or fatigue, QTc interval of 530 ms or longer, and adverse events (AE) that caused interruption of administration for more than 14 days or dose reduction of two levels or more, during the first course.

Dose delays and modifications were defined as follows: with grade 3–4 neutropenia or febrile neutropenia, dosing was interrupted until recovery to grade 1 was achieved, and the dose was reduced when grade 4 neutropenia continued for 5 days or longer or with febrile neutropenia; with grade 3–4 thrombocytopenia, dosing was interrupted until platelets recovered to 100 000/mm^3 or more; with bleeding, dosing was interrupted; with fluid retention or pleural effusion, diuretics or the use of pleural drainage was commenced, and with severe or recurrent effusion, dosing was interrupted and was restarted at a reduced dose; with a grade 3 non-hematological toxicity except anorexia or fatigue, dosing was interrupted until recovery to grade 1 was achieved; and with a grade 4 non-hematological toxicity except anorexia or fatigue, dosing was interrupted until recovery to grade 1 was achieved and was restarted at a reduced dose.

Objective response evaluation. The tumor response was evaluated using Response Criteria in Solid Tumors (RECIST) ver. 1.0[11] in patients with measurable lesions. Assessment was performed at the fourth week of the first cycle, and every 4–8 weeks thereafter.

Pharmacokinetic studies. Blood samples for pharmacokinetic study were collected before dosing, and at 30 min, 1, 1.5, 2, 3, 4, 6, 12 and 24 h on days 1, 14 and 28 of the first cycle. Samples were analyzed to determine the concentration of dasatinib and its metabolite (BMS-582691). Pharmacokinetic (PK) parameters were calculated using a non-compartmental method, including C_{max}, AUC(0–t), AUC(TAU), T_{max}, t1/2, accumulation index, LCo and Vz/F for dasatinib, and C_{max}, AUC(0–t) and T_{max} for BMS-582691.

Pharmacodynamic studies. Because a lot of evidence shows that SRC is essential to osteoclast activity[4] and dasatinib suppresses osteoclast activity *in vivo*,[12] we opted to measure the bone metabolic markers of osteolast activity as the phamacodynamic marker of dasatinib acitivity. Blood and urine samples for the study were collected during screening, and on days 14 and 28 of the first cycle. Samples were analyzed for urine type 1 collagen N-telopeptide (NTx) and serum tartrate-resistant acid phosphatase (TRACP-5b).

Results

Patient characteristics. A total of 16 patients, all of whom were treated with dasatinib, were enrolled. Patient disposition is shown in Table 1. Nine patients were treated with 100 mg, three with 150 mg and four with 200 mg once daily. All patients discontinued the study medication due to disease progression (9/16, 56%), study drug toxicity (5/16, 31%) or the patient's request (2/16, 13%).

Baseline patient characteristics are shown in Table 2. The median age was 54 years (range, 33–65), male/female ratio was 5/11 and baseline ECOG PS was 0 or 1 in all patients. All patients had experienced prior treatments including chemotherapy (14/16, 88%), surgery (14/16, 88%), radiation therapy (9/16, 56%), hormonal or immunotherapy (4/16, 25%) and the use of other agents (2/16, 13%). The median number of previous chemotherapy regimens was five (range, 2–11). The primary tumors were breast cancer in four, colon or rectal cancer in six, soft tissue sarcoma in two (one gastrointestinal stromal tumor), renal cell carcinoma (RCC) in one, head and neck cancer in one, thymoma in one and ovarian cancer in one.

Toxicities. Four patients discontinued study therapy so early that they were not evaluable for DLT. The reasons for their early discontinuation were disease progression in one, QT prolongation in one (which was later shown to be due to an electrocardiogram error) and patient request in two (these two patients refused to continue treatment after grade 1 fatigue and anorexia in one, and grade 3 fatigue and grade 2 dyspnea in the other). In total, 12 patients were evaluable for DLT and two DLT were observed in two patients. One DLT at the 100-mg level was reported as a grade 2 non-hematological toxicity (blood lactate dehydrogenase increase, cell marker [KL-6] increase, bronchial wall thickening and pleural effusion), due to which the investigator decided to discontinue the study. The other was a grade 3 non-hematological toxicity (dyspnea and general physical health deterioration) at the 200-mg level. In addition, pleural effusion of grade 2 or more was observed in four patients treated with 200 mg. These results indicated that 200 mg once daily was not acceptable. Therefore, 150 mg once daily was determined to be the MTD in this study.

A summary of overall safety is shown in Table 3. The most frequent AE (≥30%) were anorexia (69%), fatigue (69%), pleural effusion (63%), anemia (63%), constipation (56%), diarrhea (56%), vomiting (50%), aspartate aminotransferase increase

Table 1. Patient disposition

	Dasatinib			Total
	100 mg†	150 mg	200 mg†	
No. patients enrolled	9	3	4	16 (100)
No. patients treated	9	3	4	16 (100)
No. patients discontinued	9	3	4	16 (100)
Disease progression	5	2	2	9 (56)
Study drug toxicity	3	1	1	5 (31)
Discontinuation at the patient's request	1	0	1	2 (13)

†Three patients in the 100 mg cohort and one patient in the 200 mg cohort discontinued dasatinib early and were not evaluable for dose-limiting toxicity (DLT). One DLT was observed in the 100 mg cohort and one in the 200 mg cohort.

Table 2. Baseline patient characteristics

	Dasatinib			Total (%)
	100 mg	150 mg	200 mg	
Gender				
Male	2	2	1	5 (31)
Female	7	1	3	11 (69)
Age (years)				
Median	58.0	55.0	47.5	54.0
Min, Max	33, 65	39, 63	33, 53	33, 65
Age (years)				
<65	7	3	4	14 (88)
≥65	2			2 (13)
Performance status (ECOG)				
0	7	1	2	10 (63)
1	2	2	2	6 (38)
Tumor type				
Breast cancer	3		1	4 (25)
Colon cancer	1	2	1	4 (25)
Rectal cancer	1	1		2 (13)
Hypopharyngeal cancer	1			1 (6)
Renal cell carcinoma	1			1 (6)
Leiomyosarcoma	1			1 (6)
Gastrointestinal stromal tumor	1			1 (6)
Thymoma			1	1 (6)
Ovarian cancer			1	1 (6)

ECOG, Eastern Cooperative Oncology Group.

(50%), nausea (44%), headache (38%), rash (38%), dyspnea (31%), alanine aminotransferase increase (31%) and blood calcium decrease (31%). Grade 1 QTc prolongation was seen in two patients. The most frequent AE of grade 3 or more (≥10%) were anemia (19%), lymphocyte count decrease (19%), fatigue (13%) and blood magnesium increase (13%). There were no deaths within 30 days after completion of administration of the study drug. Three patients (19%) experienced serious AE: perianal abscess; open fracture; and pleural effusion (one case each). Five patients (31%) discontinued study therapy due to AE: pleural effusion (two cases); QT prolongation; exertional dyspnea; cancer pain; and anemia.

Efficacy. Antitumor efficacy is shown in Table 4. Sixteen patients were treated and six patients (38%) were not evaluable for an objective response. No patient achieved a complete or partial response. Five patients (31%) had stable disease and three patients (RCC, colon cancer and thymoma) experienced stable disease for 6 months or longer, one at each dose level. Five patients (31%) had disease progression.

Pharmacokinetics. A summary of the PK parameters of dasatinib is shown in Table 5. Dasatinib was rapidly absorbed after oral administration. Dasatinib was detectable in plasma 30 min after oral administration and its concentration reached C_{max} at a median T_{max} of 0.5–3.3 h (Fig. 1). The dasatinib C_{max} values were comparable across all dose levels and study days, with moderate to large variability (41–127% as CV%). The dasatinib AUC values were also comparable across all dose levels and study days, with moderate to large variability (33–114% as CV%), and were slightly increased with the doses administered on days 1 and 14.

The mean t1/2 of dasatinib ranged between 4.36 and 8.33 h and was similar among study days. The mean CLo ranged between 141.6 and 649.2 L/h and the mean Vz/F ranged between 606 and 9113 L, with large variability. No remarkable drug accumulation was observed after repeated dosing. The accumulation index values ranged between 0.48 and 2.30 across study days.

Pharmacodynamics. Markers of bone metabolism were measured during the first course (Table 6). The dose had no obvious effect on NTx and TRACP-5b. Mean NTx and TRACP-5b levels after multiple administrations in all patients decreased over time, with moderate to large variability. On the day before the first administration (baseline), and on days 14 and 28, the mean (SD) values across the dose levels were 66.79 (45.18), 48.00 (43.24) and 32.64 (18.07) nanomolar bone collagen equivalents/millimolar creatinine (nmol BCE/mmol Cr) for NTx, respectively, 5.13 (1.89), 4.13 (1.25) and 3.40 (0.61) U/L for TRACP-5b. Of the 13 and 12 patients who had NTx and TRACP-5b levels assessed at baseline and during the study, 12 and 10 patients experienced a decrease in each marker, respectively (Fig. 2). The maximum percentage decreases in the levels of NTx and TRACP-5 were 46.3% (−17.3–86.5, $n = 13$) and 22.2% (−16.7–51.6, $n = 12$), respectively.

Discussion

In the present study, with once daily, continuous dosing, the DLT of dasatinib were grade 2 pleural effusion, bronchial wall thickening and laboratory abnormalities at 100 mg once daily,

Table 3. Adverse events

	All (%) $n = 16$		100 mg $n = 9$		150 mg $n = 3$		200 mg $n = 4$	
	All grades	Grade ≧ 3	All grades	Grade ≧ 3	All grades	Grade ≧ 3	All grades	Grade ≧ 3
Anorexia	11 (69)	2 (13)	5		2		4	2
Fatigue	11 (69)		6		1		4	
Anemia	10 (63)	3 (19)	4		2		4	3
Pleural effusion	10 (63)	1 (6)	5		1		4	1
Constipation	9 (56)		5		2		2	
Diarrhea	9 (56)	1 (6)	3		2		4	1
Vomiting	8 (50)		4		2		2	
AST increase	8 (50)		4		2		2	
Nausea	7 (44)		5				2	
Headache	6 (38)		3		1		2	
Rash	6 (38)		2		2		2	
Dyspnea	5 (31)	1 (6)	1				4	1
ALT increase	5 (31)		3		1		1	
Blood calcium decrease	5 (31)	1 (6)	2		1		2	1
Magnesium increase	2 (13)	2 (13)	1	1			1	1

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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Table 4. Individual efficacy results

	All n = 16	100 mg n = 9	150 mg n = 3	200 mg n = 4
CR/PR	0	0	0	0
SD	5	3	1	1
PD	5	3	1	1
NE	6	3	1	2

CR, complete response; NE, not evaluable, evaluated by RECIST ver. 1.0; PD, progressive disease; PR, partial response; SD, stable disease.

which resulted in the investigator deciding to discontinue the study, and grade 3 dyspnea at 200 mg once daily. At 200 mg once daily, DLT occurred in one of three evaluable patients. However, as grade 2 pleural effusion was observed in four of four patients, the dose of 200 mg once daily was determined to be unacceptable. The MTD was consequently defined as 150 mg once daily. In other phase I studies, the MTD was 120 mg twice daily for a schedule that involved 5 days of treatment followed by 2 days of rest, 70 mg twice daily in a continuous dosing schedule[13] and 180 mg once daily in another continuous dosing schedule.[14] We do not know why the MTD was smaller than other studies, because PK in the present study showed almost the same AUC as the previous studies.

Adverse events of grade 3 or more were anemia, fatigue, lymphopenia, and blood magnesium increased. Although grade 2 or less, dyspnea occurred in four of four patients treated with 200 mg once daily, so in addition to pleural effusion, respiratory toxicity appears to be the most clinically significant adverse effect. Pleural effusion has been reported frequently with dasatinib treatment.[15,16] In a phase I study of solid cancer, dyspnea or pleural effusion occurred in five of 11 patients treated with 180 mg per day, but only one of 11 patients treated with 140 mg per day.[14] The etiology of dasatinib-induced pleural effusion is not known; however, the effusions respond to steroids, are often exudative and contain lymphocytes or neutrophils, so they might be immune mediated. The etiology of dyspnea without massive pleural effusion in the present study is not clear. The other toxicity that had the most pronounced effect on patients' ability to continue therapy was fatigue, although determining the contribution of dasatinib to fatigue is difficult. From 13 to 20% of patients with leukemia also commonly experience fatigue during dasatinib treatment, but fatigue of grade 3 or more is rare (<3%). Another serious toxicity in the present study was perianal abscess in one patient, which might have been the result of the mucosal toxicity of dasatinib. Some studies suggest that dasatinib has an immunosuppressive effect,[17] but in CML studies there is no evidence that dasatinib leads to increased infection. Hematological toxicity was not significant except for anemia in one patient, and is consistent with other studies of solid cancer, compared with more severe hematological toxicities in CML patients.

We also studied the effects of dasatinib on QT intervals. In leukemic patients, long-term treatment with dasatinib (70 mg twice daily) was associated with a 3- to 6-msec increase in the QTc interval compared with baseline. We did not see any significant QTc prolongation even following treatment exceeding 1 year, although the sample size in the present study was small.

Among 10 patients evaluable for tumor response, there was no objective response, but five patients showed SD and three showed long SD. One RCC patient maintained SD for more than a year. Renal cell carcinoma, especially of the clear cell type, overexpresses VEGF and PDGF, and VEGF/PDGF signal inhibitors such as bevacizumab, sunitinib and sorafenib have been shown to be effective for RCC. Dasatinib inhibits PDGFR, but other PDGFR inhibitors such as imatinib have not been effective for RCC. The roles of SFK in RCC have not been fully defined. The transformation of human kidney proximal tubule cells by a SRC-containing retrovirus, and stimulation of VEGF production, angiogenesis and tumor development in a RCC xenograft model have been reported.[18,19] Therefore, dasatinib might inhibit RCC progression by inhibiting SRC-associated signal pathways.

Two of the six patients with large bowel cancer showed SD, and one developed prolonged SD. SRC expression levels are higher in colon adenocarcinoma than normal mucosa, and correlate not only with tumor stage and metastatic potential, but also with poor prognosis.[20,21] The activity of YES has also been reported in premalignant tissues and in carcinoma of the colon.[22,23] Dasatinib did not inhibit the growth of colon cancer cell lines, but inhibited the metastatic potential of these cells.[24] Dasatinib sensitizes K-ras mutant colon cancer cell lines to cetuximab[25] and had synergistic activity with oxaliplatin against colon cancer cells *in vitro* and *in vivo*.[26] Several ongoing clinical studies are examining the activity of SRC inhibitors in colon cancer, as monotherapy or in combination.

One thymoma patient showed prolonged SD. There is one case report of a clinical response of thymoma to dasatinib.[27] Transgenic mice expressing high levels of LCK developed thymic tumors.[28] Dasatinib inhibits LCK at low picomolar concentrations, and inhibits T cell receptor-mediated signal transduction, cellular proliferation, cytokine production and *in vivo* T-cell responses.[29] Thymomas also frequently overexpress

Table 5. Pharmacokinetic data

Dose (mg)	Study day	n	C_{max} (ng/mL) geometric mean (CV%)	AUC† (ng•h/mL) geometric mean (CV%)	T_{max} (h) Median (min, max)	t1/2 (h) Mean (SD)	AI geometric mean (CV%)	CLo (L/h) mean(SD)	Vz/F (L) mean(SD)
100	1	9	139.83 (54)	537.98 (33)	1.0 (0.5, 4.0)	4.77 (0.61)	NA	NC	NC
	14	5	137.03 (55)	499.69 (36)	1.0 (0.5, 3.0)	5.75 (1.67)	0.81 (34)	223.0 (134.3)	1709 (2010)
	28	3	253.77 (41)	738.76 (34)	0.5 (0.5, 1.0)	4.36 (1.19)	1.12 (36)	141.6 (53.3)	606 (286)
150	1	3	127.10 (83)	544.36 (54)	1.0 (1.0, 1.0)	4.68 (0.84)	NA	NC	NC
	14	4	166.43 (109)	694.90 (77)	1.0 (1.0, 1.0)	5.04 (1.19)	1.78 (19)‡	273.3 (204.7)	1883 (1967)
	28	2	103.32 (112)	273.10 (75)	0.5 (0.5, 0.5)	8.33 (2.78)	0.48 (11)	649.2 (489.6)	5420 (5920)
200	1	4	124.48 (69)	595.62 (56)	1.3 (0.5, 3.0)	7.62 (4.11)	NA	NC	NC
	14	2	102.61 (127)	716.27 (114)	2.3 (1.5, 3.0)	7.95 (5.62)	2.30 (77)	471.6 (537.4)	9113 (12216)
	28	2	80.92 (113)	534.33 (53)	3.3 (0.5, 6.0)	7.66 (4.24)	1.72 (15)	403.6 (213.3)	5458 (6081)

†AUC (area under the plasma concentration vs time curve from time zero to infinity [INF]) for day 1 and AUC (area under the plasma concentration-time curve for a dosing interval [TAU]) for days 14 and 28. ‡n = 3. NA, not applicable; NC, not calculated.

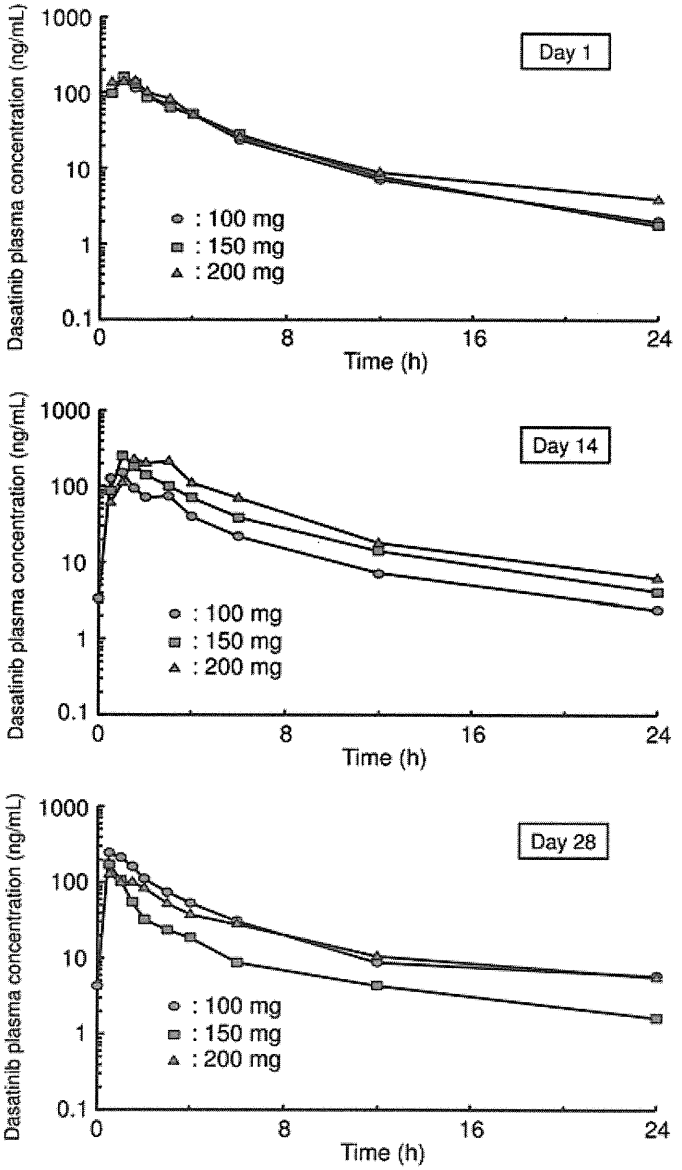

Fig. 1. Mean plasma concentration of dasatinib at days 1, 14 and 28 of the first cycle.

KIT and are sometimes associated with the *c-kit* mutation.[30] There have been reports of thymoma responding to imatinib.[31]

Recently, a better response in dasatinib-treated CML patients with large granular lymphocyte (LGL) lymphocytosis has been reported.[32] No increase of LGL was detected in the present study, although no special attention was paid to the lymphocyte subset at the time of the study. Lymphocytosis was detected in two patients, who showed lymphocyte counts of more than 5000 and 7000 at peak level. These two patients showed long SD, so it is possible that lymphocytosis with LGL expansion might also be correlated with a good response in patients with solid cancers.

Pharmacokinetic data showed that dasatinib was rapidly absorbed and metabolized. C_{max} and AUC were not significantly correlated with dosing, and varied from individual to individual. Due to the small sample size in the present study, no conclusion could be reached regarding linearity. Dasatinib did not accumulate significantly with once daily treatment, although the AUC on days 14 and 28 were slightly increased. The amounts of exposure are within the range for pharmacokinetic data for once daily treatment reported in other studies of solid cancer and CML patients.[33]

Table 6. Pharmacodynamic data

	NTx (nmol BCE/mmol Cr)			TRACP-5b (U/L)		
	n	Mean	SD	*n*	Mean	SD
ALL						
Baseline	16	66.79	45.18	16	5.13	1.89
Day 14	13	48.00	43.24	12	4.13	1.25
Day 28	7	32.64	18.07	7	3.40	0.61
100 mg						
Baseline	9	51.71	39.75	9	4.67	1.84
Day 14	6	51.55	59.74	6	4.03	1.34
Day 28	3	25.33	4.26	3	3.40	0.61
150 mg						
Baseline	3	62.57	15.78	3	5.10	1.57
Day 14	3	36.47	26.00	3	3.90	0.78
Day 28	2	40.10	16.40	2	3.60	0.14
200 mg						
Baseline	4	103.90	57.37	4	6.18	2.25
Day 14	4	51.33	30.08	3	4.57	1.75
Day 28	2	36.15	36.84	2	3.20	1.13

nmol BCE/mmol Cr, nanomolar bone collagen equivalents/millimolar creatinine; NTx, N-telopeptide; TRACP-5b, tartrate-resistant acid phosphatase.

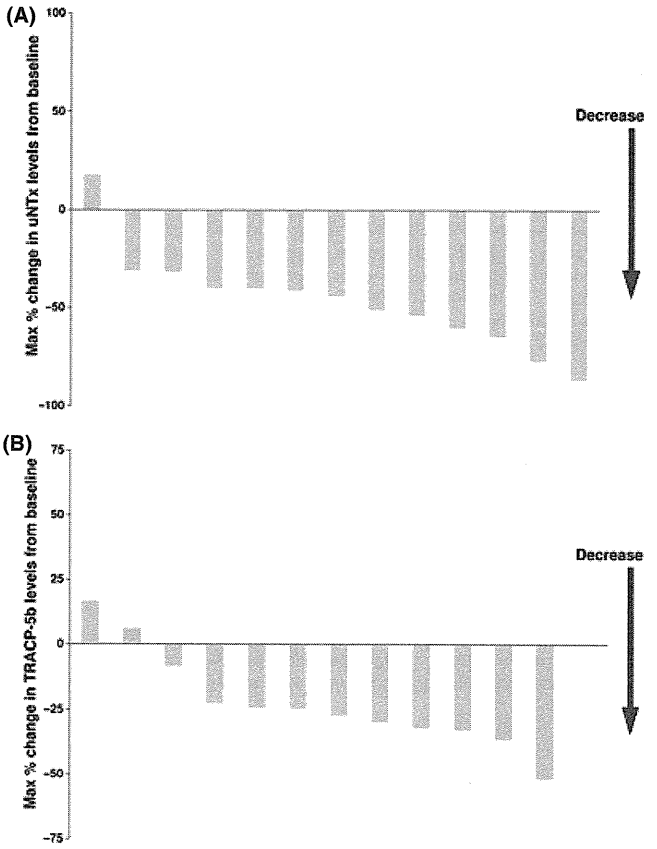

Fig. 2. Maximum changes in bone metabolic markers from baseline. Levels of urine NTX (uNTX) (a) and serum tartrate-resistant acid phosphatase (TRACP-5b) (b) were measured at baseline and during the study.

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We conducted pharmacodynamic studies of bone metabolic markers. Compared with baseline values, mean values of bone resorption markers decreased by 51% (urine NTx) and 34% (TRACP) on day 28 of dasatinib treatment. Recent advances in the treatment for metastatic solid cancers have highlighted the importance of treating bone metastasis to reduce the incidence of skeletal complications and improve patients' quality of life. Currently, bisphosphonates (BP) are commonly used as the standard for treatment of bone metastasis, and receptor activator of nuclear factor kappa B ligand antibody (denosumab) has recently been approved by the FDA as a bone-targeted agent.[34] However, the outcomes of BP or denosumab therapy leave room for improvement with regards to their efficacy, safety and convenience. In a phase II study of dasatinib for castration-resistant prostate cancer, 51% of patients receiving BP and 50% of those not receiving BP achieved a urine NTx decrease of 40% or more when given dasatinib 70 mg twice daily or 100 mg twice daily.[35] Yu *et al.* also showed that 45% of patients not receiving BP achieved a reduction in uNTx to within normal levels. The present study further confirmed the inhibitory activity of dasatinib on bone resorption in solid cancer patients without using BP, and suggests the dasatinib might be effective for bone metastases from solid cancer, as both an antitumor and bone-targeted agent.

In conclusion, dasatinib 150 mg once daily was determined to be the MTD in the present study. The safety profile of dasatinib was generally acceptable in this study population and not significantly different from that in other studies. There was no objective response, but three of 10 evaluable patients achieved prolonged SD that lasted more than 6 months.

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Disclosure Statement

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Abbreviations

AUC	area under the concentration-time curve
AUC(0–t)	AUC from time zero to the time of the last quantifiable concentration
AUC(TAU)	AUC over a dose interval
C_{max}	observed maximum concentration
CV	coefficient of variation
CLo	apparent oral clearance
t1/2	serum elimination half-life
T_{max}	time of maximum concentration
Vz/F	apparent volume of distribution for the terminal disposition phase

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PHASE I STUDIES

A phase I study of oral panobinostat (LBH589) in Japanese patients with advanced solid tumors

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Summary *Objective* The objective was to determine the maximum tolerated dose and the dose-limiting toxicity of panobinostat (LBH589) when administered as a single agent to adult patients with advanced solid tumors or cutaneous T-cell lymphoma whose disease had progressed despite standard therapy or for whom no standard therapy existed. *Methods* Panobinostat was administered orally once daily on Monday, Wednesday, and Friday of each week. A total of 13 patients were treated with one of three initial doses: 10 mg (n=3), 15 mg (n=4), or 20 mg (n=6). *Results* No dose-limiting toxicity was observed in 12 evaluable patients. The most frequently reported adverse events, regardless of whether they were related to the study drug, were diarrhea and nausea in 10 patients (76.9%). Thrombocytopenia was reported in 12 of 13 patients (92.3%). Five of 11 patients (45.4%) had stable disease. *Conclusion* Panobinostat administered orally once daily on Monday, Wednesday, and Friday of each week was well tolerated at doses up to 20 mg in Japanese patients. Dose escalation did not proceed after exploration of the 20 mg dose due to emerging global clinical data at that time.

Keywords Panobinostat · Histone deacetylase inhibitors · Phase 1 clinical trials · Cutaneous T-cell lymphoma

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Introduction

Over the past several years, deacetylase inhibitors (DACIs) have provided novel approaches to cancer treatment. For several decades, cancer has been thought of as a disease characterized by genetic defects involving gene mutations, deletions, amplifications, and chromosomal abnormalities. Recently, however, it has been well recognized that epigenetic and genetic changes play an important role in the initiation and progression of malignant neoplasms. One of the most extensively studied post-translational modifications of chromatin is the acetylation of lysine residues in histone proteins, which are regulated by histone acetyltransferases and histone deacetylase (HDAC) activity. Positively charged deacetylated histones bind tightly to the phosphate backbone of DNA and inhibit transcription. However, acetylated histones generate a more open DNA conformation, which promotes the expression of the corresponding genes [1, 2] HDACs are involved in reversible acetylation, not only of histones but also of other proteins, such as p53, NF- κB, and E2F-1, which play a key role in tumorigenesis and in the antitumor response, and of proteins that regulate DNA repair (Ku70), the cellular cytoskeleton (α-tubulin), and protein stabilization (Hsp90) [1]. At least 18 human HDACs have been identified, and they are grouped into four classes: I, II, III, and IV [3].

Dozens of structurally diverse DACIs have been identified and classified as Class I–specific inhibitors or as pan-

deacetylase (pan-DAC) inhibitors, which confer activity against both Class I and II DACs [4]. Pan-DAC inhibitors include panobinostat, vorinostat (suberoylanilide hydroxamic acid), and belinostat (PXD101). Of the pan-DAC inhibitors, vorinostat is the most extensively studied and was approved by the US Food and Drug Administration for the treatment of cutaneous T-cell lymphoma (CTCL) [3]. Recent evidence suggests that vorinostat has activity against a variety of solid and hematologic tumors [5].

Panobinostat has potent DAC inhibitory activity at low nanomolar concentrations against Class I, II, and IV purified recombinant HDAC enzymes, which suggests true pan-DAC activity [6]. In studies using enzymatic assays, IC_{50} values for panobinostat were consistently lower than those for vorinostat and belinostat; as a pan-DAC inhibitor, panobinostat was at least 10-fold more potent than vorinostat and appeared to be the most potent of the pan-DAC inhibitors in development.

Panobinostat has shown potential in both preclinical and clinical studies. Several phase I studies have been conducted to evaluate the safety, maximum tolerated dose (MTD), tolerability, and preliminary efficacy of panobinostat. In the CLBH589B2101 trial, various dosing schedules of oral panobinostat were evaluated in Western patients with advanced solid tumors or non-Hodgkin lymphoma, including CTCL. Panobinostat was well tolerated, and objective clinical responses were seen in 6 of 10 CTCL patients when administered orally on Monday, Wednesday, and Friday (MWF) of each week on a 28-day cycle. A dose of 30 mg on MWF was considered excessively toxic, and the MTD was determined to be 20 mg (given on MWF) in this Western patient population [7, 8].

On the basis of the above promising data, we conducted a phase I clinical trial to determine the MTD and dose-limiting toxicity (DLT) of panobinostat when administered orally as a single agent to Japanese patients with either advanced solid tumors or CTCL.

Patients and methods

Patient eligibility

Adult Japanese patients with histologically confirmed, advanced solid tumors or cytopathologically confirmed CTCL whose disease had progressed despite standard therapy or for whom no standard therapy existed were selected. All patients were required to have a World Health Organization performance status of ≤2 and acceptable bone marrow and organ function defined as follows: absolute neutrophil count, ≥1500/mm^3; hemoglobin, ≥9 g/dL; platelets, ≥100,000/mm^3; serum aspartate aminotransferase and alanine transaminase, ≤2.5×upper limit of normal (ULN) or ≤5.0×ULN if liver metastases present; serum total bilirubin, ≤1.5×ULN; and serum creatinine, ≤1.5×ULN. Additional ineligibility criteria included a history of primary central nervous system tumors or brain metastases, any peripheral neuropathy of grade ≥ 2 per the Common Terminology Criteria for Adverse Events (CTCAE), unresolved diarrhea of grade ≥ 2 per the CTCAE, impaired cardiac function (left ventricular ejection fraction <45%, complete left bundle branch block, obligate use of a cardiac pacemaker, congenital long QT syndrome, history or presence of significant ventricular or atrial tachyarrhythmias, clinically significant resting bradycardia [<50 beats per minute], QTcF >480 ms on screening electrocardiogram, or other clinically significant heart disease), impairment of gastrointestinal function or gastrointestinal disease, and acute or chronic liver or renal disease.

This study was approved by the institutional review board of each participating institution. All patients gave written informed consent before any screening procedures were conducted.

Trial design and treatment plan

This was a phase I, open-label, dose-escalation study of panobinostat administered orally once daily on MWF weekly on a 28-day cycle. Oral panobinostat was provided by Novartis Pharma K.K. (Tokyo, Japan).

The primary objectives were to determine the MTD and DLT of oral panobinostat when administered as a single agent to adult Japanese patients with advanced solid tumors or CTCL whose disease had progressed despite standard therapy or for whom no standard therapy existed. Secondary objectives included evaluating the safety and tolerability of oral panobinostat in Japanese patients, including acute and chronic toxicities; determining the pharmacokinetic profile of oral panobinostat in plasma; and assessing preliminary evidence of antitumor activity.

This study employed a standard "3+3" design. The starting dose was 10 mg on MWF based on the standard Japanese practice of starting at 50% of the recommended Western dose [8]. Panobinostat was administered according to provisional three-dose cohort levels: 10, 15, and 20 mg on MWF weekly. One treatment cycle consisted of 4 weeks of therapy. DLT was defined as an adverse event (AE) or abnormal laboratory value that was determined to be unrelated to disease progression, intercurrent illness, or concomitant medication use in cycle 1 and that met any one of the criteria shown in Table 1. At least three patients were assigned to each cohort, and individual cohorts were expanded to six patients after the development of one DLT Dose escalation to >20 mg on MWF was not planned in this study; therefore, even if DLT was not observed in the

Table 1 Criteria for defining dose-limiting toxicity (DLT)

Toxicity	Any of the following criteria
Hematologic[a]	CTCAE grade 3 neutropenia for >7 days
	CTCAE grade 3 thrombocytopenia for >7 days
	CTCAE grade 4 neutropenia for >7 days
	Any CTCAE grade 4 thrombocytopenia
	Neutropenic fever: ANC <1000/mm^3 and body temperature ≥38.5°C
Renal	Serum creatinine ≥2.0 × ULN to ≤3.0 × ULN for >7 days
	Any serum creatinine concentration >3 × ULN
Hepatic	Total bilirubin ≥2 × ULN to ≤3.0 × ULN for >7 days
	Any total bilirubin >3 × ULN
	CTCAE grade 3 AST or ALT for >7 days
	Any CTCAE grade 4 AST or ALT
Neurologic	More than one CTCAE grade level increase lasting >7 days
Cardiac	CTCAE grade ≥3
Other adverse events[a]	CTCAE grade 3 adverse events (excluding CTCAE grade 3 elevations in alkaline phosphatase) lasting >7 days
	CTCAE grade 4 adverse events (excluding CTCAE grade 4 elevations in alkaline phosphatase)
	CTCAE grade ≥3 vomiting or CTCAE grade 3 nausea despite the use of optimal antiemetics
	CTCAE grade ≥3 diarrhea despite the use of optimal antidiarrheal treatment
	Any other adverse event unrelated to disease progression, intercurrent illness, or concomitant medication use that did not allow administration of oral panobinostat for >25% of the total 28-day cycle

ALT alanine transaminase, *ANC* absolute neutrophil count, *AST* aspartate aminotransferase, *CTCAE* Common Terminology Criteria for Adverse Events, *ULN* upper limit of normal

[a] CTCAE grade ≥3 anemia was not considered a DLT unless judged to be a hemolytic process secondary to the study drug. CTCAE grade ≥3 lymphopenia was considered a DLT unless clinically significant

first three patients assigned to the 20-mg cohort, three patients would be enrolled at this level and a total of six patients would be evaluated. The MTD was defined as the highest dose with an observed incidence of DLT in no more than one of six patients treated at a particular dose level.

If toxicity necessitating interruption of oral panobinostat dosing was observed, re-administration began when any previously occurring nonlaboratory toxicity had resolved to a CTCAE grade ≤1. In addition, resolution of abnormalities in the following variables was required: absolute neutrophil count to ≥1000/mm^3, platelets to ≥75,000/mm^3, serum creatinine to ≤1.5 × ULN, total bilirubin to ≤1.5 × ULN, and aspartate aminotransferase and alanine transaminase to a CTCAE grade ≤1. If a patient required a dose delay of >21 days from the intended day of the next scheduled dose, the patient was withdrawn from the study.

Treatment was suspended for patients who experienced grade 3 thrombocytopenia before day 13 of a cycle or grade 4 at any time until the platelet count was ≥75,000/mm^3, at which time dosing was resumed at the next lower dose. If a patient experienced grade 3 thrombocytopenia on or after day 13, dosing was suspended until the platelet count was ≥75,000/mm^3. For patients who required a dosing suspension for >7 days, dosing resumed at the next lower dose. If the platelet count recovered to ≥75,000/mm^3 within 7 days, dosing resumed at the same dose but on a modified schedule, i.e., panobinostat was administered on MWF for 2 consecutive weeks followed by 1 week off.

The evaluable population in whom the MTD was determined (MTD-determining population) consisted of patients who had been treated with at least nine doses of panobinostat, had been observed for 28 days following the first dose, and had either completed all required safety evaluations or experienced DLT during cycle 1. Patients who did not meet these requirements were considered ineligible for this evaluation and were replaced.

Safety assessments

Safety assessments included an evaluation of AEs according to the NCI CTCAE (version 3.0), regular monitoring of laboratory variables, and a physical examination that included urinalysis, repeated evaluations of cardiac function (including electrocardiography and measurement of cardiac enzymes), and assessments of vital signs, weight, performance status, and thyroid function.

Pharmacokinetics

To determine pharmacokinetic profiles after single and repeated doses, blood samples were collected at time 0 (predose) and 0.5, 1, 2, 3, 4, 8, 24, and 48 h after the oral administration of panobinostat on days 1 and 15 of cycle.

To assess possible time-dependent changes in the pharmacokinetic profile, predose blood samples were also obtained on days 8, 9, and 22 of cycle 1; on day 15 of cycle 2; and on day 1 of cycle 3.

Pharmacokinetic parameters characterizing the disposition of oral panobinostat, such as the median time to reach the maximum plasma concentration (t_{max}), the maximum concentration (C_{max}), $t_{1/2}$, and the area under the curve (AUC), were calculated individually by using a noncompartmental method and were summarized descriptively by scheduled time point (day 1 and day 15) and initial dose cohort.

Pharmacodynamics

Complete blood counts were determined in blood drawn at baseline and on days 1 and 15 of each cycle, and the amount of fetal hemoglobin (HBF) was measured.

Antitumor activity

Tumors were evaluated on day 26 of cycle 1 and on day 1 of every even-numbered cycle (except cycle 2). Tumor response was assessed on the basis of RECIST Criteria or, in the case of patients with CTCL, on the basis of the Physician's Global Assessment of Clinical Condition (PGA), Composite Assessment of Index Lesion Disease Severity (CA), and extramedullary response [9]. Progression-free survival was defined as the time from the start date of treatment to the date of first documented progression or death due to any cause. Progression-free survival was a secondary efficacy variable for patients with a solid tumor.

Statistical analysis

The safety assessment was based on the type and frequency of AEs and on the number of abnormal laboratory values by using the CTC grade. The occurrence of DLT was also summarized by initial dose cohort. The assessment of efficacy was performed by disease type (i.e., solid tumors and CTCL).

Results

Patient demographics

Although 14 patients were enrolled in the study, only 13 patients actually received the study drug. One patient was considered eligible and enrolled; however, during the screening, his left ventricular ejection fraction was found to be 52%. Given the patient's age and the cardiotoxic potential of panobinostat, the patient was considered to be at excessive risk and was not treated. Three patients were treated at a dose of 10 mg on MWF, 4 patients at a dose of 15 mg on MWF, and 6 patients at a dose of 20 mg on MWF. Patient characteristics are summarized in Table 2. Eleven patients had solid tumors, the most frequent primary site of which was the lung (23.1%), and two patients had CTCL (one each with mycosis fungoides and unspecified peripheral T cell lymphoma). Both CTCL patients were treated at a dose of 10 mg on MWF. All patients had a performance status of ≤ 1 based on WHO criteria.

Treatment administration

Eleven patients (84.6%) were withdrawn from the study because of progressive disease. Two patients (15.4%) were withdrawn because they withdrew consent. As can be seen in Table 3, the median durations of exposure were 82.0, 51.0, and 71.5 days for the 10-, 15-, and 20-mg dose cohorts, respectively. The median duration of exposure was shorter in the 15-mg cohort than in the 10- and 20-mg cohorts, because two of the four patients at the 15-mg dose level discontinued treatment during cycle 1 due to disease progression.

Two patients (50.0%) in the 15-mg cohort and one patient (16.7%) in the 20-mg cohort required dose reductions because of AEs or laboratory test abnormalities. Seven of 13 patients required dose interruptions for the following reasons: 4 because of AEs, 3 because of laboratory test abnormalities, and 1 because of a dosing error. Among the seven patients required dose interruptions, one patient required two separate dose interruptions: one because of an AE and one because of a laboratory test abnormality. Two of these seven patients were in the 15-mg cohort, and five were in the 20-mg cohort.

DLT and MTD

Twelve patients were included in the MTD-determining population (3 patients each at the 10-mg and 15-mg dose levels and 6 patients at the 20-mg dose level). One patient in the 15-mg cohort experienced a decrease in the platelet count (grade 3) on day 15 of cycle 1. Per the protocol-specified criteria, the study drug should have been interrupted at this point; however, treatment continued until day 17, and the patient consistently experienced grade 3 thrombocytopenia for 9 days. In view of this protocol deviation, the Data Safety Monitoring Board considered this patient to be inevaluable, and the patient was therefore excluded from the MTD-determining population. A retrospective review of the data indicated that the interval from the onset of grade 3 thrombocytopenia to the nadir value of 26,000/mm^3 (grade 3) was 5 days, which would not have met DLT criteria had the protocol deviation not occurred. No DLT was observed in the MTD-determining population.

Table 2 Subject characteristics by initial dose cohort

Variable	10 mg on MWF (n=3)	15 mg on MWF (n=4)	20 mg on MWF (n=6)	Total (n=13)
Sex (n)				
Male	2	3	3	8
Female	1	1	3	5
Age (years)				
Median	62.0	61.0	61.5	62.0
Range	53–77	28–71	49–67	28–77
Weight (kg)				
Median	58.2	63.05	55.85	58.2
Range	56.0–63.9	52.9–69.5	45.4–82.0	45.4–82.0
Height (cm)				
Median	162.0	164.5	161.5	163.0
Range	156–171	143–176	147–175	143–176
Platelets (10^9/L)				
Median	376	185.5	257.5	252
Range	305–609	167–215	178–301	167–609
AST (U/L)				
Median	25	23.5	19	20
Range	14–72	16–31	16–25	14–72
ALT (U/L)				
Median	12	22	14.5	15
Range	11–59	12–37	7–21	7–59
Bilirubin (μmol/L)				
Median	6.84	12.825	11.115	10.26
Range	6.84–10.26	5.13–18.81	6.84–13.68	5.13–18.81
Creatinine (μmol/L)				
Median	53.04	65.86	66.74	62.76
Range	53.04–68.07	33.59–81.33	32.71–95.47	32.71–95.47
QT (ms)				
Median	345	360	410	390
Range	337–346	334–390	395–444	334–444
QTcF (ms)				
Median	394	394	413	403
Range	377–397	376–416	383–432	376–432
Primary site, histology/cytology [n (%)]				
Lung, adenocarcinoma	0 (0.0)	1 (25.0)	2 (33.3)	3 (23.1)
Rectum, adenocarcinoma	0 (0.0)	1 (25.0)	1 (16.7)	2 (15.4)
Non-Hodgkin lymphoma, CTCL	2 (66.7)	0 (0.0)	0 (0.0)	2 (15.4)
Colon, adenocarcinoma	0 (0.0)	0 (0.0)	1 (16.7)	1 (7.7)
Esophagus, squamous cell carcinoma	0 (0.0)	1 (25.0)	0 (0.0)	1 (7.7)
Small intestine, leiomyosarcoma	0 (0.0)	0 (0.0)	1 (16.7)	1 (7.7)
Larynx, squamous cell carcinoma	0 (0.0)	0 (0.0)	1 (16.7)	1 (7.7)
Pleura, mesothelioma	1 (33.3)	0 (0.0)	0 (0.0)	1 (7.7)
Thymus, squamous cell carcinoma	0 (0.0)	1 (25.0)	0 (0.0)	1 (7.7)
WHO performance status [n (%)]				
0	2 (66.7)	1 (25.0)	4 (66.7)	7 (53.8)
1	1 (33.3)	3 (75.0)	2 (33.3)	6 (46.2)
2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

ALT alanine transaminase, *AST* aspartate aminotransferase, *CTCL* cutaneous T-cell lymphoma, *MWF* Monday, Wednesday, and Friday, *WHO* World Health Organization