

Table 3. Hematologic toxicities (all courses)

Dose levels	Level 1 (200 mg/m <sup>2</sup> )			Level 2 (260 mg/m <sup>2</sup> )			Level 3 (300 mg/m <sup>2</sup> )			All		
	<i>n</i> = 3 (9)			<i>n</i> = 6 (23)			<i>n</i> = 3 (14)			<i>n</i> = 12 (46)		
CTCAE grade	1-2	3	4	1-2	3	4	1-2	3	4	1-2	3	4
Leucopenia	2	0	0	3	2	0	3	0	0	8	2	0
Neutropenia	1	1	0	1	3	1	1	2	0	2	6	2
Anemia	1	0	0	2	0	0	1	0	0	4	0	0
Thrombocytopenia	0	0	0	1	0	0	1	0	0	2	0	0

CTCAE, Common Terminology Criteria for Adverse Events.

Descriptive statistics were used for baseline characteristics, safety assessment, and PK variables. Regression analysis of individual  $C_{max}$  and  $AUC_{inf}$  vs. dose was performed to gain an appreciation of PK linearity. The SAS software package (ver8.2, SAS Institute, Inc., NC, USA) was used for statistical analysis.

## RESULTS

### PATIENTS AND TREATMENT

Between August 2006 and June 2007, 12 patients were enrolled and treated in this study at two participating centers in Japan. Patient characteristics are summarized in Table 2. Most patients were male (83%) with a median age of 61 (range, 45–69) years and all patients were ECOG PS 0–1. The predominant type of tumor was non-small cell lung cancer (NSCLC). Nine patients had surgery for primary tumors, seven had received more than three prior chemotherapy regimens and eight had received prior taxane-containing chemotherapy.

The patients were treated at the following dose levels: 200 mg/m<sup>2</sup> (Level 1, *n* = 3), 260 mg/m<sup>2</sup> (Level 2, *n* = 6) and 300 mg/m<sup>2</sup> (Level 3, *n* = 3). All were evaluable for safety and PK, and 11 for efficacy (one had no adequate measurable lesions for RECIST criteria).

### DLT, TOLERABILITY AND RD

No DLTs were observed through the dose escalation to the highest Level 3; therefore, the MTD was not reached methodologically. To decide on the potential RD, study sponsor, medical advisor and principal investigators jointly reviewed the reference data in the foreign studies (1,4,7) and favored 260 mg/m<sup>2</sup> from tolerability and safety perspectives, particularly the development of cumulative neurotoxicity. Additional three patients were then accrued to 260 mg/m<sup>2</sup> cohort to repeat the assessment. None of DLTs being experienced by the additional patients, 260 mg/m<sup>2</sup>, was established as RD.

### SAFETY

A total of 46 courses of ABI-007 was administered, and the median number of courses administered per patient was 3 (range, 1–11). No acute hypersensitivity reactions were observed during the infusion period. The most common toxicities were neutropenia, leucopenia, lymphopenia, alopecia and sensory neuropathy. The incidences of hematologic toxicities by dose level are shown in Table 3. Grade 3 or 4 neutropenia was often experienced in more than half of patients throughout the study; however, no febrile neutropenia was observed. The median time to onset of Grade 3 or 4 neutropenia was 15.0 (range, 8–34) days, and the median time to recovery to <Grade 2 was 6.5 (range, 3–14) days. There were no episodes of ≥Grade 2 or greater thrombocytopenia, and anemia was mostly mild. Frequent non-hematological toxicities were sensory neuropathy, alopecia, arthralgia/myalgia and rash (Table 4). The sensory neuropathy was manifested by paresthesia in a symmetric, stocking/glove distribution, and the median time to the first indication or exacerbation from the baseline was 7 days. The severity of non-hematologic toxicities was generally mild except for three cases of Grade 3 sensory neuropathy at Level 2 (*n* = 1) and Level 3 (*n* = 2), which cumulatively exacerbated from Grade 1 observed in the first week of the first course (range, 3–6 days from the administration) to Grade 3 during the third or later course (range, 3–11 courses from the administration). Among the three patients who experienced Grade 3 sensory neuropathy, one patient had received taxane-containing chemotherapy prior to the study. A variety of ocular toxicities including superficial keratopathy reported in the initial Phase I study of USA were not observed in this study. Treatment delay occurred in one patient at each Levels 2 and 3 due to the neurotoxicity, dose reduction occurred in two patients at each Levels 2 and 3 due to the neurotoxicity, and treatment was discontinued in three patients at each Levels 2 and 3, comprising five patients with treatment-related neurotoxicity and one patient with unrelated neutropenia.

Table 4. Non-hematologic toxicities (all courses)

Dose levels	Level 1 (200 mg/m <sup>2</sup> )		Level 2 (260 mg/m <sup>2</sup> )		Level 3 (300 mg/m <sup>2</sup> )		All	
	n = 3 (9)		n = 6 (23)		n = 3 (14)		n = 12 (46)	
CTCAE grade	1-2	3	1-2	3	1-2	3	1-2	3
Sensory neuropathy	1	0	5	1	1	2	7	3
Alopecia	3	0	4	0	3	0	10	0
Myalgia	0	0	6	0	3	0	9	0
Rash	2	0	4	0	1	0	7	0
Arthralgia	1	0	4	0	2	0	7	0
Asthenia	2	0	2	0	2	0	6	0
Motor neuropathy	0	0	3	0	2	0	5	0
Nausea	2	0	1	0	1	0	4	0
Anorexia	3	0	1	0	0	0	4	0
Vomiting	1	0	2	0	0	0	3	0
Diarrhea	2	0	0	0	0	0	2	0
Stomatitis	0	0	0	0	2	0	2	0

Grade 4 toxicities were not observed.

Table 5. Anti-tumor response

	Tumor type	Prior taxane therapy	Response
Level 1 (200 mg/m <sup>2</sup> )	NSCLC	+	PD
	NSCLC	+	PR
	Parotid gland	+	PD
Level 2 (260 mg/m <sup>2</sup> )	NSCLC	+	PD
	NSCLC	-	PR
	Ovary	+	PD
	NSCLC	+	PR
	Colon	-	PD
Level 3 (300 mg/m <sup>2</sup> )	Thymoma	-	SD
	Bladder	-	SD
	NSCLC	+	NE
	Pharyngeal and esophageal	+	SD

PD, progressive disease; PR, partial response; SD, stable disease; NE, not evaluable.

RESPONSE

Eleven of 12 patients were evaluable for anti-tumor response (Table 5). Partial responses were observed in three NSCLC patients. Of them, two of whom had received docetaxel-containing chemotherapy prior to the study. The first patient, entered at Level 1, had received 6 courses of

ABI-007, and the second and third patients, entered at Level 2, 11 and 6 courses, respectively. The both responders in Level 2 attained disease control until the treatment discontinuation due to the sensory neuropathy.

PHARMACOKINETICS

Blood samples for PK analysis were available from all of 12 patients following the first course of treatment. A semi-log plot of the mean values of paclitaxel concentration for each dose level vs. time is shown in Fig. 1. After 30 min infusion of ABI-007, the concentration of paclitaxel began to decrease immediately upon cessation of the infusion with *t*<sub>1/2</sub> of 17.3–27.3 h in the whole blood, which is nearly comparable with that of standard dose of solvent-based paclitaxel (6), and the decline of paclitaxel concentration from maximum was multiphasic.

The mean PK parameters of paclitaxel are summarized in Table 6. *C*<sub>max</sub>, *AUC*<sub>0–*t*</sub> and *AUC*<sub>inf</sub> of paclitaxel when administered as a 30 min infusion of ABI-007 increased with increasing dosage. *CL* and *V*<sub>z</sub> of the blood sample showed the small inter-patient variability, and the mean ± *SD* values (CV%) for *CL* and *V*<sub>z</sub> at the dose level of 260 mg/m<sup>2</sup> were 18.1 ± 2.33 (12.9 CV%) (l/h/m<sup>2</sup>) and 510 ± 96.8 (19.0 CV%) (l/m<sup>2</sup>), respectively. These values slightly decreased with increased dosage. It was considered that there was no remarkable difference in calculated values of PK parameters between whole blood and plasma. Regression analysis suggested the dose-proportionality of ABI-007 within the dose range in this study (*R*<sup>2</sup> of *C*<sub>max</sub> = 0.4470, *R*<sup>2</sup> of

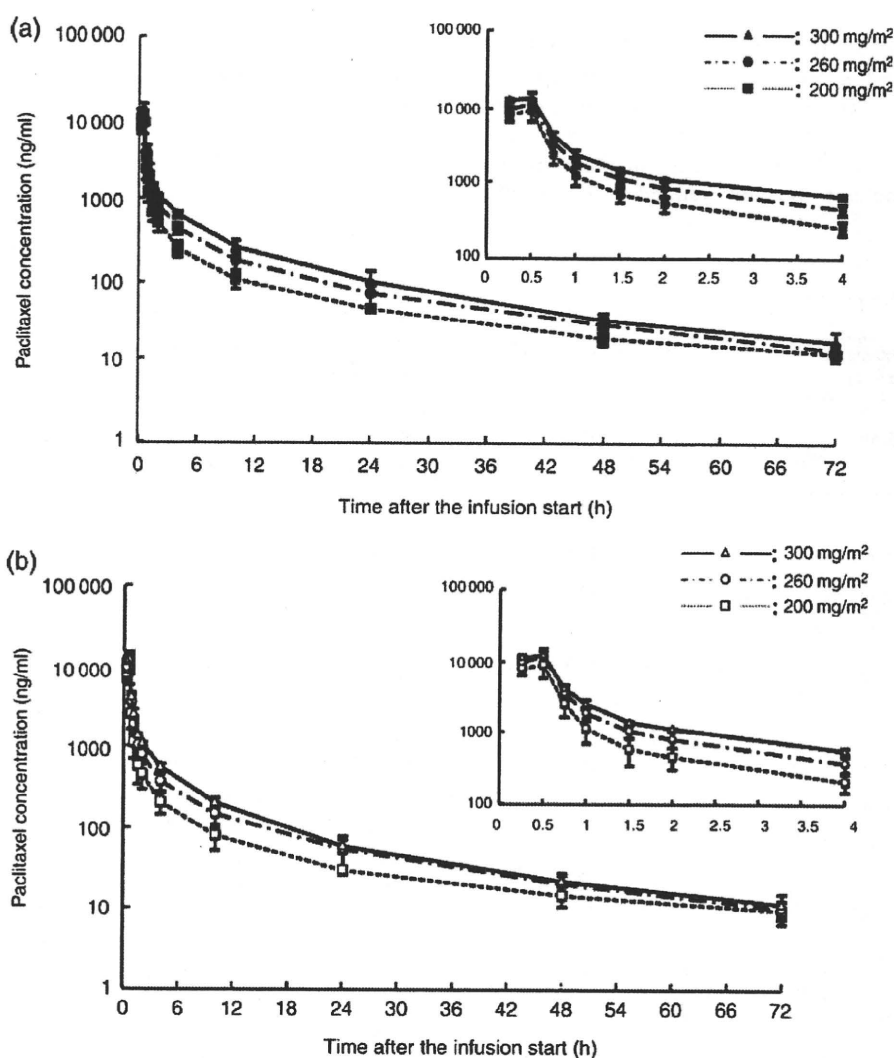


Figure 1. (a) Mean whole blood concentration–time profiles of paclitaxel. (b) Mean plasma concentration–time profiles of paclitaxel.

$AUC_{inf} = 0.7177$ ); however, it was difficult to establish the linearity due to those narrow dose range and small data size.

## DISCUSSION

In the Phase I study where ABI-007 was administered in Q3W schedule in Japanese patients, no DLT occurred at any dose level of 200, 260 and 300 mg/m<sup>2</sup>. Because MTD was not reached by the 3 + 3 rule, selection of RD was attributed to the consideration of reasonable tolerability, toxicities and PK profile. Since paclitaxel treatment was characterized for the cumulative neurotoxicity, dose selection also took into account the development of sensory neuropathy throughout the study. Consequently, 260 mg/m<sup>2</sup> was reassessed as potential RD and established as RD in the absence of applicable DLT. Outcome of sensory neuropathy in all treatment courses also provided the justification for the feasibility of

260 mg/m<sup>2</sup> (Table 7). Among 260 and 300 mg/m<sup>2</sup> cohorts, every patient experienced neuropathic events, in which Grade 3 or 4 event was more frequent in 300 mg/m<sup>2</sup> (two out of three patients) than in 260 mg/m<sup>2</sup> cohorts (one out of six patients). Moreover, all the three patients in 300 mg/m<sup>2</sup> cohort discontinued the treatment due to neuropathic events as opposed to two out of six patients in 260 mg/m<sup>2</sup> cohort.

In terms of treatment-related toxicities, Grade 3 or 4 neutropenia was experienced in 15 of 46 treatment courses (32%). Nonetheless, no febrile neutropenia was observed. Median duration of recovery from Grade 3 or 4 to <Grade 2 was 6.5 days (range, 3–14). No treatment delay was caused by neutropenia. In addition, platelet decrease  $\geq$ Grade 2 was not observed throughout the study. On the whole, hematological toxicities were mild. In regard to sensory neuropathy, the median time to the first indication or exacerbation from the baseline was 7 days, which

was relatively early to that of solvent-based paclitaxel. Especially for Grade 3 sensory neuropathy, the indication or exacerbation fell within the first week of the first course, ranging from 3 to 6 days; the time to improve from Grade 3 to Grade 2 or 1 was 21, 26 and 46 days in the respective cases. Although the improvement tended to delay when

compared with median 22 days in a previous Phase III study (4), it still remains controversial because of the great difference in the sample sizes between the two studies. Meanwhile, other non-hematological toxicities including mucositis—the DLT of the US Phase I study—were generally mild to moderate up to 300 mg/m<sup>2</sup>.

PK profiles of ABI-007 have revealed the small inter-patient variability, and the AUC and C<sub>max</sub> of paclitaxel increased with increasing the dosage. In whole blood samples, there was a significant correlation between the doses and PK parameters. The linearity was uncertain in the face of wide confidence interval (CI) with small sample size, however, presumable from the other reported data showing the linearity over a wide dose range: 80–300 mg/m<sup>2</sup> (2) and PK equality between Japanese and western population (3).

Anti-tumor response was demonstrated in the patients with NSCLC including the patients who had received prior taxane-containing therapy.

Multiple previous studies of ABI-007 also reported the promising data in the patients with NSCLC. In a Phase II trial, 260 mg/m<sup>2</sup> of ABI-007 was administered alone in the same Q3W schedule as our study in the first-line setting, overall response rate was 16.3% (95% CI, 5.24–27.31%) and the disease control rate was 48.8% (95% CI, 33.90–63.78%) (8). More recently, weekly (QW) schedule of ABI-007 was also reported: 125 mg/m<sup>2</sup> of ABI-007 was administered in monotherapy on days 1, 8 and 15 every 4 weeks, the response rate was 30% (95% CI, 16–44%) and the disease control rate was 50% (95% CI, 35–66%) (9). Despite the higher incidence of ≥Grade 3 neutropenia and sensory neuropathy relative to the Q3W schedule, QW schedule was well tolerated and active.

In conclusion, no DLT observed at any dose levels, and ABI-007 was well tolerated up to 300 mg/m<sup>2</sup> in Japanese

Table 6. PK parameters of paclitaxel

	200 mg/m <sup>2</sup> (n = 3)		260 mg/m <sup>2</sup> (n = 6)		300 mg/m <sup>2</sup> (n = 3)	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
<b>Whole blood</b>						
C <sub>max</sub> (ng/ml)	9430	28.3	11 635	13.0	13 833	15.3
AUC <sub>inf</sub> (ng h/ml)	10 360	22.0	14 593	13.7	19 138	12.2
t <sub>1/2</sub> (h)	24.3	10.9	19.5	7.9	18.3	1.9
CL (l/h/m <sup>2</sup> )	19.9	21.6	18.1	12.9	15.8	11.2
Vz (l/m <sup>2</sup> )	689	15.3	510	19.0	417	9.7
<b>Plasma</b>						
C <sub>max</sub> (ng/ml)	9040	34.0	12 000	17.6	12 700	20.5
AUC <sub>inf</sub> (ng h/ml)	9146	29.6	13 330	20.7	16 271	11.2
t <sub>1/2</sub> (h)	29.0	17.7	20.8	19.5	19.8	9.8
CL (l/h/m <sup>2</sup> )	23.1	26.4	20.2	21.5	18.6	10.6
Vz (l/m <sup>2</sup> )	935	11.7	620	36.9	527	7.0

PK, pharmacokinetic; CV, coefficient of variation; C<sub>max</sub>, maximum concentration; AUC<sub>inf</sub>, area under the concentration–time curve up to ∞ hours; t<sub>1/2</sub>, terminal elimination half-life; CL, clearance; Vz, volume of distribution based on terminal phase.

Table 7. Grade change in sensory neuropathy (all courses)

Level	Case	Before administration	Course no.										
			1	2	3	4	5	6	7	8	9	10	11
Level 1	1-2	0	0	0	1	1	1	1 <sup>a</sup>	—	—	—	—	—
Level 2	2-1	1	2	—	—	—	—	—	—	—	—	—	—
	2-2	0	1	1	1	1	1	1	1	1	2	2	3 <sup>a</sup>
	2-3	0	0	1	—	—	—	—	—	—	—	—	—
Level 3	3-1	0	1	1	2	2	2	3 <sup>a</sup>	—	—	—	—	—
	3-2	0	1	1	1	2	2 <sup>a</sup>	—	—	—	—	—	—
	3-3	0	2	2	3 <sup>a</sup>	—	—	—	—	—	—	—	—
Level 2	2-4	0	1	1	1	2	2	2 <sup>a</sup>	—	—	—	—	—
	2-5	0	1	—	—	—	—	—	—	—	—	—	—
	2-6	0	1	1	—	—	—	—	—	—	—	—	—

—, end of study.

<sup>a</sup>Study-off due to sensory neuropathy.

patients. RD in this schedule was determined to be 260 mg/m<sup>2</sup> in consideration of efficacy, toxicities and similarity of PK profile in the western studies. Additional studies of single-agent ABI-007 and platinum-based combinations are warranted.

### Acknowledgements

The preliminary results of this study were presented in part at the AACR-NCI-EORTC International Conference, San Francisco, CA, October 2007.

### Funding

This trial was funded by Taiho Pharmaceutical (Tokyo, Japan).

### Conflict of interest statement

Hironobu Minami and Tomohide Tamura receive remuneration for the lectures from Taiho Pharmaceutical (Tokyo, Japan).

### References

1. Ibrahim NK, Desai N, Legha S, Soon-Shiong P, Theriault RL, Rivera E, et al. Phase I and pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel. *Clin Cancer Res* 2002;8:1038–44.
2. Nyman DW, Campbell KJ, Hersh E, Long K, Richardson K, Trieu V, et al. Phase I and pharmacokinetics trial of ABI-007, a novel nanoparticle formulation of paclitaxel in patients with advanced nonhematologic malignancies. *J Clin Oncol* 2005;23:7785–93.
3. Sparreboom A, Scripture CD, Trieu V, Williams PJ, De T, Yang A, et al. Comparative preclinical and clinical pharmacokinetics of a cremophor-free, nanoparticle albumin-bound paclitaxel (ABI-007) and paclitaxel formulated in Cremophor (Taxol). *Clin Cancer Res* 2005;11:4136–43.
4. Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, et al. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. *J Clin Oncol* 2005;23:7794–803.
5. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* 1994;270:414–23.
6. Therasse P, Arbuck SG, Eisenhower EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
7. Ibrahim NK, Samuels B, Page R, Doval D, Patel KM, Rao SC, et al. Multicenter phase II trial of ABI-007, an albumin-bound paclitaxel, in women with metastatic breast cancer. *J Clin Oncol* 2005;23:6019–26.
8. Green MR, Manikhas GM, Orlov S, Afanasyev B, Makhson AM, Bhar P, et al. Abraxane, a novel Cremophor-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer. *Ann Oncol* 2006;17:1263–8.
9. Rizvi NA, Riely GJ, Azzoli CG, Miller VA, Ng KK, Fiore J, et al. Phase I/II trial of weekly intravenous 130-nm albumin-bound paclitaxel as initial chemotherapy in patients with stage IV non-small-cell lung cancer. *J Clin Oncol* 2008;26:639–43.

## A Phase 1 Clinical Study of Temsirolimus (CCI-779) in Japanese Patients with Advanced Solid Tumors

Yasuhito Fujisaka, Yasuhide Yamada, Noboru Yamamoto, Atsushi Horiike and Tomohide Tamura\*

Division of Internal Medicine, National Cancer Center Hospital, Tokyo, Japan

\*For reprints and all correspondence: Tomohide Tamura, Division of Internal Medicine, National Cancer Center Hospital, 5-1-1, Tsukiji Chuo-ku, Tokyo 104-0045, Japan. E-mail: ttamura@ncc.go.jp

Received November 24, 2009; accepted March 18, 2010

**Objective:** Temsirolimus (CCI-779) is a novel inhibitor of the mammalian target of rapamycin. This Phase 1 study was aimed at investigating the maximum-tolerated dose, toxicity, pharmacokinetics and antitumor activity in Japanese patients with advanced solid tumors.

**Methods:** Temsirolimus was given as a 30 min intravenous infusion once a week. Patients with solid tumors not amenable to standard forms of treatment were eligible. Dose escalation of temsirolimus was planned from 15, 45, 80 to 165 mg/m<sup>2</sup>. The pharmacokinetics of temsirolimus and sirolimus in whole blood were examined for cycles 1, 2, 4 and 5 of treatment.

**Results:** Ten patients (median age 60.5 years; range 41–69 years) with advanced solid tumors were enrolled. Their primary cancers were renal cell carcinoma (five patients), lung cancer (three patients) and colorectal cancer (two patients). The major toxicities were hypophosphatemia diarrhea, hyperglycemia, stomatitis, pyrexia, elevated aspartate aminotransferase, rash, reduced neutrophil count, elevated alanine aminotransferase, anorexia, hypertriglyceridemia and somnolence. Two of three patients who received temsirolimus 45 mg/m<sup>2</sup> developed dose-limiting toxicities of Grade 3 stomatitis (one patient) and Grade 3 diarrhea (two patients). The maximum-tolerated dose was 15 mg/m<sup>2</sup>. The peak blood concentrations of temsirolimus and sirolimus, a major active metabolite, increased in a dose-dependent manner. The area under the concentration-versus-time curve of sirolimus, but not temsirolimus, increased in a dose-dependent manner.

**Conclusions:** The recommended dose for Phase 2 clinical studies of temsirolimus in Japanese patients with advanced solid tumors is 15 mg/m<sup>2</sup> intravenously once a week.

*Key words:* Phase 1 study – CCI-779 – temsirolimus – advanced solid tumor

### INTRODUCTION

Temsirolimus (CCI-779), a novel inhibitor of mammalian target of rapamycin (mTOR), is an ester analogue of the immunosuppressive agent sirolimus (rapamycin, trade name: Rapamune®, sale: Pfizer Inc.), which was approved for the prophylaxis of organ rejection in patients receiving renal transplants in the USA. Temsirolimus inhibits several key signal transduction pathways regulating G1 phase of the cell cycle by inhibiting the activity of mTOR, a cell cycle regulatory kinase, and ultimately blocks progression from G1 to S phase of the cell cycle (1). Temsirolimus is also reported

to show an inhibitory effect on the growth of various tumor cells, especially on the tumor cells with mutation or deletion of *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor gene (2–10).

On the basis of the inhibitory effect of temsirolimus on the growth of tumor cells in preclinical studies, clinical studies of temsirolimus have been conducted in patients with a variety of malignant tumors, including advanced renal cell carcinoma (13–20,22,23). In the USA, temsirolimus has been approved for the treatment of advanced renal cell carcinoma. In Europe, temsirolimus has been approved for the

first-line treatment of patients with advanced renal cell carcinoma who have at least three of six poor prognostic factors (Trade name: Torisel<sup>®</sup>, INN: temsirolimus, sale: Pfizer Inc. (21,24)). The recommended dose of temsirolimus is 25 mg/body infused intravenously over a 30–60 min period once a week.

On the basis of the above results, a Phase 1 study was also conducted in Japan to investigate the safety, tolerability, pharmacokinetics and antitumor activity of temsirolimus in patients with advanced solid tumors. Two different temsirolimus schedules were used in US, European and international studies (daily for 5 days every second week and once a week) and showed that temsirolimus was well tolerated and had antitumor activity (14,15,22,23). The weekly administration was chosen for the conduct of this clinical study in Japan because this is the schedule that has been approved in the USA and Europe and it is a more convenient schedule for patients than daily for 5 days every second week.

## PATIENTS AND METHODS

### STUDY DESIGN

This was an open-label, single-center, Phase 1 study. The primary objective was to investigate the safety and tolerability of temsirolimus administered by a 30 min intravenous infusion once a week. The secondary objective was to collect preliminary data of the pharmacokinetics and antitumor activity of temsirolimus.

The study protocol was approved by the institutional review board of the National Cancer Center, and the study was conducted from October 2002 to March 2005 in accordance with Good Clinical Practice.

### PATIENTS

Patients were enrolled in the study only if they met the following entry criteria:

Inclusion criteria were: (i) histologically and cytologically confirmed diagnosis of advanced solid tumors; (ii) refractory to standard therapy or no appropriate therapy; (iii) measurable lesion; (iv) at least 4 weeks since any prior chemotherapy, hormonal therapy, radiation therapy and/or surgery, and any other investigational agent use (at least 6 weeks since nitrosourea and mitomycin C); (v) available to be hospitalized from the day before the first administration through the fourth week; (vi) age  $\geq 20$  and  $< 75$  years at the time of consent; (vii) neutrophil count  $\geq 1500/\text{mm}^3$ , platelet count  $\geq 100\,000/\text{mm}^3$ , hemoglobin  $\geq 8.5$  g/dl; (viii) adequate renal function: serum creatinine  $< 1.5$  mg/dl, creatinine clearance (24 h method)  $\geq 50$  ml/min; (ix) adequate hepatic function: total bilirubin  $< 2$  mg/dl, glutamic oxaloacetic transaminase (aspartate aminotransferase, AST) and glutamic pyruvic transaminase (alanine aminotransferase, ALT)  $< 3$  times the institutional upper limit of the normal range; (x) serum cholesterol  $\leq 350$  mg/dl, triglyceride  $\leq 500$  mg/dl; (xi) performance status

0–2; (xii) estimated life expectancy of at least 3 months and (xiii) written informed consent for the study.

Exclusion criteria were: (i) concomitant use of CYP3A4 inhibitors or inducers, such as anticonvulsants and rifampicin, where the treatment could not be discontinued or switched to a different medication; (ii) symptomatic brain metastasis; (iii) positive HIV antibody, HBs antigen or HCV antibody; (iv) infection requiring systemic medication; (v) complications that were difficult to control by medication or other therapies; (vi) use of immunosuppressive agents within 3 weeks prior to enrollment; (vii) regular use of steroids; (viii) concurrent angina, myocardial infarction within 6 months prior to starting study or heart disease that was difficult to control with medication or other therapies; (ix) history of hypersensitivity to diphenhydramine or its structurally related antihistamine drugs; (x) history of hypersensitivity to polysorbate 80 or polyethylene glycol contained in the diluent of the investigational drug, (xi) women who were pregnant or lactating, or not willing to use acceptable contraception during the study and for at least 3 months following the last study drug administration and (xii) inappropriate patients for the study for any other reason such as screening test results by the investigator.

### DOSAGE AND ADMINISTRATION

Temsirolimus was supplied by Wyeth K.K. (Tokyo, Japan) in 5 ml vials for intravenous injection; each contained 25 mg/ml of temsirolimus, co-packaged with a specific diluent.

Diphenhydramine, an antihistamine drug, was intravenously infused at a dose of 30 mg over 30 min  $\sim 1$  h before the temsirolimus administration to prevent hypersensitivity reactions. Approximately 30 min after the diphenhydramine infusion, temsirolimus was administered by a 30 min intravenous infusion.

The doses of temsirolimus were 15, 45, 80 or 165 mg/m<sup>2</sup>. The 15, 45 and 165 mg/m<sup>2</sup> doses correspond to flat doses of 25, 75 and 250 mg. The latter were used once a week in a Phase 2 clinical study in kidney cancer patients and tolerability and efficacy were observed at all doses (14). Thus, these doses were used in this study of Japanese patients. In addition, 80 mg/m<sup>2</sup> was used as an intermediate dose between 45 and 165 mg/m<sup>2</sup> so that there would not be a 3.7-fold increase in dose in the escalation. Temsirolimus was administered once a week as used previously (14,15,22) and at least three administrations were planned. The fourth or subsequent administrations of temsirolimus were allowed to continue until tumor progression or unacceptable toxicity occurred.

### DOSE ESCALATION METHOD

On the basis of the Guidelines for Clinical Evaluation of Anticancer Drugs (11), three patients were administered temsirolimus in each dose group. Additional patients were

treated at that dose level or dose escalation was performed according to the number of patients with unacceptable toxicity.

#### DEFINITION OF DOSE-LIMITING TOXICITY

Tolerability of temsirolimus was assessed on the basis of the safety evaluation of the initial three-weekly administrations. When the following events related to temsirolimus occurred before the fourth administration, the event was defined as unacceptable toxicity: (i) Grade 3 or 4 non-hematologic toxicity (exclusion: nausea or vomiting without the use of appropriate antiemetic drugs, and serum triglycerides <1500 mg/dl recovering to Grade 2 by the next week), (ii) Grade 4 thrombocytopenia, (iii) Grade 4 neutropenia lasting 5 days or more, (iv) Grade 4 febrile neutropenia or (v) delay of administration for 2 weeks or more due to prolonged toxicity.

The severity of toxicities was assessed according to National Cancer Institute Common Toxicity Criteria (NCI-CTC Version 2.0, 30 April 1999).

#### EFFICACY EVALUATION

On the basis of the guidelines of Response Evaluation Criteria in Solid Tumors (RECIST) (12), best overall response was assessed.

#### PHARMACOKINETICS

Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 24, 72, 96 and 168 h (just before the time of second administration) after the first administration of temsirolimus and at the time of fifth administration (168 h after the fourth administration). Blood concentrations of temsirolimus and sirolimus, a major active metabolite, were measured by Taylor Technology Inc. (NJ, USA) using a validated liquid chromatography/tandem mass spectrometry method. The limit of detection was 0.25 ng/ml for each compound. The pharmacokinetic parameters of temsirolimus and sirolimus in whole blood, including maximum concentration ( $C_{max}$ ), time to maximum concentration ( $t_{max}$ ), terminal half-life ( $t_{1/2}$ ), area under the concentration-versus-time curve (AUC), apparent clearance (corrected for unknown fraction of drug metabolized;  $CL/f_m$ ) and apparent volume of distribution at steady state ( $Vd_{ss}/f_m$ ), were calculated using a non-compartmental analysis technique.

## RESULTS

#### PATIENTS' CHARACTERISTICS

Ten patients with solid tumors were enrolled in this study (Table 1). The median age was 60.5 years with a range of 41–69 years. All patients had at least one prior chemotherapy and seven patients also had surgery for primary tumors.

Table 1. Patients' characteristics

	Number of patients (n = 10)
Sex	
Male	6
Female	4
Age (years)	
≥40 to <50	2
≥50 to <60	2
≥60 to <70	6
Median (range)	
60.5 (41–69)	10
Performance status	
0	2
1	8
Number of prior	
1	3
Chemotherapy	
2–3	2
Regimens	
≥4	5
Prior treatment	
Chemotherapy	1
Chemotherapy + radiation	2
Chemotherapy + surgery	7
Primary cancer	
Renal cell carcinoma	5
Lung cancer	1
Small cell lung cancer	1
Non-small cell lung cancer	1
Sigmoid colon cancer	1
Colorectal cancer	1

The primary cancer of five patients was renal cell carcinoma. Three patients had lung cancer and two had colon/colorectal cancer. With regard to prior chemotherapy, five patients had received four or more regimens.

#### DOSE ESCALATION AND UNACCEPTABLE TOXICITY

The starting dose of 15 mg/m<sup>2</sup> was administered to three patients. One subject discontinued temsirolimus treatment after the first administration because of Grade 4 gastrointestinal perforation. The Efficacy and Safety Evaluation Committee determined that this adverse event was not an unacceptable toxicity, and the patient was excluded from tolerability evaluation and another patient was enrolled. The other three patients in the 15 mg/m<sup>2</sup> dose group did not develop unacceptable toxicity and the escalated dose of



45 mg/m<sup>2</sup> was administered to three patients. Two of them developed unacceptable toxicity. Grade 3 stomatitis and diarrhea occurred in one patient and Grade 3 diarrhea occurred in the other patient. According to the prescribed procedure, further dose escalation was discontinued, and three additional patients were enrolled in the starting dose of 15 mg/m<sup>2</sup> to confirm tolerability. None of these three additional patients developed unacceptable toxicity. From these results, temsirolimus administered as a monotherapy once a week at an intravenous dose of 15 mg/m<sup>2</sup> was judged to be acceptable.

In the 15 mg/m<sup>2</sup> dose group, six of seven patients received three or more weekly administrations of temsirolimus. Three received 15-, 26- and 47-weekly administrations. The patient in the 45 mg/m<sup>2</sup> dose group who did not have unacceptable toxicity received four-weekly administrations of temsirolimus.

#### TOXICITIES

All patients (10/10) experienced at least one adverse event related to temsirolimus administration (Table 2). A greater percentage of patients who received temsirolimus 45 mg/m<sup>2</sup> experienced Grade 3 or 4 adverse events (100%) than those who received 15 mg/m<sup>2</sup> (29%).

Temsirolimus-related adverse events of any grade that occurred in any cycle in at least six patients were hypophosphatemia (90.0%), diarrhea (80.0%), hyperglycemia (80.0%), stomatitis (70.0%), pyrexia (70.0%), elevated AST (70.0%), rash (70.0%), reduced neutrophil count (60.0%), elevated ALT (60.0%), anorexia (60.0%), hypertriglyceridemia (60.0%) and somnolence (60.0%) (Table 2).

The adverse events of Grade 3 or higher that were related to temsirolimus treatment are as follows. In the period from the first to the third dose of temsirolimus, two patients had diarrhea and one patient each had a perforated digestive tract, stomatitis, malaise, hyperglycemia, hypokalemia or hypophosphatemia (Table 2). In the fourth and subsequent administrations, two patients had supraventricular arrhythmia and one each had pulmonary infection and hypercholesterolemia. The perforated digestive tract, malaise, hypercholesterolemia and supraventricular arrhythmia (one patient) occurred in the 15 mg/m<sup>2</sup> group and the diarrhea, stomatitis, hyperglycemia, hypophosphatemia, hypokalemia, pulmonary infection and supraventricular arrhythmia (one patient) occurred in the 45 mg/m<sup>2</sup> group. These adverse events resolved when temsirolimus treatment was stopped and the patients were treated appropriately.

For Grade 2 or lower adverse events of hemoglobin decreased, hematocrit decreased, alkaline phosphatase increased, epistaxis and onychia, the frequency of occurrence increased with the number of administrations of temsirolimus. However, for the adverse events of Grade 3 or higher, the frequency of occurrence did not increase with the number of administrations.

The reasons for discontinuing temsirolimus treatment in this trial were adverse events (four patients), progressive disease (PD; five patients) and other (one patient). Diarrhea, perforated digestive tract, stomatitis and supraventricular arrhythmia were the adverse events that led to discontinuation. No patients died in this trial.

#### ANTITUMOR ACTIVITY

All 10 patients who received temsirolimus were eligible for this study and were included in efficacy evaluation. Eight patients had stable disease (SD) and two patients had PD as their best overall responses. Of those with SD, 71% (5/7) received temsirolimus 15 mg/m<sup>2</sup> and 100% (3/3) received temsirolimus 45 mg/m<sup>2</sup>. The median length of SD was 35 days (range 20+ to 371 days, Table 3). Three patients with renal cell carcinoma who received temsirolimus 15 mg/m<sup>2</sup> had a long period of SD (113–371 days).

#### PHARMACOKINETICS

Whole blood samples were collected from seven patients in the 15 mg/m<sup>2</sup> dose group and three patients in the 45 mg/m<sup>2</sup> dose group for measurement of concentrations of temsirolimus and sirolimus. After the intravenous infusion of temsirolimus was completed, concentrations of temsirolimus in whole blood decreased more rapidly than those of sirolimus. After a single dose of temsirolimus, mean C<sub>max</sub> for temsirolimus increased in a dose-dependent manner but mean AUC did not (Table 4). Mean Vd<sub>ss</sub> for temsirolimus increased with increasing dose from 83.85 l after the 15 mg/m<sup>2</sup> dose to 162.9 l after the 45 mg/m<sup>2</sup> dose. Mean CL also increased with increasing dose (8.48 l/h after 15 mg/m<sup>2</sup> and 27.19 l/h after 45 mg/m<sup>2</sup>). Mean terminal half-life for temsirolimus was 14.77 and 13.47 h for 15 and 45 mg/m<sup>2</sup>, respectively.

After a single dose of temsirolimus, sirolimus mean C<sub>max</sub> and AUC were increased with increasing dose; however, they did not increase in a dose-proportional manner (Table 4). A similar dose-proportional increase in sirolimus was observed in the previous study (22). Mean Vd<sub>ss/f<sub>m</sub></sub> and CL/f<sub>m</sub> also appeared to increase with increasing dose. Mean terminal half-life for sirolimus was 67.04 and 59.15 h for 15 and 45 mg/m<sup>2</sup>, respectively, and, therefore, was longer than t<sub>1/2</sub> for temsirolimus. After a single dose of temsirolimus, mean AUC<sub>sum</sub> also exhibited a dose-dependent increase. Mean AUC<sub>ratio</sub> was 2.94 after the 15 mg/m<sup>2</sup> dose and 4.79 after the 45 mg/m<sup>2</sup> dose. Inter-subject variability was generally modest after single and multiple doses of temsirolimus.

#### DISCUSSION

This Phase 1 trial in Japanese patients with advanced solid tumors showed that 15 mg/m<sup>2</sup> temsirolimus infused intravenously once a week was well tolerated. The result of evaluation by dose groups demonstrated that none of the patients

**Table 2.** Temsirolimus-related adverse events, including laboratory abnormalities, reported in any cycle in at least 30% of patients who received temsirolimus

Adverse event	Temsirolimus 15 mg/m <sup>2</sup> (n = 7)		Temsirolimus 45 mg/m <sup>2</sup> (n = 3)		Total (n = 10)	
	All grades <sup>a</sup> (n)	Grades 3 and 4 <sup>a</sup> (n)	All grades <sup>a</sup> (n)	Grades 3 and 4 <sup>a</sup> (n)	All grades <sup>a</sup> (n)	Grades 3 and 4 <sup>a</sup> (n)
Any	7	2	3	3	10	5
Diarrhea	6		2	2	8	2
Nausea	2		1		3	
Stomatitis	5		2	1	7	1
Vomiting	3				3	
Malaise	2	1	3		5	1
Pyrexia	4		3		7	
Pharyngitis	3				3	
Weight loss	3		2		5	
Anorexia	3		3		6	
Dysgeusia	2		1		3	
Somnolence	4		2		6	
Headache	3		2		5	
Nasal bleeding	4		1		5	
Rash	5		2		7	
Flushing	3		2		5	
Hypertension	3		1		4	
Phlebitis	3				3	
Leukocytes decreased	2		3		5	
Lymphocytes decreased	2		1		3	
Monocytes increased	2		1		3	
Reduced neutrophil count	3		3		6	
Platelets decreased	2		2		4	
Hemoglobin decreased	3		2		5	
Hematocrit decreased	3		1		4	
Elevated ALT	4		2		6	
Elevated AST	5		2		7	
Creatinine increased	2		2		4	
LDH increased	3		2		5	
Alkaline phosphatase increased	3		1		4	
Hypercholesterolemia	4	1	1		5	1
Hyperglycemia	5		3	1	8	1
Hypertriglyceridemia	5		1		6	
Hypophosphatemia	6		3	1	9	1

In the 15 mg/m<sup>2</sup> group, one patient each had perforated digestive tract or supraventricular arrhythmia. In the 45 mg/m<sup>2</sup> group, one patient each had hypokalemia, pulmonary infection or supraventricular arrhythmia. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

<sup>a</sup>NCI-CTC Ver 2.0.

in 15 mg/m<sup>2</sup> dose group experienced unacceptable toxicity, whereas two of three patients experienced Grade 3 stomatitis and diarrhea in 45 mg/m<sup>2</sup> dose group. For single doses of 15 or 45 mg/m<sup>2</sup>, the exposure of temsirolimus and its major

active metabolite sirolimus in whole blood increased in a dose-dependent but subproportional manner. Inter-subject variability between single (the first) and multiple (the fourth) dosing periods was low. The pharmacokinetic parameters for

temsirolimus and sirolimus that were measured in this study were very similar to those measured in the Phase 1 study of patients with solid tumors treated with intravenous

temsirolimus on a weekly schedule (22) and in the Phase 2 study of patients with renal cell carcinoma treated with temsirolimus on a weekly schedule (14).

**Table 3.** Individual efficacy results of patients treated with temsirolimus

Temsirolimus dose	Primary cancer	Best overall response	Time to progression (days)
15 mg/m <sup>2</sup>	Lung cancer	PD	20
	Colorectal cancer	PD	20
	Small cell lung cancer	SD	20+
	Sigmoid colon cancer	SD	23+
	Renal cell carcinoma	SD	113
	Renal cell carcinoma	SD	244
	Renal cell carcinoma	SD	371
45 mg/m <sup>2</sup>	Renal cell carcinoma	SD	20+
	Renal cell carcinoma	SD	27+
	Non-small cell carcinoma	SD	42+

SD, stable disease; PD, progressive disease.

The common temsirolimus-related adverse events were hypophosphatemia, diarrhea, hyperglycemia, stomatitis, pyrexia, elevated AST, rash, reduced neutrophil count, elevated ALT, anorexia, hypertriglyceridemia and somnolence. These adverse events also have been reported in other studies of patients treated with temsirolimus (14,15,22,23). Because only 10 Japanese patients were treated in this study, we cannot accurately determine whether the incidence of these adverse events in this population is similar to the incidence in populations in other studies.

In the Phase 3, international, interferon- $\alpha$ -controlled study of temsirolimus conducted in patients with advanced renal cell carcinoma, the patients administered temsirolimus alone at the dose of 25 mg/body (15 mg/m<sup>2</sup>) demonstrated a significant improvement in overall survival and progression-free survival when compared with the patients administered interferon- $\alpha$  alone (15). There was no significant difference in the objective response rate (percentage of patients with complete and partial responses) for patients treated with temsirolimus or interferon- $\alpha$  but patients treated with

**Table 4.** Pharmacokinetic parameters following intravenous administration of temsirolimus

	Single dose		Multiple doses <sup>a</sup>	
	15 mg/m <sup>2</sup>	45 mg/m <sup>2</sup>	15 mg/m <sup>2</sup>	45 mg/m <sup>2b</sup>
<b>Temsirolimus (mean <math>\pm</math> SD)</b>				
<i>C</i> <sub>max</sub> (ng/ml)	1013.9 $\pm$ 316.3	1793.3 $\pm$ 421.6	912.0 $\pm$ 50.0	1580.0
<i>t</i> <sub>max</sub> (h)	0.51 $\pm$ 0	0.34 $\pm$ 0.2	0.43 $\pm$ 0.2	0.25
<i>t</i> <sub>1/2</sub> (h)	14.77 $\pm$ 0.68	13.47 $\pm$ 1.09	13.76 $\pm$ 0.15	11.65
AUC (ng h/ml) <sup>c</sup>	2873 $\pm$ 358	2750 $\pm$ 250	2203 $\pm$ 203	2403
CL (l/h)	8.48 $\pm$ 1.73	27.19 $\pm$ 6.37	11.54 $\pm$ 1.30	24.66
Vd <sub>ss</sub> (l)	83.85 $\pm$ 10.91	162.9 $\pm$ 26.67	88.29 $\pm$ 9.61	153.4
<b>Sirolimus (mean <math>\pm</math> SD)</b>				
<i>C</i> <sub>max</sub> (ng/ml)	89.1 $\pm$ 40.5	157.3 $\pm$ 37.1	118.7 $\pm$ 28.5	190.0
<i>t</i> <sub>max</sub> (h)	7.53 $\pm$ 11.3	1.87 $\pm$ 1.9	2.03 $\pm$ 1.7	0.52
<i>t</i> <sub>1/2</sub> (h)	67.04 $\pm$ 17.37	59.15 $\pm$ 28.94	71.36 $\pm$ 12.24	35.33
AUC (ng h/ml) <sup>c</sup>	8168 $\pm$ 2089	13 524 $\pm$ 9763	9061 $\pm$ 1788	7772
CL/ <i>f</i> <sub>m</sub> (l/h)	3.05 $\pm$ 0.61	7.11 $\pm$ 3.41	2.41 $\pm$ 0.90	7.37
Vd <sub>ss</sub> / <i>f</i> <sub>m</sub> (l)	189.6 $\pm$ 23.46	325.2 $\pm$ 103.4	139.0 $\pm$ 15.55	273.6
<b>Composite</b>				
AUC <sub>ratio</sub> (sirolimus:temsirolimus)	2.94 $\pm$ 1.08	4.79 $\pm$ 3.03	5.20 $\pm$ 1.69	3.35
AUC <sub>sum</sub> (ng h/ml)	11 041 $\pm$ 1935	16 274 $\pm$ 9970	13 583 $\pm$ 2715	10 469

SD, standard deviation; AUC, area under the curve.

<sup>a</sup>Following the fourth administration.

<sup>b</sup>A blood sample from only one patient was available.

<sup>c</sup>AUC for single dose means AUC<sub>0- $\infty$</sub>  and for multiple doses means AUC<sub>0- $t$</sub> .

temsirolimus had a significantly higher clinical benefit rate (percentage of patients with complete and partial responses and SD for at least 24 weeks) than did patients treated with interferon- $\alpha$ . Thus, long-term SD was an important component of the temsirolimus response in patients with advanced renal cell carcinoma. For patients treated with temsirolimus, the objective response rate was 9% and the clinical benefit rate was 32%. In this Phase 1 study, three (3) patients with renal cell carcinoma had long-term SD lasting 113–371 days. The fact that 3 of 10 Japanese patients had long-term SD and none had partial or complete responses agrees with the results obtained in the Phase 3 study and suggests that the antitumor activity of temsirolimus in Japanese cancer patients is similar to that in renal cell carcinoma patients of the international population.

From the above results, tolerability at 15 mg/m<sup>2</sup> was confirmed, and the recommended dose for Japanese patients with advanced solid tumors was determined to be temsirolimus 15 mg/m<sup>2</sup> as an intravenous infusion once a week. Further clinical studies will be required to investigate the safety and efficacy of temsirolimus in detail.

### Acknowledgements

We thank all the patients and clinical personnel who participated in this study.

### Funding

This clinical trial was supported by Wyeth K.K. (Tokyo, Japan), which was acquired by Pfizer Inc. in October 2009.

### Conflict of interest statement

None declared.

### References

- Brown EJ, Beal PA, Keith CT, Chen J, Shin TB, Schreiber SL. Control of p70 S6 kinase by kinase activity of FRAP in vivo. *Nature* 1995;377:441–6.
- Wang SI, Puc J, Li J, Bruce JN, Cairns P, Sidransky D, et al. Somatic mutations of PTEN in glioblastoma multiforme. *Cancer Res* 1997;57:4183–6.
- Cantley LC, Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci USA* 1999;96:4240–5.
- Yu K, Toral-Barza L, Discifani C, Zhang WG, Skotnicki J, Frost P, et al. mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. *Endocr Relat Cancer* 2001;8:249–58.
- Dudkin L, Dilling MB, Cheshire PJ, Harwood FC, Hollingshead M, Arbus SG, et al. Biochemical correlates of mTOR inhibition by the rapamycin ester CCI-779 and tumor growth inhibition. *Clin Cancer Res* 2001;7:1758–64.
- Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, et al. Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci USA* 2001;98:10314–9.
- Grunwald V, DeGraffenried L, Russel D, Friedrichs WE, Ray RB, Hidalgo M. Inhibitors of mTOR reverse doxorubicin resistance conferred by PTEN status in prostate cancer cells. *Cancer Res* 2002;62:6141–5.
- Georger B, Kerr K, Tang CB, Fung KM, Powell B, Sutton LN, et al. Antitumor activity of the rapamycin analog CCI-779 in human primitive neuroectodermal tumor/medulloblastoma models as single agent and in combination chemotherapy. *Cancer Res* 2001;61:1527–32.
- Podsypanina K, Lee RT, Politis C, Hennessy I, Crane A, Puc J, et al. An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten<sup>+/-</sup> mice. *Proc Natl Acad Sci USA* 2001;98:10320–5.
- DeGraffenried LA, Fulcher L, Friedrichs WE, Grunwald V, Ray RB, Hidalgo M. Reduced PTEN expression in breast cancer cells confers susceptibility to inhibitors of the PI3 kinase/Akt pathway. *Ann Oncol* 2004;15:1510–6.
- Japan Antibiotics Research Association. *Guidelines of Clinical Evaluation of Anticancer Drugs*. Tokyo: Mix 1991 (in Japanese).
- Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205–16.
- Motzer RJ, Hudes GR, Curti BD, McDermott DF, Escudier BJ, Negrier S, et al. Phase I/II trial of temsirolimus combined with interferon alfa for advanced renal cell carcinoma. *J Clin Oncol* 2007;25:3958–64.
- Atkins MB, Hidalgo M, Stadler WM, Logan TF, Dutcher JP, Hudes GR, et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J Clin Oncol* 2004;22:909–18.
- Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med* 2007;356:2271–81.
- Chan S, Scheulen ME, Johnston S, Mross K, Cardoso F, Dittrich C, et al. Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. *J Clin Oncol* 2005;23:5314–22.
- Pandya KJ, Dahlberg S, Hidalgo M, Cohen RB, Lee MW, Schiller JH, et al. A randomized, phase II trial of two dose levels of temsirolimus (CCI-779) in patients with extensive-stage small-cell lung cancer who have responding or stable disease after induction chemotherapy: a trial of the Eastern Cooperative Oncology Group (E1500). *J Thorac Oncol* 2007;2:1036–41.
- Chang SM, Wen P, Cloughesy T, Greenberg H, Schiff D, Conrad C, et al. Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme. *Invest New Drugs* 2005;23:357–61.
- Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, et al. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. *J Clin Oncol* 2005;23:5347–56.
- Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C, et al. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. *J Clin Oncol* 2009;27:3822–9.
- Wyeth Pharmaceuticals Inc. Torisel<sup>®</sup> full prescribing information. 2007. <http://www.fda.gov/cder/foi/label/2007/0220881bl.pdf>.
- Raymond E, Alexandre J, Faivre S, Vera K, Maternan E, Boni J, et al. Safety and pharmacokinetics of escalated doses of weekly intravenous infusion of CCI-779, a novel mTOR inhibitor, in patients with cancer. *J Clin Oncol* 2004;22:2336–47.
- Hidalgo M, Buckner JC, Erlichman C, Pollack MS, Boni JP, Dukart G, et al. A phase I and pharmacokinetic study of temsirolimus (CCI-779) administered intravenously daily for 5 days every 2 weeks to patients with advanced cancer. *Clin Cancer Res* 2006;12:5755–63.
- European Public Assessment Report on Torisel. 2008. <http://www.emea.europa.eu/humandocs/Humans/EPAR/torisel/torisel.htm> (22 September 2008, date last accessed).

# Association of carboxylesterase 1A genotypes with irinotecan pharmacokinetics in Japanese cancer patients

Kimie Sai,<sup>1</sup> Yoshiro Saito,<sup>2</sup> Naoko Tatewaki,<sup>3</sup> Masakiyo Hosokawa,<sup>5</sup> Nahoko Kaniwa,<sup>2</sup> Tomoko Nishimaki-Mogami,<sup>1</sup> Mikihiro Naito,<sup>1</sup> Jun-ichi Sawada,<sup>1,14</sup> Kuniaki Shirao,<sup>6,15</sup> Tetsuya Hamaguchi,<sup>6</sup> Noboru Yamamoto,<sup>6</sup> Hideo Kunitoh,<sup>6,16</sup> Tomohide Tamura,<sup>6</sup> Yasuhide Yamada,<sup>6</sup> Yuichiro Ohe,<sup>6,10</sup> Teruhiko Yoshida,<sup>7</sup> Hironobu Minami,<sup>8,17</sup> Atsushi Ohtsu,<sup>9,12</sup> Yasuhiro Matsumura,<sup>11</sup> Nagahiro Saijo,<sup>13,18</sup> & Haruhiro Okuda<sup>4</sup>

<sup>1</sup>Division of Functional Biochemistry and Genomics, <sup>2</sup>Division of Medicinal Safety Science, <sup>3</sup>Project Team for Pharmacogenetics, <sup>4</sup>Division of Organic Chemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, <sup>5</sup>Laboratory of Drug Metabolism and Biopharmaceutics, Faculty of Pharmaceutical Sciences, Chiba Institute of Science, Shiomi-cho, Choshi-City, Chiba 288-0025, <sup>6</sup>Division of Internal Medicine, National Cancer Center Hospital, <sup>7</sup>Genomics Division, National Cancer Center Research Institute, 5-1-5 Tsukiji, Chuo-ku, Tokyo 104-0045, <sup>8</sup>Division of Oncology/Hematology, <sup>9</sup>Division of GI Oncology/Digestive Endoscopy, <sup>10</sup>Division of Internal Medicine, <sup>11</sup>Investigative Treatment Division, <sup>12</sup>Research Center for Innovative Oncology, <sup>13</sup>Deputy Director, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, <sup>14</sup>Pharmaceuticals and Medical Devices Agency, 3-3-2 Kasumigaseki, Chiyoda-ku, Tokyo 100-0013, <sup>15</sup>Department of Medical Oncology, OITA University Faculty of Medicine, 1-1 Idaigaoka, Hasama-machi, Yufu 879-5593, <sup>16</sup>Department of Respiratory Medicine, Mitsui Memorial Hospital, 1 Kandaizumi-cho, Chiyoda-ku, Tokyo 101-8643, <sup>17</sup>Medical Oncology, Department of Medicine, Kobe University Hospital and Graduate School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017 and <sup>18</sup>Kinki University School of Medicine, Osaka-Sayama, Osaka 589-8511, Japan

## Correspondence

Dr Kimie Sai PhD, Division of Functional Biochemistry and Genomics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan.  
Tel.: + 81 3 3700 9478  
Fax: + 81 3 3707 6950  
E-mail: sai@nihs.go.jp

## Keywords

CES1, genetic polymorphism, haplotype, irinotecan

## Received

30 November 2009

## Accepted

14 March 2010

## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Association of UDP-glucuronosyltransferase 1A1 (UGT1A1) genetic polymorphisms \*6 and \*28 with reduced clearance of SN-38 and severe neutropenia in irinotecan therapy was demonstrated in Japanese cancer patients.
- The detailed gene structure of CES1 has been characterized.
- Possible functional SNPs in the promoter region have been reported.

## WHAT THIS STUDY ADDS

- Association of functional CES1 gene number with AUC ratio [(SN-38 + SN-38G)/irinotecan], an *in vivo* index of CES activity, was observed in patients with irinotecan monotherapy.
- No significant effects of major CES1 SNPs on irinotecan PK were detected.

## AIMS

Human carboxylesterase 1 (CES1) hydrolyzes irinotecan to produce an active metabolite SN-38 in the liver. The human CES1 gene family consists of two functional genes, CES1A1 (1A1) and CES1A2 (1A2), which are located tail-to-tail on chromosome 16q13-q22.1 (CES1A2-1A1). The pseudogene CES1A3 (1A3) and a chimeric CES1A1 variant (*var1A1*) are also found as polymorphic isoforms of 1A2 and 1A1, respectively. In this study, roles of CES1 genotypes and major SNPs in irinotecan pharmacokinetics were investigated in Japanese cancer patients.

## METHODS

CES1A diplotypes [combinations of haplotypes A (1A3-1A1), B (1A2-1A1), C (1A3-*var1A1*) and D (1A2-*var1A1*)] and the major SNPs (-75T>G and -30G>A in 1A1, and -816A>C in 1A2 and 1A3) were determined in 177 Japanese cancer patients. Associations of CES1 genotypes, number of functional CES1 genes (1A1, 1A2 and *var1A1*) and major SNPs, with the AUC ratio of (SN-38 + SN-38G)/irinotecan, a parameter of *in vivo* CES activity, were analyzed for 58 patients treated with irinotecan monotherapy.

## RESULTS

The median AUC ratio of patients having three or four functional CES1 genes (diplotypes A/B, A/D or B/C, C/D, B/B and B/D; *n* = 35) was 1.24-fold of that in patients with two functional CES1 genes (diplotypes A/A, A/C and C/C; *n* = 23) [median (25th–75th percentiles): 0.31 (0.25–0.38) vs. 0.25 (0.20–0.32), *P* = 0.0134]. No significant effects of *var1A1* and the major SNPs examined were observed.

## CONCLUSION

This study suggests a gene-dose effect of functional CES1A genes on SN-38 formation in irinotecan-treated Japanese cancer patients.

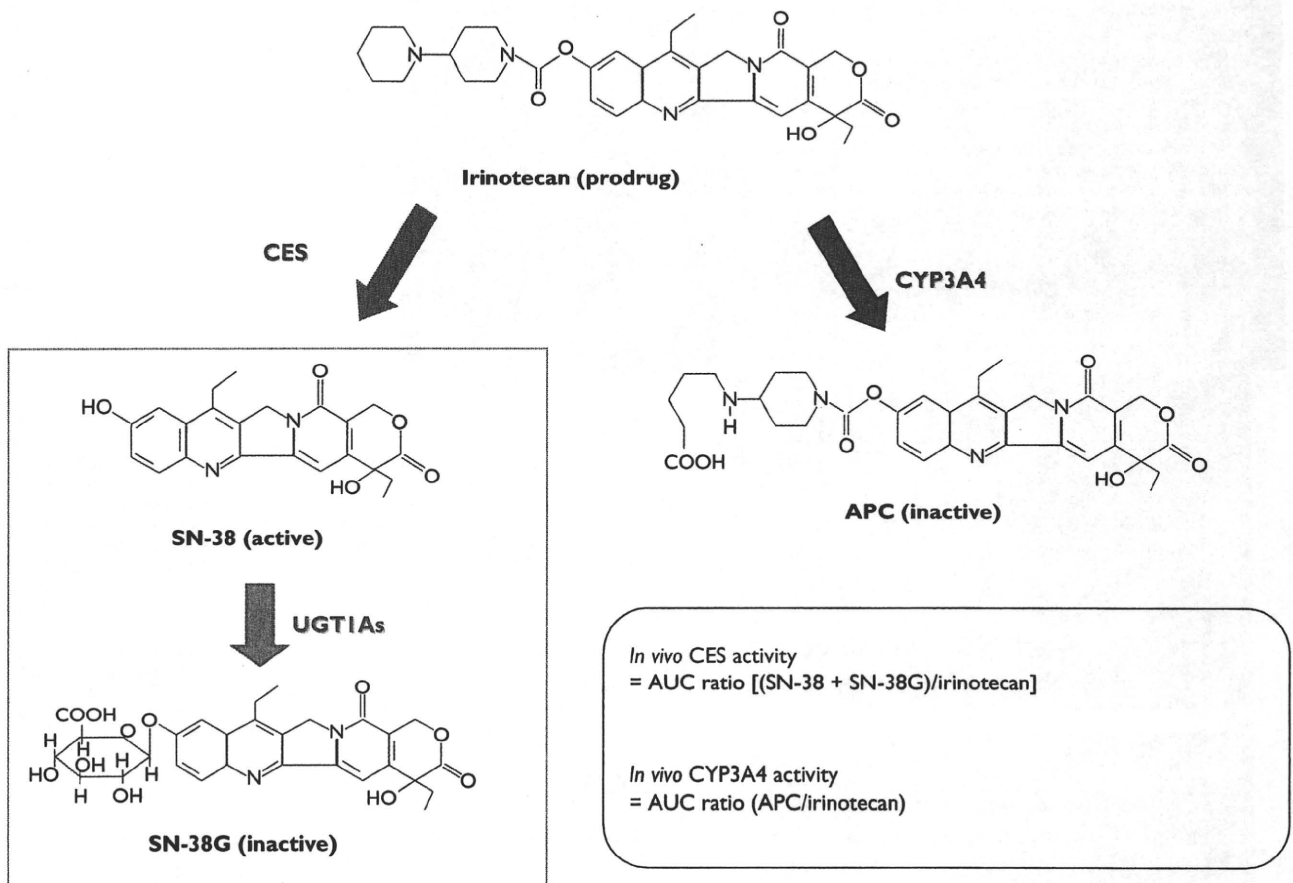
## Introduction

Human carboxylesterases (CESs) are members of the  $\alpha/\beta$ -hydrolase-fold family and are localized in the endoplasmic reticulum of many different cell types. These enzymes efficiently catalyze the hydrolysis of a variety of ester- and amide-containing chemicals as well as drugs (including prodrugs) to the respective free acids. They are involved in detoxification or metabolic activation of various drugs, environmental toxicants and carcinogens. CESs also catalyze the hydrolysis of endogenous compounds such as short- and long-chain acyl-glycerols, long-chain acyl-carnitine, and long-chain acyl-CoA esters. The two major CES families CES1 and CES2 have been identified in human tissues. CES1 is abundant in the liver and lung but not in the intestine, while CES2 is highly expressed in the intestine and kidney but has low expression in the liver and lung [1].

Human CES1 and CES2 are involved in producing a topoisomerase I inhibitor SN-38, an active metabolite of

irinotecan which is clinically used for colorectal, lung and other cancers [2]. SN-38 is further inactivated by UDP-glucuronosyltransferase 1As (UGT1As) to produce SN-38 glucuronide (SN-38G). Irinotecan is also converted by cytochrome P450 3A4 (CYP3A4) to an inactive compound 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) (Figure 1).

Recent pharmacogenetic studies on irinotecan have revealed significant associations of *UGT1A1* polymorphisms \*28 [-54\_39A(TA)<sub>6</sub>TAA>A(TA)<sub>7</sub>TAA or -40\_39insTA] and \*6 [211G>A (G71R)], the latter being specifically detected in East Asians, with reduced clearance of SN-38 resulting in severe neutropenia [3–8]. These findings have led to the clinical application of genetic testing for *UGT1A1*\*28 in the United States (since August 2005) and for *UGT1A1*\*6 and \*28 in Japan (since March 2009). In addition, possible additive effects of genotypes of the transporters for irinotecan and its metabolites, such as *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1*, have been suggested [9–12]. We previously analyzed *CES2* polymorphisms in a Japanese



**Figure 1**

Metabolic pathway of irinotecan. The prodrug irinotecan is hydrolyzed by carboxylesterase (CES) to produce an active metabolite SN-38, and subsequently detoxified by UDP-glucuronosyltransferase 1As (UGT1As) to produce an inactive metabolite SN-38 glucuronide (SN-38G). Irinotecan is also metabolized by cytochrome P450 3A4 (CYP3A4) to produce another inactive metabolite APC

population and identified minor genetic variations which were associated with lower expression/function *in vitro* and *in vivo* [13, 14]. However, major *CES2* haplotypes (\*1*b* and \*1*c*) did not affect irinotecan pharmacokinetics (PK) [14]. Since *CES1* is expressed at higher levels in the liver, a major organ for activating irinotecan, it is possible that *CES1* genotypes affect the plasma concentrations of irinotecan metabolites. However, their clinical relevance to irinotecan pharmacokinetics/pharmacodynamics has not yet been fully investigated.

Functional human *CES1* genes include *CES1A1* (1*A1*) and *CES1A2* (1*A2*), which are inversely located (tail-to-tail) on chromosome 16q13-q22.1 (1*A2*-1*A1*). Both 1*A1* and 1*A2* consist of 14 exons encoding 567 amino acids, and they have 98% homology with 5 nucleotide (4 amino acid) differences in exon 1, which encodes a signal peptide [1]. Recent studies also identified *CES1A1* variants (*var1A1*), in which exon 1 was replaced with exon 1 of *CES1A2*, and a pseudogene *CES1A3* (1*A3*; formerly referred to as *CES4*) replacing *CES1A2* [15, 16]. The 1*A3* sequence from the promoter region to exon 1 is the same as that of *CES1A2*, but contains a stop codon in exon 3. The sequence downstream from exon 11 is highly homologous with that of 1*A1* (NT\_010498) [16]. Ethnic differences in these *CES1* genes (1*A1*, *var1A1*, 1*A2* and 1*A3*) have been reported [16].

Expression levels of *CES1A2* mRNA were lower than those of *CES1A1* mRNA in several tissues. This *CES1A1* up-regulation could be mediated by additional Sp1 and C/EBP binding sites in the promoter region [17]. Transcript levels of *CES1A2* derived from *var1A1* were reported to be higher than those from the original 1*A2* [15, 16]. These findings suggest that polymorphisms in the upstream region of *CES1A1* or *var1A1* could affect their expression.

In addition to structural variations of the *CES1* gene family, several single nucleotide polymorphisms (SNPs) and small deletion/insertion variants were found. -816C in the *CES1A2* promoter region was reported to be associated with enhanced *CES1A2* expression and imidapril efficacy [18]. Furthermore, -816A>C was found to be linked with several SNPs (-62T>C, -47G>C, -46G>T, -41C>G, -40A>G, -37G>C, -34del/G and -32G>T) in the proximal promoter region, leading to two additional Sp1 binding sites, and these additional sites were suggested to increase transcription of 1*A2* [19].

In this context, this study investigated the clinical significance of *CES1* genotypes in irinotecan therapy. For this purpose, we analyzed the *CES1* genotypes (combinations of four *CES1A* isoforms) and major SNPs in the *CES1A1* exon 1 with its adjacent region and in the *CES1A2* and 1*A3* promoter regions, which could be important for *CES1* expression or function, in Japanese cancer patients treated with irinotecan, and then examined the associations of these *CES1* genotypes or SNPs with irinotecan PK.

## Methods

### Patients

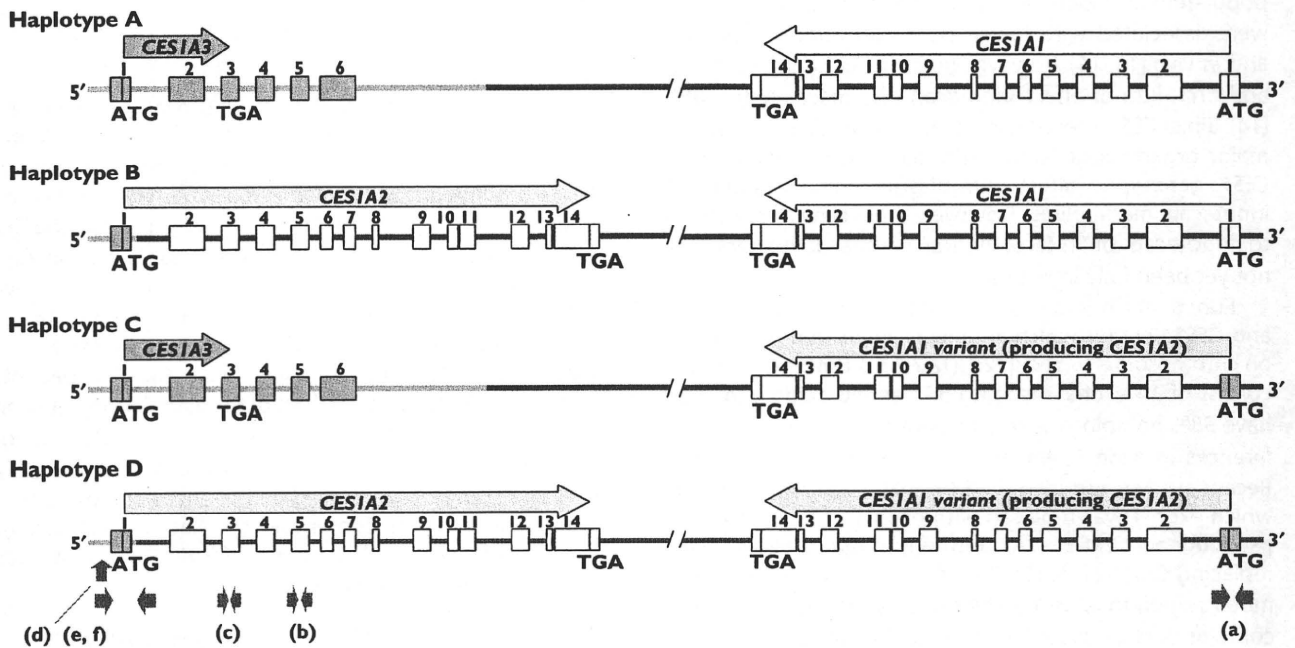
Genetic analysis of 177 Japanese cancer patients who received irinotecan therapy at the National Cancer Center in Japan was performed. The patients were the same as those described in our previous study [7], where details on eligibility criteria for irinotecan therapy, patient profiles and irinotecan regimens were described. Since the AUC ratio [(SN-38 + SN-38G) : irinotecan], a parameter of *in vivo* *CES* activity, was influenced by irinotecan regimens [14], 58 patients receiving irinotecan monotherapy (100 mg m<sup>-2</sup> weekly or 150 mg m<sup>-2</sup> biweekly) from the 177 patients were primarily used for analysis of the association between *CES1* genotypes and irinotecan PK parameters. The patient set was the same as used in our previous study on *CES2* [14]. 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.

### Determination of *CES1* genotypes and SNPs

For describing the *CES1* gene family, haplotypes A to D designated by Fukami *et al.* [16] were used (Figure 2): haplotype A, *CES1A3-CES1A1* (1*A3*-1*A1*); haplotype B, *CES1A2-CES1A1* (1*A2*-1*A1*); haplotype C, *CES1A3-CES1A1* variant (1*A3*-*var1A1*); and haplotype D, *CES1A2-CES1A1* variant (1*A2*-*var1A1*). To determine the diplotypes, combinations of haplotypes A to D, we sequenced 1*A1*/*var1A1* exon 1 and its flanking region and the 1*A2*/1*A3* promoter region of 177 patients. These regions are indicated in Figure 2, and a list of primers/probes is shown in Table 1.

For discrimination between 1*A1* and *var1A1*, their exon 1s and flanking regions were sequenced (Figure 2a). Briefly, the first PCR was performed using 25 ng of genomic DNA with 0.625 units of Ex-Taq (Takara Bio. Inc., Shiga, Japan) and 0.2 μM of primers, Ces1-FP and Ces1-RP (Table 1a, first PCR). The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. Then, the second PCR was performed with the primers, Ces1\_seqF and Ces1\_seqR (Table 1a, second PCR) under the same reaction conditions described above. The PCR products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequencing primers listed in Table 1a (sequencing). Excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3730 DNA Analyzer (Applied Biosystems). The conditions of the PCR and sequencing procedures described in the following section were the same as described above unless otherwise noted.

1*A2* and 1*A3* were discriminated by the restriction fragment length polymorphism (RFLP) method for exon 5



**Figure 2**

*CES1* gene structure and haplotypes. The regions used for haplotype determination in this study are indicated with arrows (a-f)

reported by Fukami *et al.* [16] (Figure 2b). Briefly, the PCR was performed using a primer set (1A-int4F and 1A-int5AS) (Table 1b), and then the PCR products were digested with *PvuII* to produce *CES1A3*-derived fragments (409 bp and 248 bp). UV intensity of the fragments stained with ethidium bromide was measured after electrophoresis (2% agarose gel). The number of 1A3 (0, 1 or 2) was also confirmed by direct sequencing of exon 5 using the same primer set. To verify that the 1A3 sequence is derived from the pseudogene, we confirmed the existence of a stop codon at codon 105 of 1A3 exon 3 (Figure 2c) in 11 randomly selected patients (heterozygous or homozygous) by amplification and sequencing using primers listed in Table 1c.

Genotyping for -816A>C in the 1A2 and 1A3 promoter region (Figure 2d) was conducted by the TaqMan method of Geshi *et al.* [18] (Table 1d) in all patients. We also examined attribution of -816C to 1A2 or 1A3 by specific amplifications from 5'-regions to intron 1 of the 1A2 and 1A3 (Figure 2e,f) in 23 randomly selected heterozygous patients. For specific amplifications, primers CES1A3-1A2\_F1 and CES1A2 R1 for *CES1A2* (Table 1e) and primers CES1A3-1A2\_F1 and CES1A3 R1 for 1A3 (Table 1f, first PCR) were used with 0.05 U  $\mu\text{l}^{-1}$  LA-Taq with GC buffer I (Takara Bio. Inc.); and for 1A3, the second PCR using primers CES1A3-1A2\_F2 and CES1A3 R2 (Table 1f, second PCR) was also conducted with 0.05 U  $\mu\text{l}^{-1}$  Ex-taq. Then, direct sequencing of the 1A2 and 1A3 PCR products was per-

formed. Complete linkage among -816A>C and several SNPs in the proximal promoter region (between -62 to -32) [19] was confirmed for 11 randomly selected subjects.

All variations were confirmed by sequencing PCR products generated from new amplifications from genomic DNA. GenBank NT\_010498.15 was used as the reference sequence for *CES1A1*, *CES1A3* and the promoter region of *CES1A2*, and AB119998.1 was used for exon 1 and its downstream region of *CES1A2*. The translational initiation site was designated as +1 to describe the polymorphism positions. Diplotype configuration was estimated with the LDSUPPORT software [20]. The diplotypes A/D and B/C could not be distinguished.

#### Pharmacokinetic data and association analysis

The area under the concentration-time curve (AUC) values for irinotecan and its metabolites, SN-38, SN-38G and APC, were previously obtained [4, 21]. The AUC ratio of SN-38 plus SN-38G to irinotecan [ $\text{AUC}_{\text{SN-38 + SN-38G}}/\text{AUC}_{\text{irinotecan}}$ ] was used as a parameter reflecting *in vivo* CES activity [14]. The AUC ratio of APC to irinotecan [ $\text{AUC}_{\text{APC}}/\text{AUC}_{\text{irinotecan}}$ ] was used as a parameter for *in vivo* CYP3A4 activity [21].

Statistical significance (two-sided,  $P < 0.05$ ) for associations between AUC ratios (or AUC/dose) and *CES1* genotypes or SNPs was determined by the Mann-Whitney test or the Jonckheere-Terpstra (JT) test using Prism version 4.0 (GraphPad Prism Software Inc. San Diego, CA, USA) and StatXact version 6.0 (Cytel Inc., Cambridge, MA). Correla-



**Table 1**

Primers and probes used in this study

Region (indicated in Figure 2)	Primer	Primer sequence	Reference	
<b>(a) CES1A1 exon 1 and promoter region</b>	<b>First PCR</b>	Ces1-FP	5'-CCAGGCAAAACCTAGGAGTG-3'	This study
		Ces1-RP	5'-AGTACAGGGCGATCTCAGGA-3'	
	<b>Second PCR</b>	Ces1_seqF	5'-GTATTCCTTAGCCAGCGTA-3'	
		Ces1_seqR	5'-CAGAGCCGGACCTGTTGT-3'	
	<b>Sequencing</b>	Ces1_SF2	5'-AGAGCCTGGAAAAGCTATGAAAA-3'	
		Ces1_SR	5'-TTCTACGCATCTGCGCCACC-3'	
<b>(b) CES1A1, 1A2 and 1A3 exon 5 PCR and sequencing</b>	1A-int4F	5'-GCTCAGTAAATAGTTGCCAGTT-3'	[16]	
	1A-int5AS	5'-TCTCATCAGCATCACATCAAG-3'		
<b>(c) CES1A3 exon 3 PCR and sequencing</b>	CES1A3-15183F	5'-CAGGGAAGATCGTTGATTTGGTTT-3'	This study	
		5'-TTCTTCCACCACTAACATTATG-3'		
	CES1A3-15823R	5'-AAGATGTTTCATTAAGATGCACAG-3'		
		Sequencing (additional primer)		
<b>(d) CES1A2 and 1A3 -816A&gt;C genotyping PCR</b>	F	5'-CCTTAATTTGGTGATTTACATTGC-3'	[18]	
	R	5'-CAAGACATGGTTCAGCTTCTCAAG-3'		
	FAM	5'-CATCACCCCTACTGC-3'		
	VIC	5'-CATCACACCTACTGCT-3'		
<b>(e) CES1A2 promoter region PCR</b>	CES1A3-CES1A2_F1	5'-ATGATTTCCAGCTTCATCTACA-3'	This study	
		CES1A2_R1		5'-GAGAGAACGTTCCCATGTCTTT-3'
<b>(f) CES1A3 promoter region</b>	<b>First PCR</b>	CES1A3-CES1A2_F1	5'-ATGATTTCCAGCTTCATCTACA-3'	This study
		CES1A3_R1	5'-GCTTGAGTTTTCTTACAGACA-3'	
	<b>Second PCR</b>	CES1A3-CES1A2_F2	5'-AACAGTTTATAACCTGTTATTTT-3'	
		CES1A3_R2	5'-TGCTTTGGATAAGACAAGATGTT-3'	
	<b>Sequencing of CES1A2/1A3 promoter region</b>	CES1A3-CES1A2_F2	5'-AACAGTTTATAACCTGTTATTTT-3'	
		CES1A3-CES1A2_R1	5'-CACACTTCCAATCTCAGGTA-3'	
		CES1A3-CES1A2_F3	5'-TTATGCCACAAGCAGTTGGGCG-3'	
CES1A3-CES1A2_R2		5'-TCCAAGTCCAATCCAAGTACGGA-3'		

NT\_010498.15 was used as the reference sequence for CES1A1, CES1A3 and the promoter region of CES1A2, and AB119998.1 was used for exon 1 and its downstream region of CES1A2.

tions between the AUC ratios  $[AUC_{(SN-3B + SN-38G)} / AUC_{Irinotecan}]$  and  $[AUC_{APC} / AUC_{Irinotecan}]$  were analyzed by Spearman's rank correlation test. Multiplicity adjustment was not applied to bivariate analysis, and contributions of the candidate genetic markers to the AUC ratios  $[AUC_{(SN-3B + SN-38G)} / AUC_{Irinotecan}]$  were further determined by multiple regression analysis after logarithmic transformation of the AUC ratio. The variables examined were age, sex, body surface area, history of smoking or drinking, performance status, serum biochemistry (GOT, ALP, creatinine) at baseline, CES1 genotypes and SNPs, CES2\*2 [100C>T(R34W)] or \*5 [1A>T (M1L)] [13, 14], UGT1A1\*6 or \*28 [7, 8], and the transporter haplotypes, ABCB1\*2 [2677G>T(A893A)], ABCC2\*1A (-1774delG), ABCG2#IIB [421C>A (Q141K) and IVS12+49G>T] and SLC01A1\*15-17 [521T>C (V174A)] [10]. The variables in the final models were selected by the forward and backward stepwise procedure at a significance level of 0.10 using JMP version 7.0.0 (SAS Institute, Inc., Cary, NC, USA). UGT1A1\*6 or \*28 was grouped as '+' for stratifying patients: for example, homozygous UGT1A1 \*6 or \*28 was depicted as UGT+/-.

## Results

### Genotypes and SNPs of CES1 gene family in Japanese

Frequencies of individual CES1 genes and CES1 diplotypes stratified according to the number of functional CES1 genes are summarized in Table 2. The frequencies of the patients with two, three and four functional CES1 genes were 44%, 47% and 9%, respectively, in all 177 patients.

By sequencing 1A1 and var1A1 exon 1s and their flanking region, we detected four novel variations; three in the 5'-flanking region and one in the 5'-untranslated region (5'-UTR) (Table 3): -258C>T (allele frequency: 0.014), -233C>A (0.003), -161A>G (0.006) and -30G>A (0.042). Eleven nucleotide substitutions from the 5'-UTR to intron 1 at allele frequencies of 0.294–0.299 were closely linked with var1A1 (Table 3). The SNP -816A>C found in the 1A2 and 1A3 promoter regions was genotyped by a TaqMan method [18], and the allele frequency of -816A>C in 177 subjects was 0.249 (Table 4). It was noted that -816C was detected only in patients with 1A3 (1A3/1A2 and 1A3/1A3),

**Table 2**Frequency of *CES1* genes and diplotypes in Japanese cancer patients

<i>CES1</i> diplotype	Number of <i>CES1</i> gene				Total*	Frequency (n = 177)†		Frequency (monotherapy: n = 58)†	
	1A1	var1A1	1A2	1A3					
A/A	2	0	0	2	2	0.203	0.441	0.138	0.397
A/C	1	1	0	2		0.220		0.241	
C/C	0	2	0	2		0.017		0.017	
A/B	2	0	1	1	3	0.237	0.469	0.293	0.534
A/D or B/C	1	1	1	1		0.192		0.190	
C/D	0	2	1	1		0.040		0.052	
B/B	2	0	2	0	4	0.040	0.090	0.017	0.069
B/D	1	1	2	0		0.034		0.052	
D/D	0	2	2	0		0.017		0.000	
Frequency (n = 354)*	0.703	0.297	0.325	0.675					
(monotherapy: n = 116)‡	0.690	0.310	0.336	0.664					

\*Number of functional genes. †Number of subjects. ‡Number of chromosomes.

but not in the 1A2 homozygotes (1A2/1A2). In the 1A2/1A3 patients, 38 of the 39 patients having -816C were heterozygous for -816C (Table 4). These findings suggested a close association between -816C with 1A3. Following specific amplifications of the regions from 5'-regions to intron 1 in 1A2 and 1A3 (Figure 2e,f) of 23 patients randomly selected from the 38 patients with -816A/C and 1A2/1A3, we confirmed that -816C resided in the 1A3 gene (data not shown). Thus, -816A>C is the major SNP of 1A3 but very rare in 1A2. In addition, the SNPs, -62T>C, -47G>C, -46G>T, -41C>G, -40A>G, -37G>C, -34del/G and -32G>T, in the proximal promoter region reported to be linked with -816A>C [19] were found to be completely linked with 1A3 (data not shown).

#### Association of *CES1* genotypes with *in vivo* *CES* activity

***CES1* diplotypes** In patients treated with irinotecan monotherapy, we found the AUC ratios of patients with haplotypes A or C (having the 1A3 pseudogene) were lower than those without A or C, indicating functional *CES1* gene number dependency. The median AUC ratio of patients having three or four functional *CES1* genes was 1.24-fold of that in patients with two functional *CES1* genes [median (25th–75th percentiles): 0.31 (0.25–0.38) vs. 0.25 (0.20–0.32),  $P = 0.0134$ , Mann-Whitney test] (Figure 3a). No significant differences were observed between 1A1 and var1A1 (among 1A1/1A1, var1A1/1A1 and var1A1/var1A1). As we previously reported, the *CES2* variations, *CES2*\*5 [1A>T(M1L)] and *CES2*\*2 [100C>T(R34W)] [13, 14] showed low *CES* activity as indicated in Figure 3a.

Platinum-containing regimens themselves enhance renal excretion of irinotecan and its metabolites, especially SN-38G. No significant effect of *CES1* gene number on the AUC ratio was observed. However, it was noted that the median renal excretion ratio [(SN-38 + SN-38G)/irinotecan] in patients with four functional *CES1* genes was 1.37-fold higher than that in patients with two or three

functional genes ( $P = 0.0217$ , Mann-Whitney test) (data not shown).

To exclude the possibility that the higher AUC ratio observed above (Figure 3a) was biased by CYP3A4, another metabolic enzyme for irinotecan, we analyzed the association between the (SN-38 + SN-38G)/irinotecan AUC ratio and the APC/irinotecan AUC ratio, an *in vivo* parameter of CYP3A4 activity [21], in patients treated with irinotecan monotherapy. The result showed no correlation between the two parameters (Spearman  $r = 0.126$ ,  $P = 0.345$ ).

***CES1* SNPs** Next, associations of the two 1A1 SNPs, -75G>T and -30G>A (Table 3) and 1A3-816A>C with the AUC ratio [(SN-38 + SN-38G)/irinotecan] were analyzed. The effects of the SNPs were analyzed in patients stratified by the functional *CES1* gene number and also in all the patients receiving monotherapy. A -75G>T-dependent increase in the AUC ratio was observed in the whole group of patients ( $P = 0.027$ , JT test) (Figure 3b), and this trend was remarkable in patients with three or four functional *CES1* genes. No significant effect of -30G>A was observed (Figure 3c). As for -816C in 1A3, no association between this SNP and the AUC ratio was evident in patients with two or three functional *CES1* genes (Figure 3d). In the platinum-containing regimens, no significant effects of these SNPs on the AUC ratio or the renal recovery ratio were observed (data not shown).

**Multivariate analysis** The contribution of *CES1* genotypes to the AUC ratio was further analyzed by multivariate analysis, using the patient background factors and polymorphisms including the haplotypes of *CES2*, *UGT1A1* and transporters as variables [7, 8, 10, 13, 14]. The final model revealed a significant association of the functional *CES1* gene number ( $n = 3$  or 4) with the AUC ratio. Contributions of smoking history, irinotecan dose, hepatic and renal function were also detected while that of *ABCB1*\*2 (+/+) was

**Table 3**  
Summary of genetic variations of *CES1A1* and *var 1A1* exon 1s and their flanking regions detected in this study

SNP identification			Position		From the translational initiation site or the nearest exon	Nucleotide change and flanking sequences (5' to 3')	Amino acid change	Allele frequency (n = 354)*	CES1A1 variant (CES1A2 type)
This study	NCBI (dbSNP)	JSNP	Location	NT_010498.15					
MPJ6_CS1001†			5'-flank	9481424	-258	tgggcaagttaacagctctCTTgtaactgacagtagagtc		0.014	
MPJ6_CS1002†			5'-flank	9481399	-233	aicgacagtagagctccagaCAggtttgatgaaagaggta		0.003	
MPJ6_CS1003†			5'-flank	9481327	-161	tagaacccaggagatctgAGggaaaggagggtttttctg		0.006	
MPJ6_CS1004	rs3815583	IMS-JST175949	Exon1(5'-UTR)	9481241	-75	aacttggcgggctgggcG/Tccagggtctggacagacagi		0.41	var1A1
MPJ6_CS1005	rs28429139		Exon1(5'-UTR)	9481212	-46	ggacagacagtcctctgaaAGctgtcacagagacctcgaag		0.299	var1A1
MPJ6_CS1006	rs28494177		Exon1(5'-UTR)	9481205	-39	acagtcctctgaactgacAGgagacctcgaagcccccag		0.299	var1A1
MPJ6_CS1007†			Exon1(5'-UTR)	9481196	-30	ctgaactgacagagacctcG/Acaggcccgagaaactgtgc		0.042	var1A1
MPJ6_CS1008	rs28520463		Exon1(5'-UTR)	9481187	-21	acagagacctcgaagcccccG/Cagaaactgtccctccag		0.297	var1A1
MPJ6_CS1009	rs28499065		Exon1(5'-UTR)	9481186	-20	cagagacctcgaagcccccAGGgaactgtccctccaga		0.297	var1A1
MPJ6_CS1010	rs28515828		Exon1(5'-UTR)	9481168	-2	ccctccacagatggtctcccaC/Ggagtggtctcgtgcttta		0.299	var1A1
MPJ6_CS1011			Exon 1	9481156	11	tccagtggtggtccctgctcG/Cgaccttactctggccactc	Arg4Pro	0.297	var1A1
MPJ6_CS1012			Exon 1	9481152	15	ccagatggtcctgctgctCTTtattcttggcactctctc	Ala5Ala	0.297	var1A1
MPJ6_CS1013			Exon 1	9481151	16	ccagatggtcctgctgctCTTtattcttggcactctctc	Phe6Leu	0.297	var1A1
MPJ6_CS1014			Exon 1	9481148	19	cgatggtcctgctgcttTA/Gtcttggcactctctctgct	Ile7Val	0.297	var1A1
MPJ6_CS1015	rs28563878		Exon 1	9481133	34	tgcttttactggcactctCT/Gctgttcccggttgggg	Ser12Ala	0.297	var1A1
MPJ6_CS1016	rs12149359		Intron 1	9481099	IVS1+16	ttgggggagctctcttgaAA/Gtcaaaatcggggcactttt		0.294	var1A1

\*Number of chromosomes. †Novel variation detected in this study.

**Table 4**Frequency of *CES1A2*/(*1A3*) promoter SNP -816A>C in Japanese cancer patients

<i>CES1A2</i> and <i>1A3</i>	-816A>C	Number of subjects	Allele frequency
<b>1A2/1A2</b>	A/A	16	0/32 (0%)
	A/C	0	
	C/C	0	
<b>1A2/1A3</b>	A/A	44	40/166 (24.1%)
	A/C	38	
	C/C	1	
<b>1A3/1A3</b>	A/A	41	48/156 (30.8%)
	A/C	26	
	C/C	11	
<b>Total</b>		177	88/354 (24.9%)

not significant (Table 5). The *CES1* genotypes explained 22.6% of variability in the final model among all the variables and 11.3% of total variability in the AUC ratio.

#### Effects of *CES1* genotypes on SN-38 AUC and toxicity

To clarify the clinical importance of *CES1* genotyping for irinotecan therapy, the effects of *CES1* genotypes or SNPs on AUC levels of the active metabolite SN-38 and neutropenia were examined in the non-*UGT*+/+ patients. In this non-*UGT*+/+ population, significantly higher AUC ratios of (SN-38 + SN-38G)/irinotecan were also observed in the patients with three or four functional *CES1* genes ( $P = 0.0234$ , Mann-Whitney test) as observed in all the patients treated with irinotecan monotherapy (Figure 3a). With increased number of functional *CES1* genes, an increasing trend of SN-38 AUC/dose was observed in patients receiving irinotecan monotherapy (1.4-fold for four genes vs. two genes;  $P = 0.080$ , JT test) (Figure 4). However, multiple regression analysis revealed no statistically significant contribution of *CES1* genotypes to SN-38 AUC/dose although *UGT1A1*\*6 or \*28 and *ABCB1*\*2/\*2 showed significant contributions [10]. Regarding neutropenia, a higher incidence (though statistically insignificant) for grade 3/4 neutropenia in patients with four functional *CES1* genes was observed (50% for four genes and 16% for two or three genes,  $P = 0.09$ , Fisher's exact test). The effects of the SNPs (-75G>T, -30G>A and -816A>C) on SN-38 AUC or incidence grade 3/4 neutropenia were not significant (data not shown). In platinum-containing regimens, no significant effects of the *CES1* genotypes on SN-38 AUC/dose or incidence of grade 3/4 neutropenia were detected in the non-*UGT*+/+ patients (data not shown).

## Discussion

Recent pharmacogenetic studies on irinotecan have shown the clinical significance of *UGT1A1* \*6 and \*28 in Japanese

patients [7,8] and *UGT1A1*\*28 in Caucasians [5,6] for severe neutropenia. Subsequent studies have revealed additional genetic factors including transporters [10–12]. However, the clinical importance of genotypes of the irinotecan-activating enzymes *CES1* and *CES2* is still uncertain.

Since the hydrolytic activity of *CES2* for irinotecan was reported to be much higher than that of *CES1* [2], most studies have focused on the clinical significance of *CES2* polymorphisms in irinotecan therapy [13, 14, 22]. We previously identified minor *CES2* genetic variations in Japanese, including *CES2*\*2 [100C>T (R34W)] and *CES2*\*5 [1A>T (M1L)] which caused low *in vitro* expression/function of *CES2* [13, 14] and also exhibited reduced *in vivo* *CES* activity in irinotecan-treated patients [14] (also see Figure 3a). However, the major *CES2* haplotypes in Japanese, \*1b (IVS10-108G>A and 1749A>G, frequency = 0.233) and \*1c (-363C>G, IVS10-108G>A and IVS10-87G>A, frequency = 0.027), did not show any significant effects on irinotecan PK [14]. No clinical significance of *CES2* polymorphisms has been reported in Caucasians [22]. Neither *CES1* nor *CES2* SNPs affecting their mRNA expression in normal colonic mucosa were found in European and African populations [23]. Since precise structures of the *CES1* genes and their promoter regions had not been elucidated, evaluation of the roles of the *CES1* genotypes in irinotecan therapy has been rather difficult.

In the present study, the frequencies of individual *CES1* genes (*1A1*, *var1A1*, *1A2* and *1A3*) (Table 2) were almost comparable with the previous report in the Japanese population (0.748, 0.252, 0.313 and 0.687, respectively) [16]. To our knowledge, the present study is the first report suggesting a possible effect of *CES1* genotypes on irinotecan PK. This study showed that the AUC ratio [(SN-38 + SN-38G)/irinotecan], and probably *in vivo* *CES* activity, was elevated depending on the number of functional *CES1* genes (*1A1*, *var1A1* and *1A2*) in patients treated by irinotecan monotherapy (100 or 150 mg m<sup>-2</sup> irinotecan) (Figure 3a). This gene-dose effect was not clearly shown in the platinum-containing combination therapy (60–70 mg m<sup>-2</sup> irinotecan), where renal excretion of irinotecan and its metabolites (especially SN-38G) is highly enhanced by a large volume of infusion fluid. However, the median renal excretion ratio [(SN-38 + SN-38G)/irinotecan] in patients with four functional genes was 1.37-fold higher than that in patients with two or three functional genes in the platinum-containing therapy (data not shown), supporting a partial but significant contribution of the *CES1*s to activate irinotecan. The present study showed no significant differences in the AUC ratios between *1A1* and *var1A1* (Figure 3a), indicating a common upstream region may be involved in regulation of gene expression of *1A1* and *var1A1*. The previous reports showed the expression levels of *CES1A2* were lower than those of *CES1A1* [17] and suggested that *CES1A2* mRNA was derived mainly from transcription of *var1A1* rather than the original *1A2* [15, 16]. The present study, on the other hand, has suggested that the