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Phase I study of irinotecan and doxifluridine for metastatic colorectal cancer focusing on the *UGT1A1*28* polymorphism

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Although individuals carrying the *UGT1A1* allele **28* have an increased risk of severe toxicities associated with irinotecan, no phase I study has been conducted based on the polymorphism. This report presents the recommended doses of irinotecan for patients with the respective genotypes. Twenty-seven patients with advanced colorectal cancer were enrolled in this study, and the *UGT1A1*28* polymorphism was genotyped before chemotherapy. One course of chemotherapy consisted of irinotecan infused once every 2 weeks at 70, 100, 120, and 150 mg/m^2 at dose levels 1, 2, 3, and 4, respectively, and doxifluridine was administered orally. This treatment continued for at least 12 weeks. The dose-limiting toxicity was determined as grade 3 hematological and non-hematological toxicities for the TA_6/TA_6 (6/6) and TA_6/TA_7 (6/7) genotypes. The pharmacokinetics of irinotecan, SN-38, and SN-38 glucuronide, was assessed at dose level 2. Eighteen and nine patients had the 6/6 and 6/7 genotypes, respectively. The maximum tolerated dose (MTD) was not observed up to dose level 4 in patients with the 6/6 genotype. In contrast, MTD was observed at dose level 2 (100 mg/m^2) in patients with the 6/7 genotype. Patients with the 6/7 genotype had a significantly higher area under the plasma time–concentration curve $_{0\text{-}\infty}$ SN-38 (P = 0.022) and biliary index (P = 0.030) than those with 6/6. The recommended starting doses of biweekly irinotecan for phase II/III were 150 mg/m^2 for patients with the *UGT1A1* 6/6 genotype and 70 mg/m^2 for those with the 6/7 genotype, respectively. The gene polymorphism should be considered when determining the precise recommended doses to be administered in phase I studies. (*Cancer Sci* 2010; 101: 722–727)

Irinotecan with fluoropyrimidine has been approved worldwide as one of the first-line therapies for metastatic colorectal cancer.[1–3] Although prolonged survival has been noted with the use of these drugs, severe diarrhea and neutropenia have also been reported in 20%–35% of patients treated. Recent studies have revealed that the risk of severe toxicity might be predicted by determining the genetic variation of irinotecan metabolism. Irinotecan is activated by hydrolysis to SN-38, a potent topoisomerase I inhibitor[4] that is primarily inactivated through biotransformation into SN-38 glucuronide (SN-38G) by *UGT1A1*.[5] The toxicity of irinotecan has been reported to correlate with the polymorphism of the number of TA repeats in TATA box of the promoter region of the *UGT1A1* gene (*UGT1A1*28*) that affects the transcriptional efficiency.[6] Because of the clinical importance of the glucuronidation pathway in irinotecan treatment, *UGT1A1*28* was chosen as a candidate predictor of severe toxicity.[7–9] According to cumulative evidence, an advisory meeting by the subcommittee of the Food and Drug Administration Center or Drug Evaluation and Research was held in November 2004 to consider the role of *UGT1A1*28* genotyping in the administration of irinotecan (http://www.fda.gov/). In 2005, the US labeling of irinotecan was updated in order to provide pharmacogenetic information for patients known to be homozygous for the *UGT1A1*28* allele, with a dose reduction of irinotecan to be considered when administered alone or in combination with other agents. However, no phase I dose escalation study has been performed to find the optimal doses of irinotecan based on the *UGT1A1* polymorphism. The presents study describes the results of a phase I study of irinotecan and Doxifluridine (5′-DFUR) focusing on the polymorphism to determine the maximum tolerated dose (MTD) and the recommend dose (RD) of irinotecan for the respective *UGT1A1* genotypes.

The Irinotecan combined with fluorouracil and leucovorin (FOLFIRI) regimen has been approved as the first-line chemotherapy for advanced colorectal cancer,[3] but the inconvenience and morbidity associated with long-term central venous access has prompted the development of alternative regimens. 5′-DFUR (an intermediate form of capecitabine) is an oral fluoropyrimidine that was rationally designed to generate fluorouracil (FU), preferentially at the tumor site via an enzymatic process where pyrimidine phosphorylases, which are present at higher concentrations in tumor tissues in comparison to normal tissues, thus leading to higher FU concentrations within tumor cells.[10] Experimental data related to the cytotoxicity of 5′-DFUR on human bone marrow stem cells and human tumor cell lines confirm its cytotoxic selectivity for human tumor cells.[11] Furthermore, a randomized phase III study provided the results of a comparison of 5′-DFUR and FU supporting the better therapeutic index of 5′-DFUR.[12] In the present study, 5′-DFUR was selected to be combined with irinotecan because low bone marrow suppression, a better therapeutic index, and the improved quality of life of patients have been associated with it.

The aim of the present study was to confirm the RD of irinotecan when combined with a fluoropyrimidine in patients with advanced colorectal cancer.

Materials and Methods

Patients. Patients were eligible for this study if they met the following criteria: proven unresectable or recurrent colorectal cancer; aged between 20 and 75 years; no major surgery, radiotherapy, or chemotherapy within 4 weeks prior to the study; an Eastern Cooperative Oncology Group performance status of 0–2; predicted life expectancy of at least 3 months; adequate baseline organ functions, defined as a leukocyte count of at least 4000/μL, neutrophil count of at least 2000/μL, platelet count of at least 100 000/μL, hemoglobin of at least 9 g/dL, and aspartate

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aminotransferase and alanine aminotransferase levels three times or less than the upper limit of the institutional reference range; total bilirubin below 1.5 mg/dL; and serum creatinine below 1.5 mg/dL. Patients were ineligible if they had any of the following conditions: serious infectious disease or other severe complications (e.g. pulmonary fibrosis/interstitial pneumonia, uncontrollable diabetes); watery diarrhea, paralytic ileus, or intestinal obstruction; massive pleural effusion or ascitic fluid; symptomatic brain metastases; active concurrent malignancies; pregnancy or lactation, or trying to get pregnant; a history of drug allergy; and prior treatment with irinotecan. The study was conducted according to the Declaration of Helsinki and approved by the Institutional Review Board of Yamaguchi University Hospital and Ethical Review Committee of Gene Analysis Research of Yamaguchi University School of Medicine and University Hospital (Yamaguchi, Japan). All of the patients gave their written, informed consent to participate in the study.

Pretreatment evaluation and follow up. The pretreatment evaluation included obtaining a complete medical history, physical examination, chest X-ray, electrocardiography, and imaging of measurable disease, determination of the complete blood cell count, and biochemical screening. During treatment, patients were monitored for clinical toxicities, complete blood cell count, serum chemistry, and physical condition before each biweekly dose of chemotherapy. Adverse events were evaluated according to the Common Terminology Criteria for Adverse Events v3.0. In addition, the target lesions were measured using computed tomography, which was performed before each treatment course and at the end of treatment. The clinical response was evaluated in accordance with the Response Evaluation Criteria in Solid Tumor.[13]

Treatment plan. Patients received treatment for 12 weeks. Irinotecan was administered once every 2 weeks in 500 mL normal saline or dextrose via 120-min intravenous infusion on days 1, 15, 29, 43, 57, and 71. 5′-DFUR was given as 200-mg capsules; two capsules were administered orally in the morning and evening after a meal on five consecutive days followed by a 2-day washout during the 12-week treatment period. The prophylactic use of granulocyte colony-stimulating factor was not allowed. In the case of intolerable toxicity, disease progression, or patient refusal, the study treatment was discontinued.

If any toxicity required a dosing delay of more than 4 weeks, the patient was withdrawn from the study due to toxicity. In patients who experienced dose-limiting toxicity (DLT) or required a dosing delay of more than 2 weeks, irinotecan was administered at one level lower than the original dose. In patients who experienced DLT after the dose reduction, the protocol treatment was stopped. DLT was defined as any grade 3 or 4 non-hematological toxicity (except nausea, vomiting, and alopecia), hematological toxicity, or discontinuation of treatment due to treatment-related toxicity during six courses of treatment.

Dose-escalation schedule. The initial dose of 5′-DFUR was fixed at 576 mg/m^2 per day at a dose of 800 mg/body for patients with ≥1.39 m^2 of body surface area, or 600 mg/body for those with <1.39 m^2, and irinotecan doses of 70, 100, 120, and 150 mg/m^2 were studied. Cohorts of three patients were to be entered at each dose level, starting at dose level 1. If any DLT was observed in any of the first three patients, an additional three patients were enrolled at the same dose level. If three or more patients at any dose level experienced the same DLT, the dose was determined to have reached the MTD, and the dose level below the MTD was considered to be the recommended dose for further studies.

***UGT1A1*28* and **6* genotyping.** Genomic DNA was extracted from peripheral blood anticoagulated with EDTA-2Na using a conventional sodium iodide (NaI) method.[14] The TA index of the *UGT1A1* promoter was genotyped by fragment sizing. Polymerase chain reaction (PCR) was performed in a total volume of 10 μL containing template DNA (80 ng/μL), according to the manufacturer's instructions (Ex *Taq*; TaKaRa, Tokyo, Japan). The primers used were a forward primer that was modified by the addition of a 5′ fluorescent label FAM and an unlabeled reverse primer (UGT–FAMF1; FAM 5′-GTGACACAGTCAAA CATTAACTTGGT-3′, UGT–R1; 5′-GCCTTTGCTCCTGCCA GAGGTT-3′). The amplification was performed with a Gene Amp PCR System PC808 (ASTEC, Tokyo, Japan), with initial denaturation at 95°C for 2 min, followed by 27 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s. The PCR product (TA_6, 94 bp; TA_7, 96 bp) and Hi-Di formamide (including the internal size standard [GeneScan 500, Applied Biosystems, Foster City, CA, USA]) were mixed. The samples were then run in the ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Fragment sizes were determined by comparison with the internal standard GeneScan 500 using the local Southern algorithm and analyzed by GeneMapper software version 3.5 (Applied Biosystems).

To confirm the genotype data with fragment size analysis, direct sequencing was performed as follows: 2.5 μL PCR products were incubated with 1 μL ExoSAP-IT (Amersham Bioscience, Tokyo, Japan) for 20 min at 37°C and then for 20 min at 80°C. Sequencing reactions were then carried out using a BigDye terminator cycle sequencing kit (Applied Biosystems, USA). After purification with an ethanol, the reaction products were subjected to an ABI 3100 Avant Genetic Analyzer (Applied Biosystems, USA). Alleles with 6 and 7 TA repeats are reported as TAn, and genotypes are assigned based on the number of TA repeats in each allele (i.e. 6/6, 6/7, and 7/7).

Polymorphisms in the *UGT1A1*6* gene, G211A (Gly71Arg, rs4148323), were genotyped using the *Taq*Man technique, as described previously.[15] The primer set used for the amplification was *Taq*Man SNP genotyping assays C 559715 20 (Applied Biosystems). The reactions were carried out according to the manufacturer's instructions. The end-point reading of the fluorescence generated during the PCR amplification was performed using an ABI Prism 7900HT (Applied Biosystems), and genotype assignments were obtained with sequence detection system software (Applied Biosystems). Results were plotted on a 2-D scatter plot of the wild-type allele *versus* the polymorphic allele. Variant alleles were defined as A for G211A.

***UGT1A1*28* polymorphism and selection of patients.** The genotypes of the *UGT1A1*28* polymorphism were determined as described earlier. Patients with the 6/6 or 6/7 genotype were independently enrolled in the phase I study. No patients with the 7/7 genotype were observed in the phase I study. The frequency of TA_7/TA_7 was only 1.7% in 463 Japanese colorectal cancer patients (Shoichi Hazama *et al.*, personal communication, 2004) and patients who had bilirubin levels of 1.5 mg/dL or higher were not included in the study, which would partly explain why the homozygous patients were not observed.

Pharmacokinetics of irinotecan, SN-38, and SN-38G. Venous blood samples for the pharmacokinetic analysis were collected on day 1 from patients at irinotecan dose level 2 (100 mg/m^2) in a 5-mL glass tube containing lithium heparin as the anticoagulant. The samples were obtained at the following time points: before drug administration; 1 h during infusion; and 0, 1, 2, 4, 8, 18, 24, and 48 h after the end of the irinotecan infusion. Blood was immediately processed to the plasma by centrifugation for 5 min at 400*g* (4°C) and then stored at −80°C until analysis. Total irinotecan and its metabolite concentrations in the plasma were measured by high-performance liquid chromatography, as described previously.[16] The lower limits of quantification were 25 ng/mL for irinotecan, 1 ng/mL for SN-38, and 2 ng/mL for SN-38G.

The area under the plasma time–concentration curve (AUC; ng·h/mL) was calculated using the logarithmic-linear trapezoidal algorithm to the last data point, with extrapolation-to-time infinity using the estimated value of the slope of the terminal

Table 1. Patients' characteristics

	UGT1A1 polymorphism	TA_6/TA_6	TA_6/TA_7	Total
		18	9	27
Sex	Males	10	5	15
	Females	8	4	12
Age		61 (44–75)	60 (50–75)	61 (44–75)
ECOG PS	0	12	6	18
	1	6	3	9
Total bilirubin	0–0.5	17	2	19
level (mg/dL)	0.6–0.9	1	7	8
	1.0–	0	0	0
Primary	Colon	9	6	15
	Rectum	9	3	12
Metastatic site	Liver	10	6	16
	Lung	8	4	12
	Lymph node	6	2	8
	Other	5	2	7
Prior CT	No prior CT	3	2	5
	Fluoropyrimidine based	15	7	22

CT, chemotherapy; ECOG PS, Eastern Cooperative Oncology Group performance status.

logarithmic-linear disposition phase. The degree of glucuronidation of SN38 to SN38G in the plasma was defined as the ratio of SN38G AUC/SN38 AUC (glucuronidation ratio [GR]). The biliary index (BI) was defined as the product of the irinotecan AUC and the ratio of the SN38 AUC over the SN38G AUC.

Statistical analyses were performed by Student's *t*-test, and a value of $P < 0.05$ was the criteria for significance. Estimated values of the pharmacokinetic parameters were reported as mean ± SE.

Results

Patient demographic characteristics. Twenty-seven patients (15 males and 12 females) with metastatic colorectal cancer were enrolled in this trial. The patients' demographic characteristics are listed in Table 1. Of the 27 patients, 18 had the *UGT1A1*1* (wild) allele (*UGT1A1* polymorphism TA_6/TA_6) and nine had the *UGT1A1*28* (variant) allele (*UGT1A1* polymorphism TA_6/TA_7). The median age was 61 years, ranging from 44 to 75 years. Twenty-two patients (81%) had received one or more prior chemotherapy regimens with previous fluoropyrimidine-based treatment.

Dose-escalation results. The total number of patients and those with DLT are listed in Table 2. DLT were observed in two patients with the *UGT1A1*1/*1* genotype. One patient experienced grade 3 neutropenia during the first course of treatment (dose level 3), and the other patient experienced grade 4 leucopenia, grade 4 neutropenia, grade 3 diarrhea, and grade 3 nausea during the first course of treatment (dose level 3). At this dose level, two of the six patients developed DLT. At dose level 4, neither DLT nor grade 3 or 4 toxicity were observed. The scheduled dose levels were completed, and the dose did not reach the MTD until level 4. An additional three patients were treated at this dose level to confirm its feasibility. Ultimately, one of the six patients experienced DLT with grade 3 neutropenia. Therefore, dose level 4 was determined to be the recommended dose for the phase I trial of the combination therapy for patients with the *UGT1A1*1/*1* genotype.

Table 2. Dose-escalation schema and incidence of DLT

Dose level	CPT-11 (mg/m^2)	5′-DFUR (mg/m^2)	Patients (*n*)	DLT
TA_6/TA_6				
1	70	576	3	0
2	100	576	3	0
3	120	576	6	2
4	150	576	6†	1†
TA_6/TA_7				
1	70	576	6	2
2	100	576	3	3

†Three additional patients were treated to confirm its feasibility. CPT-11, irinotecan; 5′-DFUR, doxifluridine; DLT, dose-limiting toxicity.

DLT were observed in five patients with the *UGT1A1*1/*28* genotype. One patient experienced grade 3 fatigue during the first course of treatment, and another patient experienced grade 3 neutropenia and grade 3 diarrhea during the first course of treatment (dose level 1). At this dose level, two of the six patients developed DLT. Dose level 2 was determined to be the MTD. At this dose level, all three patients developed DLT. One patient experienced grade 3 diarrhea, while one patient experienced grade 3 neutropenia, and the remaining patient experienced grade 3 leucopenia, grade 3 neutropenia, grade 3 nausea, and grade 3 vomiting during the first course of treatment. Therefore, dose level 1 was determined to be the recommended dose for the phase I trial of the combination therapy for patients with the *UGT1A1*1/*28* genotype.

Toxicity at the *UGT1A1*1/*1* genotype. All adverse events reported in the 18 patients with the *UGT1A1*1/*1* genotype are shown in Tables 3 and 4. The common hematological adverse events were leucopenia (56%) and neutropenia (50%). However, the toxicity was not severe in this genotype, with only three (17%) of the 18 patients developing grade 3 or 4 neutropenia, and only one (6%) of the 18 patients developing grade 3 or 4 leucopenia. For non-hematological toxicity, only one of the 18 patients experienced greater than grade 3 nausea, and only one of the 18 patients experienced greater than grade 3 diarrhea during all courses of treatment. One patient (at level 3) out of two **6* heterozygotes in this group suffered from grade 4 neutropenia and grade 3 diarrhea.

Toxicity at the *UGT1A1*1/*28*28* genotype. All adverse events reported in the nine patients with the *UGT1A1*1/*28*28* genotype are shown in Tables 3 and 4. Although the dose level was low (dose level 1 or 2), the common hematological adverse events were neutropenia (56%) and leucopenia (56%). The toxicity was frequent in this genotype, with three (33%) of the nine patients developing grade 3 or 4 neutropenia, and one (11%) of the nine patients developing grade 3 or 4 leucopenia. For non-hematological toxicity, two (22%) of the nine patients experienced greater than grade 3 diarrhea, one (11%) of the nine patients experienced greater than grade 3 nausea, only one (11%) of the nine patients experienced greater than grade 3 vomiting, and only one (11%) of the nine patients experienced greater than grade 3 fatigue during all courses of treatment. Only one *UGT1A1*6 he*terozygote (double heterozygous for **28* and **6*) in this group suffered from grade 3 neutropenia and diarrhea at level 1.

Efficacy. Tumor response was not the primary end-point in this phase I study; however, evidence of antitumor activity was observed. Twenty-six of the 27 patients were assessable for tumor response; partial responses (PR) were achieved in eight patients (Table 5).

Pharmacokinetics of irinotecan, SN-38, and SN-38G. The association of each *UGT1A* genotype with irinotecan pharmacokinetics was examined in patients with TA_6/TA_6 and TA_6/TA_7 at the dose of 100 mg/m^2 (level 2) irinotecan (Table 6). Patients with the heterozygous genotype (TA_6/TA_7) had significantly higher $AUC_{0-\infty}$ SN-38, maximum concentration of SN-38, half-life period of SN-38, and lower total clearance of SN-38 values compared to the wild-type genotype (TA_6/TA_6). The BI of

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Table 3. Hematological toxicity

Dose level	CPT-11 (mg/m^2)	5′-DFUR (mg/m^2)	Patients (*n*)	WBC (grade 1/2/3/4)	Neutrophil (grade 1/2/3/4)	Platelet (grade 1/2/3/4)
TA_6/TA_6						
1	70	576	3	0/1/0/0	0/1/0/0	0/0/0/0
2	100	576	3	2/0/0/0	2/0/0/0	0/0/0/0
3	120	576	6	2/1/0/1	1/0/1/1	0/0/0/0
4	150	576	6	2/1/0/0	1/1/1/0	0/0/0/0
TA_6/TA_6						
1	70	576	6	1/1/0/0	0/1/1/0	0/0/0/0
2	100	576	3	0/2/1/0	0/1/2/0	0/0/0/0

CPT-11, irinotecan; 5′-DFUR, doxifluridine WBC, white blood count.

Table 4. Non-hematological toxicity

Dose level	Patients (*n*)	Nausea (grade 1/2/3/4)	Fatigue (grade 1/2/3/4)	Diarrhea (grade 1/2/3/4)	Vomiting (grade 1/2/3/4)	Alopecia (grade 1/2/3/4)
TA_6/TA_6						
1	3	0/1/0/0	10/0/0	0/1/0/0	0/1/0/0	0/0/0/0
2	3	1/0/0/0	1/0/0/0	1/1/0/0	0/0/0/0	0/0/0/0
3	6	4/1/1/0	2/0/0/0	0/0/1/0	0/2/0/0	0/1/0/0
4	6	4/0/0/0	1/1/0/0	1/1/0/0	2/0/0/0	3/1/0/0
TA_6/TA_7						
1	6	3/0/0/0	0/2/1/0	1/1/1/0	1/2/0/0	1/0/0/0
2	3	0/1/1/0	1/0/0/0	0/1/1/0	1/0/1/0	0/1/0/0

Table 5. Clinical response

Dose level	Patients (*n*)	CR	PR	SD	PD	Response rate (%)	Disease control rate (%)
TA_6/TA_6							
1	3	0	0	1	2	0	33
2	3	0	2	1	0	67	100
3	6	0	2	3	1	33	83
4	6	0	2	4	0	33	100
Total	18	0	6	9	3	33	83
TA_6/TA_7							
1	6	0	2	3	0 (1)	33	83
2	3	0	0	3	0	0	100
Total	9	0	2	6	0 (1)	22	89

CR, complete response; NE, not evaluated; PD, progressive disease; PR, partial response; SD, stable disease.

Table 6. Pharmacokinetics parameters

	Genotype	C_{max} (ng/mL)	$T_{1/2}$ (h)	AUC 0–∞ (ng/h/mL)	CL_{total} (L/m^2/h)
CPT-11	TA_6/TA_6	929.2 ± 114.4	11.5 ± 2.9	6647.0 ± 1475.4	16.4 ± 3.0
	TA_6/TA_7	1011.0 ± 174.7	11.7 ± 4.3	6789.7 ± 938.2	14.9 ± 1.2
	6/6 *vs* 6/7	$P = 0.535$	$P = 0.968$	$P = 0.932$	$P = 0.673$
SN-38G	TA_6/TA_6	50.9 ± 10.3	19.1 ± 3.2	857.3 ± 319.5	149.1 ± 45.2
	TA_6/TA_7	53.9 ± 11.1	19.3 ± 2.9	1054.0 ± 103.2	96.9 ± 10.3
	6/6 *vs* 6/7	$P = 0.851$	$P = 0.962$	$P = 0.590$	$P = 0.323$
SN-38	TA_6/TA_6	12.4 ± 0.5	10.5 ± 1.1	115.3 ± 16.8	904.0 ± 128.7
	TA_6/TA_7	25.4 ± 0.9	19.7 ± 1.8	462.3 ± 94.4	235.3 ± 47.6
	6/6 *vs* 6/7	$P = 0.0003$	$P = 0.013$	$P = 0.022$	$P = 0.008$

Values are given as mean ± SE. These data were collected from patients at level 2 at which the irinotecan dose was 100 mg/m^2.AUC, area under the plasma concentration–time curve; CL, clearance; C_{max}, maximum drug concentration; CPT-11, irinotecan; SN-38G, SN-38 glucuronide; $T_{1/2}$, half-life period.

patients with TA_6/TA_7 (2979 ± 594) was significantly higher ($P = 0.030$) than that of patients with TA_6/TA_6 (973 ± 126). Although there was no significant difference ($P = 0.145$), the GR was three times lower in the TA_6/TA_7 group (2.38 ± 0.3) compared to the TA_6/TA_6 group (7.58 ± 2.9).

Discussion

It is important to identify patients genetically predisposed to severe toxicity of therapeutic agents. The effect of the *UGT1A1*28* genotype on neutropenia has already been observed,[7,9,17] but the recommended doses of irinotecan for each genotype had not been established. While the recommended dose of irinotecan alone should be investigated, irinotecan combined with fluorouracil was recommended at that time.[1,2] We decided that the Ethical Review Committee would find it difficult to approve the irinotecan alone regimen, so the present phase I study of irinotecan/5′-DFUR therapy focusing on the genetic *UGT1A1* polymorphism was conducted in patients with metastatic colorectal cancer. In patients with TA_6/TA_6, only three of the 12 patients experienced DLT at dose levels 3 and 4. However, in patients with TA_6/TA_7, two of the six patients at dose level 1 and all three patients at dose level 2 suffered from DLT, respectively (Table 2). Surprisingly, the recommended dose of irinotecan was determined to be 70 and 150 mg/m^2 for patients with heterozygous allele TA_6/TA_7 and those with TA_6/TA_6, respectively. This is the first report to confirm the RD of irinotecan according to the *UGT1A1*28* genotype. The results of the pharmacokinetic analysis supported the profiles of toxicity (Table 6). Patients with TA_6/TA_7 had significantly higher $AUC_{0-\infty}$ SN-38, C_{max} SN-38, $T_{1/2}$ SN-38, and lower CL_{total} SN-38 values compared with TA_6/TA_6. The BI of patients with TA_6/TA_7 was higher than that of patients with TA_6/TA_6.

Recently, a novel prospective dose-finding study of irinotecan alone based on *UGT1A1*6* and **28* genotyping (UGT0601) was published and the results discussed at the annual meeting of the Japanese Society of Clinical Oncology in 2008, as well as ESMO in 2008[18] and ASCO in 2009.[19] The authors reported that the RD in heterozygotes of **6* or **28* was determined to be 150 mg/m^2 (approval dose in Japan), and the MTD was determined to be150 mg/m^2 in homozygotes (**28/*28*, **6/*6*, and **28/*6*). However, the incidences of grade 3/4 neutropenia at 150 mg/m^2 during the first cycle were 9.8% (4/41), 18.8% (3/16), and 62.5% (10/16) in wild-type, heterozygote, and homozygote patients, respectively. The second administration was delayed 7 days or more in most homozygous patients (63% at 150 mg/m^2). One patient with **28/*28* homozygotes died of septic shock during the second cycle. These findings suggest that it is difficult to recommend 150 mg/m^2 biweekly, and the initial dosage and administration should be considered carefully for homozygous patients. Another *UGT1A1* genotype-directed phase I study of irinotecan combined with capecitabine[20] was presented at the ASCO conference in 2009. The authors concluded that the RD of irinotecan was 350 mg/m^2 for wild-type and heterozygous patients, and 200 mg/m^2 for homozygote patients, with capecitabine every 3 weeks.

The difference between our study and the UGT0601 study is the RD for heterozygotes. The reasons can be summarized as follows. First, the DLT was defined in our study as any grade 3 or 4 non-hematological and hematological toxicity, different from the ordinary phase I in the points of hematological toxicities because grade 4 neutropenia is usually DLT. Second, treatment-related toxicities were collected during six courses of treatment. In Japan, chemotherapy of colorectal cancer is performed by not only the specialist of clinical oncology, but also by surgeons in clinical practice. We determined that a safer regimen is necessary for the clinical setting. The incidences of grade 3/4 neutropenia in the UGT0601 study seemed to be more frequent in heterozygote (18.8%) than wild-type patients (9.8%).

The role of the *UGT1A1*28* allele in the toxicity and pharmacokinetics of irinotecan is considerably different between Asians and Caucasians. Only the **28* homozygote seemed to be associated with neutropenia in Caucasians,[9,21–23] whereas the **28* heterozygote has also been shown strongly associated with severe toxicity in Japanese patients.[7] The results of the present study reveal that SN-38 glucuronidation was highly impaired in heterozygote patients, as previously reported in Japan.[7,17] This ethnic difference can be associated with other genetic variants of UGT1A family polymorphisms, such as *UGT1A1*60*, **6*, *UGT1A7*3*, and *UGT1A9*22* that are shown in linkage disequilibrium with *UGT1A1*28*.[24–29] These variants can affect SN-38 glucuronidation and have been suggested to be associated with severe irinotecan-related toxicity. Specifically, *UGT1A1*6* might be useful for predicting toxicity in Asian patients treated with irinotecan-based chemotherapy.[7,18,19,24,30] In our study, two of three of **6* heterozygote patients suffered from grade 3/4 toxicity.

A PR to irinotecan plus 5′-DFUR was achieved in six of 18 patients with TA_6/TA_6 and two of nine patients with TA_6/TA_7. Specifically, PR was achieved in two of six patients with TA_6/TA_7 at a low dose of irinotecan (70 mg/m^2), suggesting a potential usefulness of our RD for those patients. Global standard intensive chemotherapy for colorectal cancer has been established around the world. This regimen can be useful for patients with poor performance status or for elderly patients.

In summary, this combination therapy can be used safely for patients with colorectal cancer according to the (TA)nTAA promoter polymorphism of *UGT1A1*. Other kinds of genetic polymorphisms of the *UGT1A* gene family should be considered in order to obtain more precise information to predict toxicities in individual patients. Further phase II and III studies should therefore be conducted to establish the predictive usefulness of those polymorphisms and the therapeutic efficacy of irinotecan plus 5′-DFUR at the RD.

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References

1 Douillard JY, Cunningham D, Roth AD *et al.* Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000; **355**: 1041–7.
2 Saltz LB, Cox JV, Blanke C *et al.* Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 2000; **343**: 905–14.
3 Tournigand C, Andre T, Achille E *et al.* FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004; **22**: 229–37.
4 Kawato Y, Aonuma M, Hirota Y, Kuga H, Sato K. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res* 1991; **51**: 4187–91.
5 Iyer L, King CD, Whitington PF *et al.* Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest* 1998; **101**: 847–54.
6 Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc Natl Acad Sci U S A* 1998; **95**: 8170–4.
7 Ando Y, Saka H, Ando M *et al.* Polymorphisms of UDP-glucuronosyltransferasegene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 2000; **60**: 6921–6.
8 Massacesi C, Terrazzino S, Marcucci F *et al.* Uridine diphosphate glucuronosyl transferase 1A1 promoter polymorphism predicts the risk of gastrointestinal toxicity and fatigue induced by irinotecan-based chemotherapy. *Cancer* 2006; **106**: 1007–16.

doi: 10.1111/j.1349-7006.2009.01428.x

9 Innocenti F, Undevia SD, Iyer L *et al.* Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol* 2004; **22**: 1382–8.
10 Miwa M, Ura M, Nishida M *et al.* Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. *Eur J Cancer* 1998; **34**: 1274–81.
11 Armstrong RD, Cadman E. 5'-Deoxy-5-fluorouridine selective toxicity for human tumor cells compared to human bone marrow. *Cancer Res* 1983; **43**: 2525–8.
12 Alberto P, Mermillod B, Germano G *et al.* A randomized comparison of doxifluridine and fluorouracil in colorectal carcinoma. *Eur J Cancer Clin Oncol* 1988; **24**: 559–63.
13 Therasse P, Arbuck SG, Eisenhauer EA *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.
14 Wang L, Hirayasu K, Ishizawa M, Kobayashi Y. Purification of genomic DNA from human whole blood by isopropanol-fractionation with concentrated Nal and SDS. *Nucleic Acids Res* 1994; **22**: 1774–5.
15 Okayama N, Hamanaka Y, Suehiro Y, Hasui Y, Nakamura J, Hinoda Y. Association of interleukin-10 promoter single nucleotide polymorphisms -819 T/C and -592 A/C with aging. *J Gerontol A Biol Sci Med Sci* 2005; **60**: 1525–9.
16 Sparreboom A, de Bruijn P, de Jonge MJ *et al.* Liquid chromatographic determination of irinotecan and three major metabolites in human plasma, urine and feces. *J Chromatogr B Biomed Sci Appl* 1998; **712**: 225–35.
17 Araki K, Fujita K, Ando Y *et al.* Pharmacogenetic impact of polymorphisms in the coding region of the UGT1A1 gene on SN-38 glucuronidation in Japanese patients with cancer. *Cancer Sci* 2006; **97**: 1255–9.
18 Ura T, Satoh T, Tsujinaka T *et al.* A genotype-directed dose-finding study of irinotecan based on UGT1A1 *28 and *6 polymorphisms in Japanese patients with gastrointestinal cancer (UGT0601). *Ann Oncol* 2008; **19** (Suppl): abstr 406P.
19 Esaki T, Satoh T, Ura T *et al.* A prospective PGx and PK/PD dose-finding study of irinotecan based on UGT1A1*6 and *28 genotyping (UGT0601). *J Clin Oncol* 2009; **27** (Suppl): abstr e14560.
20 Kim T, Sym S, Lee S *et al.* A UGT1A1 genotype-directed phase I study of irinotecan (CPT-11) combined with fixed dose of capecitabine in patients with metastatic colorectal cancer (mCRC). *J Clin Oncol* 2009; **27** (Suppl): abstr 2554.
21 Toffoli G, Cecchin E, Corona G *et al.* The role of UGT1A1*28 polymorphism in the pharmacodynamics and pharmacokinetics of irinotecan in patients with metastatic colorectal cancer. *J Clin Oncol* 2006; **24**: 3061–8.
22 Mathijssen RH, Marsh S, Karlsson MO *et al.* Irinotecan pathway genotype analysis to predict pharmacokinetics. *Clin Cancer Res* 2003; **9**: 3246–53.
23 Stewart CF, Panetta JC, O'Shaughnessy MA *et al.* UGT1A1 promoter genotype correlates with SN-38 pharmacokinetics, but not severe toxicity in patients receiving low-dose irinotecan. *J Clin Oncol* 2007; **25**: 2594–600.
24 Han JY, Lim HS, Shin ES *et al.* Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. *J Clin Oncol* 2006; **24**: 2237–44.
25 Minami H, Sai K, Saeki M *et al.* Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1*6 and *28. *Pharmacogenet Genomics* 2007; **17**: 497–504.
26 Saito Y, Sai K, Maekawa K *et al.* Close association of UGT1A9 IVS1 + 399C>T with UGT1A1*28, *6, or *60 haplotype and its apparent influence on 7-ethyl-10-hydroxycamptothecin (SN-38) glucuronidation in Japanese. *Drug Metab Dispos* 2009; **37**: 272–6.
27 Yamamoto N, Takahashi T, Kunikane H *et al.* Phase I/II pharmacokinetic and pharmacogenomic study of UGT1A1 polymorphism in elderly patients with advanced non-small cell lung cancer treated with irinotecan. *Clin Pharmacol Ther* 2009; **85**: 149–54.
28 Lankisch TO, Schulz C, Zwingers T *et al.* Gilbert's Syndrome and irinotecan toxicity: combination with UDP-glucuronosyltransferase 1A7 variants increases risk. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 695–701.
29 Fujita K, Ando Y, Nagashima F *et al.* Genetic linkage of UGT1A7 and UGT1A9 polymorphisms to UGT1A1*6 is associated with reduced activity for SN-38 in Japanese patients with cancer. *Cancer Chemother Pharmacol* 2007; **60**: 515–22.
30 Sai K, Saito Y, Sakamoto H *et al.* Importance of UDP-glucuronosyltransferase 1A1*6 for irinotecan toxicities in Japanese cancer patients. *Cancer Lett* 2008; **261**: 165–71.