UDP-glucuronosyltransferase (UGT) 1A1*28 Polymorphism-directed Phase II Study of Irinotecan with 5'-deoxy-5-fluorouridine (5'-DFUR) for Metastatic Colorectal Cancer

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Abstract. *Aim: We performed a phase II study of irinotecan with 5'-deoxy-5-fluorouridine (5'-DFUR) for metastatic colorectal cancer based on UDP-glucuronosyltransferase (UGT) 1A1 polymorphism. Patients and Methods: A total of 28 patients were enrolled. The dose of irinotecan was 150 mg/m^2 for patients with the *1/*1 wild-type genotype, and 70 mg/m^2 for those with the *1/*28 mutated genotype. The primary end-point was the response rate (RR); secondary end-points were safety, time to treatment failure (TTF), and overall survival (OS). Results: In 28 patients total, genotype was wild-type in 22 and mutated in six. The RR was *1/*1 (22.7%; wild-type) vs. *1/*28 (16.7%; mutated); the median TTF was 5 months vs. 4.5 months, and the median OS was 13 months vs. 17.5 months, respectively. None of these differences were significant. Toxicities of grade 3 or higher were neutropenia (9.0% vs. 0%, respectively) and diarrhea (13.6% vs. 0%, respectively). Conclusion: This genotype-oriented therapy was effective and safe, and thus appears useful for patients who have complications or advanced age.*

Colorectal cancer (CRC) is one of the most common types of cancer worldwide and remains the third leading cause of cancer-related death in Japan.

Irinotecan-containing regimens have been approved as the standard therapy for metastatic CRC (1-3). Irinotecan is a prodrug that is converted to its active metabolite, SN-38, by carboxylesterase. SN-38 is in turn converted to an inactive metabolite, SN-38G, by uridine UDP-glucuronosyltransferase (UGT). Adverse events associated with irinotecan therapy, such as myelosuppression and diarrhea, are significantly correlated with the area under the curve (AUC) of irinotecan, SN-38, and SN-38G (4-6). The toxicity of irinotecan is reported to be associated with the *UGT1A1**28 polymorphism, a polymorphism affecting the number of TA repeats in the TATA box of the promoter region of the *UGT1A1* gene (7). This polymorphism impairs transcriptional efficiency, leading to reduced glucuronidation, higher levels of SN-38 and SN-38G, and therefore increased toxicity of irinotecan. Because of the clinical importance of the glucuronidation pathway in irinotecan treatment, *UGT1A1**28 has been selected as a candidate predictor of severe toxicity (8-10).

The regimen of irinotecan combined with fluorouracil and leucovorin (FOLFIRI) has been approved as first-line chemotherapy for advanced CRC (3). However, the inconvenience and morbidity associated with long-term central venous access has prompted the development of alternative regimens. 5'-deoxy-5-fluorouridine (5'-DFUR) (an intermediate form of capecitabine) is an oral fluoropyrimidine that was designed to generate fluorouracil (FU), so as to produce higher FU concentrations within tumor cells than in normal tissues (9-11). 5'-DFUR was selected to be combined with irinotecan because when compared with other chemotherapy agents, it has been associated with only mild bone marrow suppression, a greater therapeutic index, and improved quality of life (12-13).

We have already published a phase I study examining the relationship of *UGT1A1* polymorphism and toxicity to irinotecan and 5'-DFUR for metastatic CRC. This phase I study determined the maximum tolerated dose (MTD) and

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the recommended doses (RD) for patients with the *UGT1A1* *1/*1 and *1/*28 genotypes. The RD of bi-weekly irinotecan administration was 150 mg/m^2 for patients with the *1/*1 (wild-type) genotype and 70 mg/m^2 for patients with the mutated *1/*28 genotype (14).

To our knowledge, this is the first phase II study to evaluate efficacy and safety of irinotecan in combination with 5'-DFUR in which the phase II study was based on the RD shown by a phase I study examining response in patients with the *UGT1A1**28 mutation.

Patients and Methods

Study design. This was a multicenter, open-label, phase II study conducted by the Departments of Digestive Surgery and Surgical Oncology (Surgery II) at the Yamaguchi University Graduate School of Medicine (Yamaguchi, Japan). Its objective was to evaluate the relationship between *UGT1A1* polymorphism and the efficacy and safety of combination treatment of irinotecan with 5'-DFUR. The primary end-point was the estimated response rate (RR) for patients previously untreated with irinotecan or oxaliplatin for advanced CRC. The secondary end-points were overall survival (OS), time-to-treatment failure (TTF), and drug-induced toxicities.

Patient eligibility. The main eligibility criteria included the following: demonstrated unresectable or recurrent CRC; measurable lesions according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, version (1.0); Eastern Cooperative Oncology Group performance status (ECOG PS) of 1 or less; no previous chemotherapy or one prior treatment with fluoropyrimidine; no chemotherapy within 4 weeks prior to the study; adequate baseline organ function, defined as a leukocyte count of at least 3,500/μl, neutrophil count of at least 1,500/μl, platelet count of at least 100,000/μl, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels no more than twice the upper limit of the institutional reference range; total bilirubin below 1.5 mg/dl; serum creatinine below 1.5 mg/dl; predicted life expectancy of at least three months; age between 20 and 75 years; and written, informed consent to participate in the study.

The exclusion criteria were: contraindications to irinotecan; prior treatment with irinotecan or oxaliplatin; major complications or active concurrent malignancies; brain metastasis requiring treatment; mental illness; diabetes treated with insulin; a history of drug allergy; difficulty ingesting 5'-DFUR; pregnancy or lactation, or intending to conceive; and being assessed by the physician in charge as not being appropriate to enter the study.

The study was conducted according to the Declaration of Helsinki and was approved by the Institutional Review Board of Yamaguchi University Hospital and the Ethical Review Committee for Gene Analysis Research of Yamaguchi University School of Medicine and University Hospital. The Institutional Review Board of each Author's institution approved the protocol. The study protocol was registered at the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN 000005011).

Pretreatment evaluation and follow-up. The pretreatment evaluation included a complete medical history, physical examination, and imaging of measurable disease. We also determined the complete blood cell count, serum chemistry, and physical condition before each bi-weekly dose of chemotherapy. Adverse events were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 3.0 (15). The target lesions were measured using computed tomography (CT), which was performed every four weeks and at the end of treatment. Clinical response was evaluated in accordance with the Response Evaluation Criteria In Solid Tumors (RECIST), version 1.0 (16).

UGT1A1 genotyping. Genomic DNA was extracted from peripheral blood anticoagulated with EDTA-2Na using a conventional sodium iodide (NaI) method (17). The TA index of the *UGT1A1* promoter was genotyped by fragment sizing. We used 10 μl of polymerase chain reaction (PCR) solution 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' and UGT–R1: 5'-GCCTTTGCTCCTGCCA GAGGTT-3', respectively). Amplification was performed with a Gene Amp PCR System PC808 (ASTEC, Tokyo, Japan). The PCR products (TA6, 94 bp; TA7, 96 bp) were mixed with Hi-Di formamide (including the internal size standard, GeneScan 500; Applied Biosystems, Foster City, CA, USA). The samples were then run in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Fragment sizes were determined by comparison with the internal standard Gene-Scan 500 using the Local Southern algorithm and analyzed by GeneMapper software version 3.5 (Applied Biosystems). Alleles with 6 and 7 TA repeats were reported as TAn, and genotypes were assigned based on the number of TA repeats in each allele (*i.e.* 6/6, 6/7, and 7/7).

Treatment plan. Irinotecan was administered once every two weeks (day 1) in 500 ml normal saline or dextrose *via* a 90-min intravenous infusion. The irinotecan dose administered was 150 mg/m^2 for patients with wild-type *1/*1 genotype and 70 mg/m^2 for patients with mutated *1/*28 genotype, as determined by our phase I trial. 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 two-day washout (14). This treatment cycle was repeated every two weeks until disease progression, patient unwillingness to participate further, unacceptable toxicity, or death. The prophylactic use of granulocyte colony-stimulating factor was not allowed. One cycle was defined as administration of bi-weekly irinotecan (Figure 1).

Appropriate dose interruptions/reductions were implemented in the event of specific toxicities, depending on their nature and intensity. The next course of treatment began only when the neutrophil count was >1,500/μl, the platelet count was >100,000/dl, and any other treatment-related toxicities were grade 1 or milder; otherwise, treatment was withheld for up to a week. In addition, the second time this toxicity occurred, treatment was restarted with a 20% dose reduction of irinotecan. If adverse events did not improve to grade 0 or 1 after three weeks, patients were excluded from the study. In the case of disease progression, or patients declining further participation, grade 4 hematological toxicity or diarrhea, grade 3 or 4 non-hematological toxicity (except nausea, vomiting, or alopecia), and necessity for dose reduction of irinotecan more than twice, patients were excluded from the study.

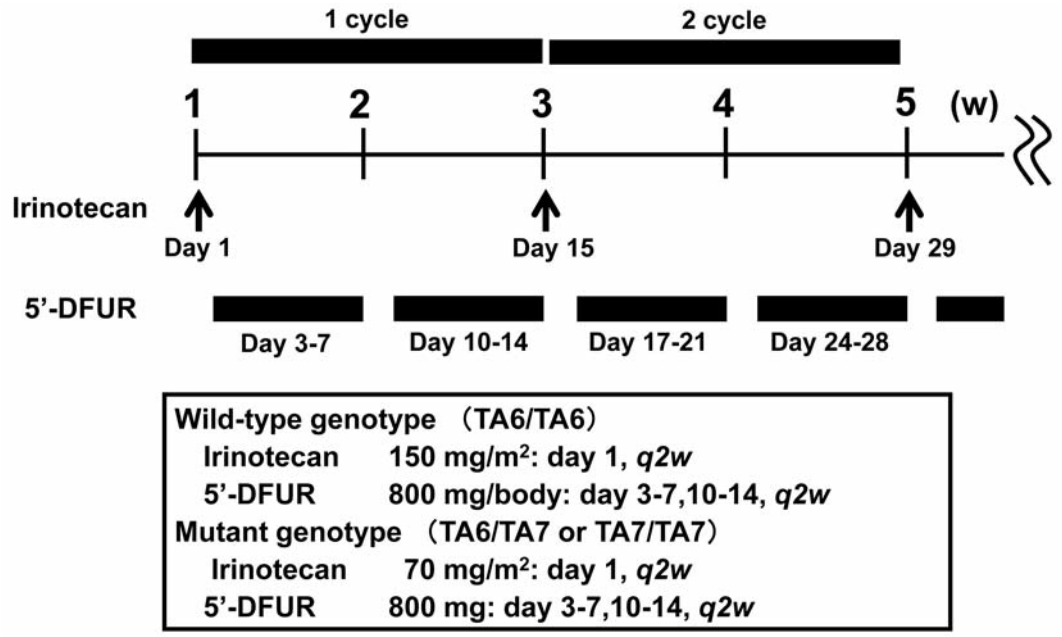

Figure 1. *Treatment schedule. This figure shows the treatment scheme in this study. Irinotecan was administered bi-weekly. The irinotecan dose was 150 mg/m^2 for patients with wild-type genotype and 70 mg/m^2 for those with mutated genotype. 5'-deoxy-5-fluorouridine (5'-DFUR) was given as 200-mg capsules; two capsules were administered orally in the morning and evening after a meal on five consecutive days followed by a two-day washout. One cycle was defined as administration of bi-weekly irinotecan.*

Study assessments. Complete and partial responses required subsequent confirmation of the response after an interval of at least four weeks. OS was defined as the time from registration to death from any cause. TTF was defined as time from registration to time when the protocol treatment was discontinued.

Statistical analysis. The primary endpoint of this study was RR based on RECIST criteria. All analyses were performed on an intention-to-treat basis. Patients alive at the final survival analysis or those whose disease had not progressed at the time of the final analysis were censored using the last contact date. Survival curves were drawn by the Kaplan-Meier method. To assess the relationship between toxicity and *UGT1A1* polymorphism, the chi-square test or Fisher's exact test were used. The log-rank test was used to assess the relationship between TTF and OS and *UGT1A1* polymorphism. *p*-Values <0.05 were considered statistically significant.

Results

Patients' characteristics. From October 2005 to September 2007, 28 patients (20 men and 8 women) with metastatic CRC were enrolled in this trial. Their characteristics are listed in Table I. Out of the 28 patients, 22 had the *UGT1A1**1 (wild-type) allele (*UGT1A1* polymorphism TA6/TA6) and six had the *UGT1A1**28 (mutant) allele (*UGT1A1* polymorphism TA6/TA7). Age ranged from 47 to 79 years, with a median age of 66 years. PS was 0 in 69% of patients and 1 in the others. Fifteen patients (47%) had received previous fluoropyrimidine-based treatment (eight patients as adjuvant chemotherapy, seven as first-line chemotherapy). All patients were evaluated for toxicity and for response to treatment. A median of 12 cycles of combination therapy (range 1-43 cycles) was administered. A total of 23 patients (82%) completed at least six cycles of therapy.

Efficacy. In the group as a whole, six patients had partial response, 17 had stable disease, two had progressive disease, and three were not evaluated. The overall RR was 21.4% and the disease control rate (DCR, defined as the percentage of patients who have achieved complete response (CR), partial response (PR) and stable disease (SD) to a therapeutic intervention) was 82.1%. In the 22 patients with wild-type genotype, five had PR, 12 had SD, the RR was 22.7%, and the DCR was 77.2%. In the six patients with the mutated genotype, one had PR, five had SD, the RR was 16.7%, and the DCR was 100% (Table II). In the group as a whole, the median OS was 15.2 months (Figure 2) and the median TTF was 5 months (Figure 3). When results were analyzed for the *UGT1A1* genotype (wild-type *vs*. mutant), the median OS was 13 months *vs*. 17.5 months and the median TTF was 5 months *vs*. 4.5 months, and neither of these differences were statistically significant (Figures 2 and 3).

Toxicity. Adverse events of grade 3 or higher were reported in eight patients (29%). The most common hematological adverse events were neutropenia (50%) and leucopenia

Table I. *Patients' characteristics.*

	UGT1A1 polymorphism		
	Wild type 22	Mutant type 6	Total 28
Gender			
Males	17	3	20
Females	5	3	8
Age, year	68 (49-79)	61 (47-75)	66 (47-79)
ECOG PS			
0	10	3	13
1	12	3	15
Incipient/recurrent			
Incipient	14	4	18
Recurrent	8	2	10
Prior chemotherapy			
No	10	3	13
Yes	12	3	15
Metastatic site			
Liver	14	4	18
Lung	8	3	11
Lymph node	8	0	8
Peritoneal	4	0	4
Other	2	1	3

ECOG PS, Eastern Cooperative Oncology Group performance status; UGT1A1: UDP-glucuronosyltransferase (UGT) 1A1.

(54%). Only three patients (10.7%) developed neutropenia of grade 3 or higher, and only two patients (7.1%) developed leucopenia of grade 3 or higher (Table III).

Non-hematological toxicities of grade 3 or higher were as follows: diarrhea (10.7%), anorexia (7.1%), general fatigue (3.6%), and nausea (3.6%) (Table IV). Regarding patients with the wild-type genotype, seven (32%) experienced an adverse event of grade 3 or higher. The most common hematological adverse events were neutropenia (55%) and leucopenia (59%). Three patients (13.6%) developed neutropenia of grade 3 or higher and two (9.0%) developed leucopenia of grade 3 or higher. Non-hematological toxicities of grade 3 or higher were anorexia (4.5%), general fatigue (4.5%), and diarrhea (13.6%) (Tables III and IV).

Among patients with the mutant genotype, one (16.7%) experienced an adverse event of grade 3 or higher. The most common hematological adverse events were neutropenia (33%) and leucopenia (33%). No patients developed neutropenia or leucopenia of grade 3 or higher. Non-hematological toxicities of grade 3 or higher were anorexia (16.7%) and nausea (16.7%); none of the patients with this mutation experienced diarrhea of this severity (Tables III and IV). Although the comparisons did not achieve statistical significance, toxicities in those with the *1*28 genotype who received 70 mg/m^2 of irinotecan tended to be of lower grade than in those with the *1*1 genotype who received 150 mg/m^2 of irinotecan.

Table II. *Response rate. The lesions were measured according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, version 1.0 (16).*

UGT1A1 genotype	PR	SD	PD	NE†	RR (%)	DCR (%)
Wild-type (n=22)	5	12	2	3	22.7	77.2
Mutant (n=6)	1	5	0	0	16.7	100
All (n=28)	6	17	2	3	21.4	82.1

PR, Partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; DCR, desease control rate; *UGT1A1*: UDP-glucuronosyltransferase (UGT) 1A1. †Three patients discontinued the study because of side-effects (two cases) and changing to the other therapy (one case).

Discussion

Irinotecan is a key agent in the treatment of metastatic CRC. Because the toxicity of irinotecan has been reported to correlate with the *UGT1A1**28 polymorphism, this mutation has yet been selected as a candidate predictor of severe toxicity (18). However, no prospective genotype-directed phase II studies of irinotecan for CRC have been published. To our knowledge, this is the first phase II study to use the doses of irinotecan recommended by a phase I study assessing the relationship between *UGT1A1**28 genotype and irinotecan dose required (14).

In the present study, 22 patients had the wild-type genotype and six the mutant genotype. The RR was 22.7% in the former group *vs.* 16.7% in the latter group, and the DCR was 77.2% *vs.* 100%, respectively, although these differences were not significant. The median TTF was five months for those with the wild-type genotype *vs.* 4.5 months in the mutant genotype, and the median OS was 13 months *vs.* 17.5 months, respectively, again showing no significant differences.

We evaluated the association between the *UGT1A1**28 polymorphism and the toxicity of irinotecan in combination with 5'-DFUR. The most common irinotecan-induced toxicities of grade 3 or higher (wild-type *vs.* mutant) were neutropenia (9.0% *vs.* 0%), leucopenia (13.6% *vs.* 0%), and diarrhea (13.6% *vs.* 0%). Toxicities in those with the mutant genotype who received 70 mg/m^2 of irinotecan tended to be milder than those with wild-type genotype who received 150 mg/m^2 of irinotecan. These data suggest that irinotecan and 5'-DFUR combination therapy can be safely administered to patients with the *UGT1A1**28 polymorphism.

In previous reports of the FOLFIRI regimen, the RR ranged from 34% to 56% and the median OS from 14 to 21.5 months (1-3). The combination of irinotecan and 5'-DFUR is also reported to produce a similar RR and median OS to FOLFIRI: RR ranged from 36% to 40% and median OS from

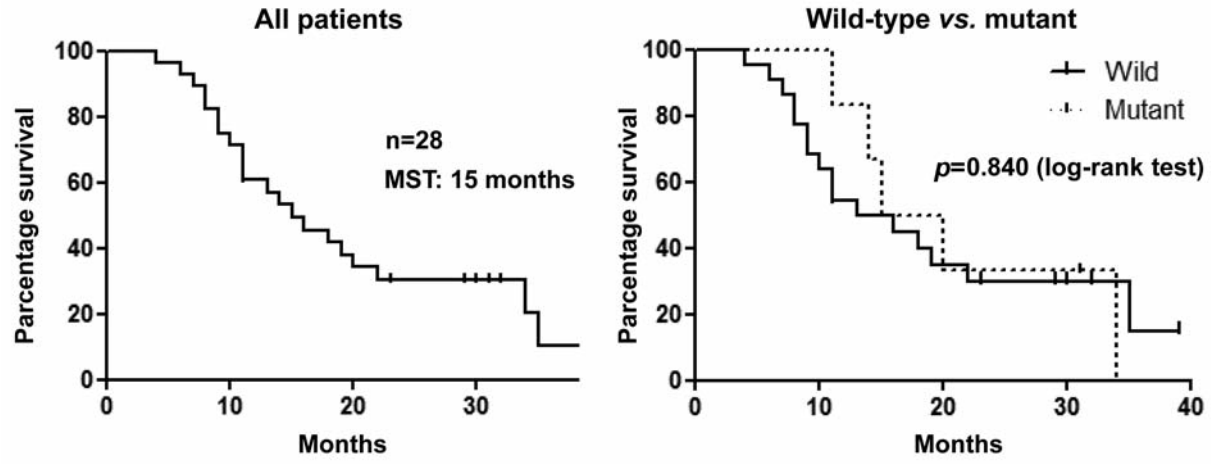

Figure 2. *Overall survival (OS) rate. Among all enrolled patients, the median survival time was 15 months. When results were analyzed by UDP-glucuronosyltransferase (UGT) 1A1 genotype (wild-type vs. mutant), the median survival time was 13 months vs. 17.5 months, respectively, a difference that did not reach statistical significance.*

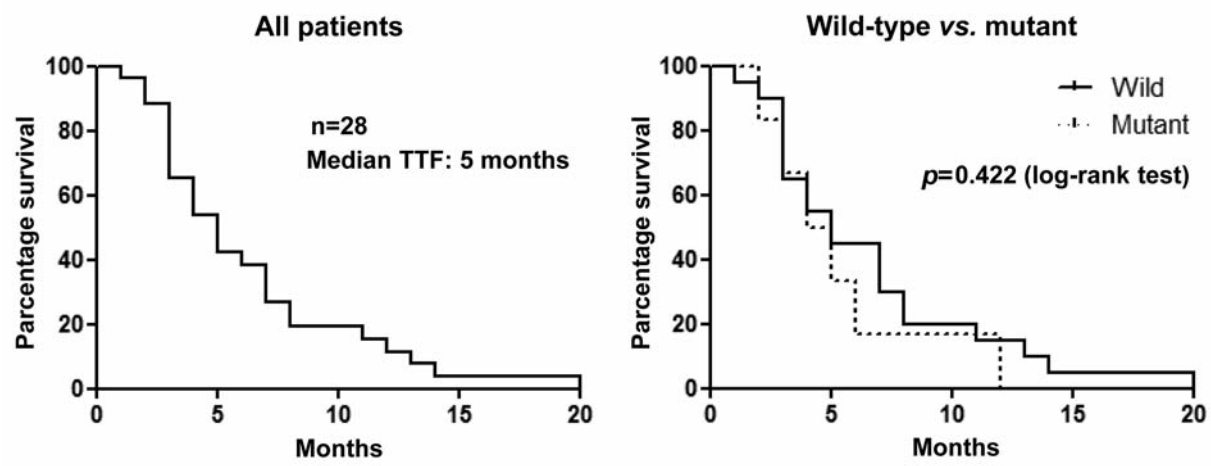

Figure 3. *Time-to-treatment failure (TTF). Among all enrolled patients, the median TTF was five months. When results were analyzed by UDP-glucuronosyltransferase (UGT)-1A1 genotype (wild-type vs. mutant), the median TTF was 5 months vs. 4.5 months, respectively, a difference that did not reach statistical significance.*

15 to 20.5 months (19, 20). In the present study, the RR was lower than what is reported for other irinotecan-based chemotherapy regimens. One possible reason for this is that half of the enrolled patients had received previous fluoropyrimidine-based treatment as first-line chemotherapy. However, for both genotypes, the present regimen maintained a high DCR and similar median OS compared with other reports (1-3, 21, 22).

The RD for irinotecan is controversial. Dose-finding studies of irinotecan both in combination with 5-fluoropyrimidine and alone have recently been reported. Toffoli *et al*. reported a MTD of 310 mg/m^2 for patients with the *1/*28 genotype and 370 mg/m^2 for those with the *1/*1 genotype (23). Marcuello *et al*. evaluated the FOLFIRI regimen and reported that the dose of irinotecan was escalated to 450 mg/m^2 in patients with the *1/*1 genotype, to 390 mg/m^2 in those with the *1/*28 genotype, and to 150 mg/m^2 in those with the *28/*28 genotype (24). They commented that the RD of irinotecan in FOLFIRI was considerably lower for patients with the *1*28 genotype than the 180 mg/m^2 usually used for those with *1/*1 genotypes, although the RD was not defined. Yamashita *et al*. reported a phase I/II study in which the dose of irinotecan in FOLFIRI could be escalated to 180 mg/m^2 in Japanese patients with CRC (25). In their report, 25 patients received FOLFIRI at the RD, and in terms of outcome, grade 3 or 4 neutropenia occurred in 44% and the RR was 24%.

Table III. *Hematotoxicity. Adverse events were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v.3.0 (15).*

UGT1A1 genotype	Adverse event	Cases		Grade				≥G3
		n	%	1	2	3	4	(%)
Wild-type (n=22)	Neutropenia	12	55	2	7	2	1	13.6
	Leukopenia	13	59	2	9	1	1	9
Mutant (n=6)	Neutropenia	2	33	2	0	0	0	0
	Leukopenia	2	33	1	1	0	0	0
All cases (n=28)	Neutropenia	14	50	4	7	2	1	10.7
	Leukopenia	15	54	3	10	1	1	7.1

UGT1A1: UDP-glucuronosyltransferase (UGT) 1A1.

Table IV. *Non-Hematotoxicity. Adverse events were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v.3.0 (15).*

UGT1A1 genotype	Adverse event	Cases		Grade				≥G3
		n	%	1	2	3	4	(%)
Wild-type (n=22)	Anorexia	5	23	2	2	1	0	4.5
	Diarrhea	10	45	4	3	3	0	13.6
	Fatigue	9	41	5	3	1	0	4.5
	Hair loss	9	41	8	1	-	-	-
	Nausea	6	27	3	2	0	0	0
	Headache	0	0	0	0	0	0	0
Mutant (n=6)	Anorexia	4	67	3	0	1	0	16.7
	Diarrhea	1	17	1	0	0	0	0
	Fatigue	1	17	1	0	0	0	0
	Hair loss	2	33	2	0	-	-	-
	Nausea	1	17	1	0	1	0	16.7
	Headache	1	17	1	0	0	0	0
All cases (n=28)	Anorexia	9	32	5	2	2	0	7.1
	Diarrhea	11	39	5	3	3	0	10.7
	Fatigue	10	36	6	3	1	0	3.6
	Hair loss	11	39	10	1	-	-	-
	Nausea	7	25	4	2	1	0	3.6
	Headache	1	3.6	1	0	0	0	0

UGT1A1: UDP-glucuronosyltransferase (UGT) 1A1.

None of these three studies reported progression-free survival (PFS) or OS, yet many investigators and physicians now believe that the most important factor in response to cancer therapy is OS, rather than the RR. It is also considered very important that key agents for treating mCRC, such as fluoropyrimidine, oxaliplatin, irinotecan and molecular targeted agents, can be administered safely and on a long-term basis, without reducing patient quality of life (26).

We determined that a safer regimen than those currently available is necessary for the clinical setting, and accordingly performed phase I and II studies designed to take into account the effect of the *UGT1A1**28 polymorphism. The present regimen of irinotecan and 5'-DFUR, in which the irinotecan dose varied based on *UGT1A1* status, was associated with high DCR and OS, and appears safe. We, therefore, believe it could be useful as a standard therapy for CRC, particularly for patients who struggle to tolerate intensive therapy because of complications or advanced age.

A recent report stated that Asian patients have a high frequency of the unfavorable genotype *UGT1A1**6, which is a predictive factor of severe toxicity to irinotecan (14). Such ethnic differences have been associated with other UGT1A

family polymorphisms, such as *UGT1A1*60, *UGT1A7*3, and *UGT1A9*22, which were found to be in linkage disequilibrium with *UGT1A1*28 (27-32). The evaluation of such other *UGT1A* polymorphisms before irinotecan treatment may allow us to accurately predict severe toxicities and perform chemotherapy more safely.

In conclusion, this novel phase II study of irinotecan and 5'-DFUR combination therapy was based on the RD of irinotecan previously established for those with the *UGT1A1*28 genotype. It showed favorable DCR and OS, and acceptable toxicity. This genotype-oriented regimen might therefore be useful as a standard therapy for CRC, particularly for patients who do not tolerate intensive therapy well.

Disclosure

The Authors have no conflicts of interest.

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