Phase I/II Study of FOLFIRI in Japanese Patients with Advanced Colorectal Cancer

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Objective: This phase I/II study determined the recommended dose of FOLFIRI (irinotecan, infusional 5-fluorouracil and leucovorin) for Japanese patients with advanced colorectal cancer, and evaluated safety at the recommended dose in patients without the UDP-glucuronosyltransferase 1A1*28 allele which caused reduced enzyme expression.

Methods: The phase I part assessed the maximum tolerated dose of FOLFIRI to determine the recommended doses of irinotecan and infusional 5-fluorouracil. The doses were escalated from 150 to 180 mg/m² (irinotecan) and 2000 to 2400 mg/m² (5-fluorouracil). UDP-glucuronosyltransferase 1A1*6 and *28, and pharmacokinetics of irinotecan were observationally examined. In the phase II part, patients without the UDP-glucuronosyltransferase 1A1*28 allele received FOLFIRI at the recommended dose to evaluate safety.

Results: Among 15 patients in the phase I part, dose-limiting toxicity (diarrhea) occurred in one patient who received 150 mg/m² irinotecan and 2400 mg/m² infusional 5-fluorouracil. The respective recommended doses were 180 and 2400 mg/m² for irinotecan and infusional 5-fluorouracil, without reaching the maximum tolerated dose. Twenty-five patients received FOLFIRI at the recommended doses. Grade 3 or 4 neutropenia occurred in 44%, and Grade 3 diarrhea in 4%.

Conclusions: This phase I/II study demonstrates that the recommended doses of irinotecan and infusional 5-fluorouracil in FOLFIRI for Japanese patients with advanced colorectal cancer who do not possess the UDP-glucuronosyltransferase 1A1*28 allele are 180 and 2400 mg/m², respectively. Toxicities occurring at the recommended doses are manageable in these patients.

Key words: FOLFIRI – recommended dose – Japanese – safety – UGT1A1 genotyping

INTRODUCTION

FOLFIRI, infusional 5-fluorouracil (5-FU) and l-leucovorin (l-LV) plus irinotecan, was developed in Europe and is now widely used as one of the standard treatment regimens for advanced colorectal cancer (CRC) (1,2). The doses of irinotecan and infusional 5-FU in the FOLFIRI regimen used in Western countries are 180 mg/m² and 2400–3000 mg/m², respectively, repeated every 2 weeks (1,2). In Japanese patients, however, the maximum tolerated doses (MTD) of irinotecan and infusional 5-FU in FOLFIRI remain uncertain. We routinely use irinotecan at a dose of 150 mg/m² in FOLFIRI. This dose has been approved for irinotecan monotherapy every 2 weeks by the Japanese Ministry of Health, Labour and Welfare.
Several lines of evidence have linked irinotecan toxicity to the \textit{UGT1A1}*28 allele. Patients homozygous for \textit{UGT1A1}*28 carry a significantly higher risk of severe irinotecan-related adverse events than those who do not possess this genotype \cite{3,4} because \textit{UGT1A1}*28 decreases \textit{UGT1A1} protein expression and reduces the glucuronidation capacity for \textit{SN-38}. In Asians, a specific mutation, \textit{UGT1A1}*6 \cite{5}, has been proved to reduce the catalytic activity of \textit{UGT1A1} \cite{6,7}. The \textit{UGT1A1}*28/*28, *6/*6 and *6/*28 genotypes have been shown to be related to severe neutropenia in Asian populations \cite{8-10}.

We performed a dose-finding phase I study of irinotecan and continuous infusional 5-FU in the FOLFIRI regimen in Japanese patients with advanced CRC. We also observationally examined the \textit{UGT1A1} genotyping and irinotecan pharmacokinetics in the phase I part to investigate the relation between them and FOLFIRI-related toxicity. Then, we evaluated the safety and efficacy of FOLFIRI at the recommended dose (RD) in patients without the \textit{UGT1A1}*28 allele. Here, we report the results of the first Japanese phase I and II study of FOLFIRI with \textit{UGT1A1} genotyping.

**PATIENTS AND METHODS**

**ELIGIBILITY**

This study enrolled patients with histologically confirmed advanced CRC. Eligibility criteria included an age of ≤75 years; an Eastern Cooperative Oncology Group (ECOG) scale performance status of 0 or 1; no previous chemotherapy for at least 4 weeks; no previous irinotecan-based chemotherapy; adequate bone marrow (absolute neutrophil count ≥2000/µl, platelet count ≥100 000/µl), liver (serum total bilirubin ≤ upper limit of normal (ULN), serum aspartate aminotransferase and alanine aminotransferase ≤3.0 × ULN) and renal (serum creatinine ≤1.5 × ULN) functions; no severe medical conditions; no brain metastasis and no prior radiotherapy of the pelvis. The Institutional Review Board of Saitama Medical University approved the study protocol. Patients signed written informed consent for their peripheral blood samples and medical information to be used for research purposes.

**STUDY OBJECTIVES**

**PHASE I PART**

The primary objective of the phase I part of this study was to assess the MTD and dose-limiting toxicity (DLT) of irinotecan and infusional 5-FU in FOLFIRI during the first course of treatment in patients with advanced CRC and thereby determine the RD. \textit{UGT1A1}*28 and *6 and the pharmacokinetics of irinotecan, the active metabolite of irinotecan \textit{SN-38} and the inactive metabolite \textit{SN-38} glucuronide (\textit{SN-38G}) were observationally examined to evaluate the relations of \textit{UGT1A1} genotype to irinotecan pharmacokinetics and irinotecan-induced adverse events. Pharmacogenetic and pharmacokinetic information were not reflected for patient enrollment and dose escalation in the phase I part.

**PHASE II PART**

In the subsequent phase II part of the study, we excluded patients who had at least one \textit{UGT1A1}*28 allele, because we had considered that these patients were at higher risk in irinotecan-induced severe toxicities based on the report by Ando et al. \cite{3}. The primary objective of the phase II part was to evaluate the safety of FOLFIRI at the RD. The secondary objective was to assess response.

**TREATMENT AND DOSE ESCALATION**

FOLFIRI comprised a 2-h intravenous infusion of \textit{l-LV} (200 mg/m²) and a 90-min intravenous infusion of irinotecan (each level as described below) on day 1, followed by an intravenous bolus injection of 5-FU (400 mg/m²) and a 46-h intravenous infusion of 5-FU (each level as described below); treatment was repeated every 2 weeks. Two sessions of treatment were counted as one course. The starting doses (Level 1) of irinotecan and infusional 5-FU were 150 and 2000 mg/m², respectively. The dose of infusional 5-FU was increased to 2400 mg/m² (Level 2). Then, the doses of irinotecan and infusional 5-FU were elevated to 180 and 2400 mg/m², respectively (Level 3). If patients did not tolerate Level 1, the dose of irinotecan was decreased to 120 mg/m² (Level 0).

DLT was defined as Grade 4 neutropenia lasting for more than 5 days, neutropenic fever (Grade 3 or 4 neutropenia with fever ≥38.5°C), Grade 4 thrombocytopenia, Grade 3 thrombocytopenia with hemorrhage or Grade 3 or higher non-hematologic toxicity during the first course of treatment. Three patients were initially enrolled at each dose level. If none of the first three patients had DLT, the dose was escalated, and three additional patients received the next dose level. If one of the three patients had DLT, then three additional patients were enrolled at the same dose level, and escalation to the next dose level was continued if only one of the six patients had DLT. If DLT occurred in more than one of the first three patients or more than one of six patients treated at any given dose level, dose escalation was stopped, and that level was defined as MTD. If DLT occurred at dose Level 1, then the dose level was decreased to Level 0. If DLT occurred at dose Level 0, the study was stopped. Patients who received Level 0 and had DLT were treated with FOLFIRI at an irinotecan dose of 100 mg/m² (Level −1) after resolution of toxicity as evaluated by the physician in charge. In principle, the RD was defined as the dose one level below the MTD, but toxic effects occurring in later courses were also considered. Six patients were enrolled at the RD.
After determining the RD in the phase I part of the study, additional patients received FOLFIRI at the RD in the phase II part.

Toxicity was assessed weekly during the first course and every 2 weeks during the second and subsequent courses according to the National Cancer Institute Common Toxicity Criteria version 2.0 (http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcv20_4-30-992.pdf). Chemotherapy was delayed until recovery if the leukocyte count was <3000/μl, the platelet count was 100 000/μl or if clinically significant, persistent non-hematologic toxicity occurred. Tumor response was evaluated every two courses according to the standard World Health Organization response criteria (11). Treatment was continued until disease progression, unacceptable toxicity or the patient’s refusal of further treatment.

**UGT1A1 Genotyping**

Genomic DNA was extracted from 200 μl of peripheral blood, which had been stored at −80°C until analysis, with the use of a QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany). The polymorphism UGT1A1*6 was analyzed by the polymerase chain reaction-restriction fragment length polymorphism method as described elsewhere (12). UGT1A1*28 was determined by the direct sequencing method as described by Fujita et al. (12).

**Pharmacokinetic Analysis of Irinotecan and Its Metabolites**

Blood samples for pharmacokinetic analysis were obtained during the first treatment with FOLFIRI in the patients who agreed to have blood sampling for pharmacokinetics. The samples were taken from the arm opposite the infusion site at the beginning of irinotecan infusion and at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 h after the end of the 1.5 h infusion. The samples were immediately centrifuged, and the plasma was stored at −80°C until analysis. Total (lactone and carboxylate) plasma concentrations of irinotecan, SN-38 and SN-38G were analyzed by reverse-phase high-performance liquid chromatography as described by Araki et al. (8). The lower limits of quantification were 5 ng/ml (7.4 nM) for irinotecan and 0.5 ng/ml (1.2 and 0.88 nM) for SN-38 and SN-38G. The intra-assay and inter-assay coefficients of variation for irinotecan and its metabolites were <10%.

**Pharmacokinetic Parameters**

The plasma concentration–time data of irinotecan and its metabolites were analyzed by a standard non-compartmental method, using WinNonlin version 5.2 software (Pharsight Corporation, Mountain View, CA, USA). The area under the plasma concentration–time curve (AUC) for time zero to the last sampling was calculated with the linear trapezoidal rule (until the peak plasma concentration) and linear-log trapezoidal rule (until the last quantifiable concentration).

**Statistical Design in Phase II Part**

In the phase II part of this study, we set a response rate of 25% as the target activity level and chose 5% as the lowest response rate of interest. According to Simon’s two-stage minimax design with an α level of 10 and 90% power (13), we planned to enroll at least 20 patients at the RD, with at least one response among 13 patients in the first step being required for this regimen to be considered worthy of further evaluation. The exact confidence interval for the response rate was calculated based on binomial distribution.

**RESULTS**

**Patient Characteristics**

Between March 2005 and April 2007, a total of 34 patients were enrolled into this phase I/II study, 15 in phase I and 19 in phase II. The baseline characteristics of the patients are shown in Table 1. The median age was 61 years (range, 35–75). Twenty-three (67%) patients had received at least one prior regimen of chemotherapy.

| Table 1. Baseline characteristics of all patients enrolled in the present phase I/II study |
| Characteristics                                                                 | Number of patients |
| Age (years)                                                                     | 61 (35–75)*       |
| Gender                                                                         |                  |
| Male                                                                           | 21                |
| Female                                                                         | 13                |
| ECOG performance status                                                        |                  |
| 0                                                                              | 31                |
| 1                                                                              | 3                 |
| Primary tumor                                                                  |                  |
| Colon                                                                          | 21                |
| Rectum                                                                         | 13                |
| Number of metastases                                                          |                  |
| 1                                                                              | 20                |
| 2                                                                              | 10                |
| 3                                                                              | 4                 |
| Number of prior chemotherapy regimens                                          |                  |
| 0                                                                              | 11                |
| 1                                                                              | 21                |
| ≥2                                                                             | 2                 |

ECOG, Eastern Cooperative Oncology Group.
*Median (range).
The results of the phase I part are shown in Table 2. There was no DLT at dose Level 1. At dose Level 2, one of the first three patients had leg edema diagnosed to be caused by deep vein thrombosis (DVT), initially designated as DLT. Three additional patients then received the same dose level. One patient had Grade 3 diarrhea. Although two of the first six patients who received dose Level 2 were initially judged to have DLT, further careful follow-up examinations of the patient considered to have DVT revealed no definite evidence of DVT; this reaction was therefore not considered DLT. We concluded that only one of the six patients given dose Level 2 had DLT and escalated the dose to Level 3. At dose Level 3, no DLT occurred in the first three patients. At that time, we obtained information regarding another ongoing phase I study of FOLFIRI in Japan. In that study, three of six patients who received irinotecan 180 mg/m² and infusional 5-FU 3000 mg/m² had DLT. This dose level was regarded to be the MTD, and the RD of irinotecan was determined to be the same as our dose Level 3 (14). On the basis of this information, we estimated that the RD was dose Level 3 and decided to confirm this by assigning three additional patients to this dose Level. None of the six patients given dose Level 3 had DLT. We therefore considered dose Level 3 the RD for the next phase II part of this study.

Among the 15 patients participating in the phase I study, the \textit{UGT1A1} genotype was \textit{UGT1A1}*1/*1 in 10 patients, \textit{UGT1A1}*1/*6 in 2, \textit{UGT1A1}*6/*6 in 2 and \textit{UGT1A1}*6/*28 in 1. The patient with \textit{UGT1A1}*6/*28 genotype had Grade 4 neutropenia after receiving irinotecan at a dose of 150 mg/m². The pharmacokinetics of irinotecan, SN-38 and SN-38G were examined in eight patients. The average ratio of the AUC of SN-38 to that of SN-38G (AUC\text{SN-38}/AUC\text{SN-38G}) in the patients with \textit{UGT1A1}*6/*28 and \textit{UGT1A1}*6/*6 was 1.78. The average AUC\text{SN-38}/AUC\text{SN-38G} in the patients with \textit{UGT1A1}*1/*1 was 0.65.

### PHASE II PART

In the phase II part, we evaluated the safety and efficacy of FOLFIRI at the RD in 25 patients: 6 who received the RD in phase I and 19 who were newly enrolled. One patient was homozygous for \textit{UGT1A1}*6, and four were heterozygous for \textit{UGT1A1}*6. No patient harboring the \textit{UGT1A1}*28 allele was included.

Twenty-five patients received a median of 4.5 courses (eight sessions) of treatment. Toxic effects occurring during any course of treatment are shown in Table 3. Grade 3 or 4 neutropenia occurred in 11 patients (44%), but only one had febrile neutropenia. Nausea and fatigue were common non-hematologic toxic effects, but most cases were Grade 1 or 2. The dose of irinotecan had to be reduced because of toxicity.
in nine patients (36%); the dose was reduced during the first or second course in eight of these patients. Treatment was delayed during the first two courses in 12 patients (48%). The reasons for treatment delay were neutropenia or leukopenia in 10 patients, anorexia in 2, diarrhea in 2 and infectious colitis in 1. Tumor response is shown in Table 4. Tumor response was assessable in 22 patients. In the other three patients, tumor response could not be assessed because of early discontinuation of treatment. The objective response rate was 24% (95% confidence interval: 9.4–45.1%) with no complete response and six partial responses.

One patient had multiple metastases to the liver, lung and abdominal lymph nodes. The metastases to the lung and abdominal lymph nodes disappeared after eight and a half courses of treatment. In addition, the liver metastasis shrank and could be resected curatively. The major reasons for treatment discontinuation were progressive disease in 18 patients and toxicity or the patient’s refusal to continue treatment in 5.

## DISCUSSION

This study evaluated the DLT and MTD of the FOLFIRI regimen in Japanese patients with advanced CRC. Observational UGT1A1 genotyping and pharmacokinetic analysis were also performed in the phase I part, but these lines of information were not reflected for both patient enrollment and dose escalation. We estimated that the RDs of irinotecan and infusional 5-FU for FOLFIRI in Japanese patients were 180 and 2400 mg/m², respectively, similar to the RDs in Western countries (1,15).

The incidence of Grade 3 or 4 neutropenia was higher, but that of diarrhea was lower than the incidences in previous studies conducted in Western countries (Table 3) (1,2). Treatment was frequently delayed because of neutropenia but could be continued after dose reduction. Febrile neutropenia occurred in only one patient in the phase II part. Our results suggest that toxic effects associated with the RD of FOLFIRI as determined in this study were manageable in patients without the UGT1A1*28 allele.

The doses of irinotecan and infusional 5-FU did not reach the MTD, and only one patient had DLT (Grade 3 diarrhea) at dose Level 2 (irinotecan 150 mg/m² and infusional 5-FU 2400 mg/m²). Therefore, the question remains whether the doses of irinotecan and infusional 5-FU could have been escalated much higher. Previous clinical studies, without UGT1A1 genotyping, reported that irinotecan could be administered in a dose around 260 mg/m². However, these studies did not show a clear advantage of using a higher dose of irinotecan with respect to efficacy and recommended 180–200 mg/m² of irinotecan on the basis of toxicity and compliance (15,16).

Although we analyzed UGT1A1 genotypes, prior stratification had not been applied for dose escalation based on UGT1A1 genotypes or AUC<sub>SN-38/AUC<sub>SN-38G</sub>. UGT1A1 genotyping and pharmacokinetic data were available for 8 of 12 patients who received dose Levels 2 or 3. The patient with UGT1A1*6/*28 who had Grade 4 neutropenia during the first course could continue FOLFIRI treatment after reducing the dose of irinotecan to 100 mg/m². AUC<sub>SN-38/AUC<sub>SN-38G</sub> decreased from 2.16 to 1.56 after dose reduction. The patient had a partial response, with no further severe myelosuppression. One patient with UGT1A1*1/*1 genotype had DLT. Although there was no clear-cut relation between UGT1A1 genotype and DLT because of the small number of patients, our results suggest that patients harboring UGT1A1*6/*28 should be cautiously treated with FOLFIRI and dose reduction might be considered. Previous studies have recommended that caution is exercised when patients with UGT1A1*6/*28, *6/*6 or *28/*28 receive FOLFIRI (8–10). However, no confirmatory dose adjustment study in this population exists and optimal dose remains to be explored.
A genotype-driven phase I study of irinotecan included in the FOLFIRI regimen given to Western patients without UGT1A1*28/*28 demonstrated a higher MTD in those with UGT1A1*1/*1 or UGT1A1*1/*28 genotype and a dose-dependent tumor response (17). In Japan and other Asian countries, dose escalation studies of irinotecan in FOLFIRI should be performed taking into account UGT1A1*28 as well as the *6 genotypes, since the RD may be influenced by the presence of these variant alleles (8–10). The RD of irinotecan for patients without UGT1A1*28/*28, *6/*6 or *6/*28 might be higher even among Asians. However, we could not plan the genotype-driven phase I study of irinotecan in the FOLFIRI regimen at that time, because there was limited information regarding the effects of UGT1A1*6 and *28 on the irinotecan-related toxicities.

In the phase II part of this study, we excluded patients who had at least one UGT1A1*28 allele, because we had considered at that time that these patients were at a higher risk of irinotecan-induced severe toxicities based on the report by Ando et al. (3). So, we demonstrated the feasibility of FOLFIRI at RDs in a limited population.

The RDs of FOLFIRI in Japanese patients were proved to be consistent with those in Western countries. This finding implies that it may be possible for Japanese patients to participate in global trial(s) to evaluate any investigational new agent combined with FOLFIRI.

In conclusion, this phase I/II study demonstrates that the RDs of irinotecan and infusional 5-FU in FOLFIRI for Japanese patients without the UGT1A1*28 allele are determined to be 180 and 2400 mg/m², respectively. Toxic effects at these doses are manageable based on this protocol setting.

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**Conflict of interest statement**

None declared.

**References**


