Value of plasma SN-38 levels and DPD activity in irinotecan-based individualized chemotherapy for advanced colorectal cancer with heterozygous type UGT1A1*6 or UGT1A1*28

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Purpose: The relationship between the pharmacokinetics of irinotecan and outcomes of advanced colorectal cancer is unclear, and few studies have examined individualized irinotecan-based chemotherapy depending on plasma 7-ethyl-10-hydroxy camptothecin (SN-38) levels and dihydropyrimidine dehydrogenase (DPD) activity, particularly for the UGT1A1*6 or UGT1A1*28 heterozygous type.

Methods: This study retrospectively explored the relationship among plasma SN-38 level 1.5 hours after critical enzyme for irinotecan (CPT-11) administration (CSN-38 1.5h), plasma SN-38 level 49 hours after CPT-11 administration (C_SN-38 49h), DPD activity, and clinical outcomes for the UGT1A1*6 and UGT1A1*28 heterozygous types.

Results: C_SN-38 1.5h and C_SN-38 49h of the UGT1A1*6 or UGT1A1*28 heterozygous type were close to those of UGT1A1*6 and UGT1A1*28 wild-types; some of those with relatively high C_SN-38 49h levels obtained better median progression-free survival (mPFS), whereas others with higher C_SN-38 49h concentrations showed a relatively high incidence of adverse reactions possibly because of the decreased activity of DPD.

Conclusion: Increasing the dosage of CPT-11 according to C_SN-38 1.5h may improve the efficacy in patients with lower C_SN-38 1.5h levels. For cases with comparably low DPD activity, advisable primary and subsequent dose adjustment of 5-fluorouracil based on plasma 5-fluorouracil levels may be a practical strategy for reducing the occurrence of adverse reactions for personalized treatment of the UGT1A1*6 or UGT1A1*28 heterozygous type.

Keywords: irinotecan, pharmacokinetics, enzyme activity, uridine diphosphate glucuronosyltransferase 1A1, colorectal cancer

Introduction

Single-nucleotide polymorphisms (SNPs) in drug metabolizing enzymes have an considerable effect on drug absorption, metabolism, distribution, and excretion and can lead to completely different efficacies and/or adverse reactions (ADRs).³,⁴ Uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1), which converts 7-ethyl-10-hydroxy camptothecin (SN-38) to SN-38 glucuronide (SN-38G), is a crical enzyme for irinotecan (CPT-11), which is the first-line drug for treating metastatic colorectal cancer (mCRC). Previous studies demonstrated that the incidence of life-threatening ADRs is often linked to mutant homozygotes in UGT1A1*6 and *28, which reduce or inhibit UGT1A1 activity and increase plasma SN-38 concentrations;³⁴ however, the incidence of the homozygous genotype is <10% in Asian population.⁵,⁶ Thus, in addi-
tion to screening for homozygous genotypes that may cause serious ADRs, the main purpose of CPT-11 individualized therapy for these patients is to elucidate whether the pharmacokinetics of CPT-11 is correlated with clinical outcomes so that the dose can be adjusted within a relatively short period to achieve better results.

There may be a widely variable range of UGT1A1 activity for UGT1A1*6 and/or *28 heterozygous types (including *1/*28-*1/*1, *1/*1-*1/*28, and *1/*28-*1/*1/*6 genotypes). Theoretically, to achieve personalized administration, the best strategy is to relate CPT-11 pharmacokinetics parameters with the UGT1A1*6 and *28 genotypes, rather than relying on one factor. However, the relationship between plasma SN-38 levels or concentration–time curve (area under the curve [AUC]) and clinical efficacy remains unclear, which may be related to the different distribution of UGT1A1*28 and UGT1A1*6 genotypes between population and poor clinical operations of calculating SN-38/SN-38G AUC for the following CPT-11 dosage. Thus, the maximum tolerated dose (MTD) is determined only by dose escalation.10,11 Our previous studies showed that plasma SN-38 level 1.5 hours after CPT-11 administration (C_{SN-38 1.5h}) was related to progression-free survival (PFS) for UGT1A1*6 and *28 wild-types and to better clinical efficacy for relatively high C_{SN-38 1.5h}.10,11

In addition, CPT-11 is routinely combined with 5-fluorouracil (5-FU) as a first-line treatment for mCRC, and 80%–85% of 5-FU is metabolized to inactive dihydro fluorouracil (DHFU) by dihydropyrimidine dehydrogenase (DPD) in the liver. Serious ADRs such as neutropenia, diarrhea, and oral mucositis, which are similar to those caused by CPT-11, occur in cases of partial or total deficiency of DPD activity, leading to inhibition of plasma 5-FU clearance; accordingly, the identification of CPT-11-associated ADRs may be affected. Therefore, it is important to detect DPD activities before FOLFIRI chemotherapy, which can reduce the probability of 5-FU-related ADRs by decreasing the 5-FU dosage for those with lower DPD activities to improve the effectiveness of CPT-11 individualized medication.

Assessing the SNPs UGT1A1*6 and *28 and DPD activities simultaneously is a feasible strategy for dosage personalization of CPT-11, although few studies have examined this approach.13 Thus, we retrospectively explored the correlation between clinical parameters such as C_{SN-38 1.5h}, plasma SN-38 level 49 hours after CPT-11 administration (C_{SN-38 49h}), DPD activity, and outcomes (efficacy and ADRs) to provide a basis for individualized CPT-11 administration according to plasma SN-38 levels and DPD activity for patients with the UGT1A1*28 or *6 heterozygous genotypes.

**Methods**

**Patient’s eligibility**

The SNPs of UGT1A1*6 and *28 were detected in 550 cases before the first chemotherapy treatment from December 2012 to May 2014, and 499 cases met the following inclusion criteria: previously untreated local advanced or mCRC with measurable lesions verified by pathological and imaging data, East Cooperative Oncology Group (ECOG) physical status score of 0–2 points, life expectancy greater than 3 months, no chemotherapy contraindication, written informed consents, serum bilirubin levels, and transaminase levels limited to 1.5- and 5-fold the normal levels, and ability to undergo administration of at least three cycles of FOLFIRI chemotherapy, as well as one assessment. Patients with complete or incomplete intestinal obstruction, chronic enteritis, a history of extensive colectomy, severe allergy to CPT-11 or 5-FU, other malignant tumors and central nervous system metastases, previously treated measurable lesions such as by radiotherapy or local interventional therapy, major organ dysfunction, and poor compliance and pregnancy were ruled out. A total of 234 cases confirmed with UGT1A1*28 and/or *6 heterozygous genotype were analyzed, which include those from the Zhongshan Hospital (54 cases), Cancer Medical Center (43 cases) affiliated with Fudan University Shanghai Medical College, Ruijin Hospital (41 cases), Renji Hospital (36 cases), and General Hospital (30 cases) affiliated with Shanghai Jiaotong University Medical of School and Shanghai Tenth People's Hospital (20 cases) affiliated with Tongji University (Table 1).

**SNPs analysis for UGT1A1**

Plasma genomic DNA was collected using a DNA purification kit (Qiagen, Hilden, Germany), and gene fragments containing UGT1A1*6 and *28 polymorphism sites were amplified by PCR (25 µL): 2 µL of 10× PCR buffer (15 mM MgCl₂), 2 µL of dNTP (2.5 mM), 0.5 µL of sense and antisense primers (10 µM), 0.2 µL of Taq DNA polymerase (5 U/µL), 1 µL of DNA templates, and 18.8 µL of double-distilled water (ddH₂O). The primer pairs for *6 and *28 polymorphism points in the UGT1A1 gene were designed as follows: upstream, 5′-TCCCTGCTACCTTTTGT-GAC-3′; downstream, 5′-AGCAGGCCCAGGACAAGT-3′. The conditions of PCR amplification were as follows: initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 25 seconds, extension at 72°C for 50 seconds, and then extension at 72°C for 7 minutes. Next, 5 µL of eligible PCR samples
tion products were directly sequenced with a DNA sequencer. The PCR enzymatic hydrolysates, 1 µL of sequencing reagent was maintained at 4°C after the reaction. Finally, the reaction at 80°C for 15 minutes. Approximately 3 µL of positive PCR amplification was detected using the Shimadzu UFLC chromatographic system. The mobile phase consisted of acetonitrile:0.05 M NaHPO4 salt solution:triethylamine (72.5:27.5:0.5, v:v:v) with a flow rate of 2.6 mL/min and was adjusted to pH 5.0 by phosphate.

**Detection of plasma SN-38 levels**

SN-38 and internal standard were dissolved in 50% methanol at a concentration of 1.0 mg/mL and stored at −80°C. To draw a calibration curve, an appropriate volume of standard working solution was added to 180 µL aliquots of blank human plasma ranging from 5 to 1,500 ng/mL. All samples were mixed with 100 µL of 7% perchloric acid, vortexed for 3 minutes, and centrifuged at 15,800 × g for 10 minutes. Plasma SN-38 levels were detected using the Shimadzu UFLC chromatographic system as described earlier. Data were processed with Shimadzu LC-Solution chromatography software (version 1.21, SP1).

**Evaluation and follow-up**

The first evaluation was conducted after three cycles of chemotherapy according to evaluation criteria for the curative effect of solid tumor (Response Evaluation Criteria in Solid Tumors).
Tumors, edition 1.1) for all patients. Efficacy reconfirmation was evaluated 4 weeks later for those who achieved complete or partial remission. ADRs were graded under the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0). Patients could be administered other treatments such as second-line chemotherapy with or without molecular targeted drugs and best support care after progression and were visited every 3 months for survival analysis. The median follow-up time was 15 months (range, 8–22 months).

**Statistical methods**

The measurement data were presented as the mean±SD, and the enumeration data were expressed as a rate or composition using SPSS® statistic software (version 19.0; IBM Corporation, Armonk, NY, USA). The Student’s t-test and Log-rank test were used to determine the differences in survival and efficacy between groups. ADRs were graded under the Common Terminology Criteria for Adverse Events (version 3.0 (CTCAE v3.0). Patients could be administered other treatments such as second-line chemotherapy with or without molecular targeted drugs and best support care after progression and were visited every 3 months for survival analysis. The median follow-up time was 15 months (range, 8–22 months).

**Results**

**SNPs and proportion for UGT1A1*6 and/or *28 heterozygous genotype**

The sequencing results are shown in Figure 1A–F for the 234 cases with the UGT1A1*6 and/or *28 heterozygous genotype (accounting for 46.89%), including 116 cases of the *1/*1-*1/*6 genotype, 98 cases of the *1/*28-*1/*6 genotype, and 20 cases of the *1/*28-*1/*6 genotype. The constituent ratio was shown in Figure 1G.

**C_{SN-38} 1.5h and C_{SN-38} 49h between UGT1A1*6 and *28 wild-type and UGT1A1*6 and/or *28 heterozygous genotype**

As shown in Figure 2A, the C_{SN-38} 1.5h values of the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes were 61.74±11.49 and 63.10±10.29 ng/mL, respectively, which were not significantly different from that of the *1/*1-*1/*1 genotype (60.84±11.13 ng/mL, P=0.57 and 0.13), but were significantly lower than that of the *1/*28-*1/*6 genotype (75.10±23.16 ng/mL, P<0.001). The same results were observed for C_{SN-38} 49h (Figure 2B).

Moreover, there were no significant differences in gender, age, ECOG performance status, location of the primary tumor, TMN staging, median of chemotherapeutic cycles, initial doses of CPT-11, and DPD activity between the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes (Table 1).

**Regression analysis of C_{SN-38} 1.5h and C_{SN-38} 49h with clinical parameters**

Stepwise regression analysis was conducted for the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes with C_{SN-38} 1.5h and C_{SN-38} 49h serving as dependent variables, and the initial doses of CPT-11, serum bilirubin levels before and after treatment, chemotherapeutic cycles, short-term response, PFS, overall survival (OS), and ADRs were independent variables. We found that C_{SN-38} 1.5h was related to PFS (t=16.81, P<0.001), whereas C_{SN-38} 49h was related to bone marrow hypocellularity, increased alanine aminotransferase, and diarrhea in the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes (t=8.82, P<0.001; t=5.02, P<0.001; and t=4.84, P<0.001, respectively; Table 2).

**Median PFS (mPFS) of corresponding C_{SN-38} 1.5h and C_{SN-38} 49h subgroups in *1/*28-*1/*1 and *1/*1-*1/*6 genotypes**

As shown in Figure 3, the mPFS of the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes were 6.73±0.18 and 6.80±0.32 months, respectively, with no significant difference between groups (χ²=2.11, P=0.57). However, a comparison of the C_{SN-38} 1.5h >51.82 ng/mL and C_{SN-38} 49h >14.34 ng/mL subgroups with the ≤51.82 and≤14.34 ng/mL subgroups, respectively, grouped according to the adjusted predictive values and standard errors of the plasma SN-38 levels in the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes revealed that the mPFS of the C_{SN-38} 1.5h >51.82 ng/mL subgroup was significantly longer than that of the ≤51.82 ng/mL subgroup (6.83±0.17 vs 5.63±0.31 months, 6.93± 0.48 vs 6.63±0.13 months, 7.27±0.35 vs 6.70±0.21 months, P=0.05 and P=0.59).

**ADRs between corresponding C_{SN-38} 1.5h and C_{SN-38} 49h subgroups in *1/*28-*1/*1 and *1/*1-*1/*6 genotypes**

Given the relationship between C_{SN-38} 49h and bone marrow hypocellularity in the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes, the incidence of ADRs was compared between the C_{SN-38} 49h >14.34 and ≤14.34 ng/mL subgroups; the results showed that the incidence of bone marrow hypocellularity, diarrhea, increased alanine aminotransferase, nausea, and oral mucositis in the C_{SN-38} 49h >14.34 ng/mL subgroup was significantly lower than that of the C_{SN-38} 49h ≤14.34 ng/mL subgroup.
significantly higher than that in the ≤14.34 ng/mL subgroup (P<0.001, P<0.001, P<0.001, and P<0.001); however, the difference between the CSN38-1.5h>51.82 and ≤51.82 ng/mL subgroups was not significant (P=0.04, P=0.24, P=0.97, P=0.12, and P=0.27; A, B).

**DPD activities between CSN-38 49h >14.34 and ≤14.34 ng/mL subgroups**

Comparison of the DPD activities between the CSN-38 49h >14.34 and ≤14.34 ng/mL subgroups showed that the enzyme activities of the former were clearly lower than those of the latter (3.24±1.02 vs 4.93±2.08, F=11.20, P=0.001; Figure 2C).

**mPFS of DPD activities between >4.13 and ≤4.13 subsets in CSN-38 49h >14.34 and ≤14.34 ng/mL subgroups**

By setting DPD activities as dependent variables and clinical parameters such as short-term response, PFS, OS, and ADRs as independent variables, stepwise regression indicated that DPD activities were related to the bone marrow hypocellularity and increased alanine aminotransferase (t=−3.03 and t=−2.75, P=0.003 and P=0.007; Table 2), and the mPFS of DPD activities of the >4.13 and ≤4.13 subsets divided based on the adjusted predictive values and stand errors did not greatly differ in the CSN38-49h >14.34 ng/mL subgroup (6.83±0.33 vs 7.27±0.53 months, χ²=0.07, P=0.85; Figure 3).

**Discussion**

Dosage individualization of chemotherapeutic drugs is an important factor in personalized cancer treatment, and it has been widely acknowledged in mCRC that CPT-11-associated life-threatening ADRs can be avoided by screening out the UGT1A1 homozygous genotype before administration of CPT-11-based chemotherapy;14,15 however, meta-analysis and studies did not confirm the relationship between the UGT1A1*6 and *28 genotypes and clinical outcomes,3,16–19 but individual dose adjustment is difficult based only on the UGT1A1 genotype. Moreover, most Asian populations have wild-type UGT1A1 or a heterozygous genotype, and the risks of CPT-11-associated serious ADRs are much lower than those for the homozygous genotype according to some meta-analyses, as SN-38 glucuronidation of the former two has been completely saturated.20 Therefore, the main purpose of personalized CPT-11 administration is to

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**Figure 1** Sequencing results of UGT1A1*28 and *6 SNPs and distributive characteristics of different SNP combinations for mCRC patients.

**Notes:** DNA sequencing for wild-type UGT1A1*28 (A) and UGT1A1*6 (D), heterozygous type UGT1A1*28 (B) and UGT1A1*6 (E), and homozygous type UGT1A1*28 (C) and UGT1A1*6 (E) by FinchTV® software. (G) The pie chart gives the proportion of the different combinations of wild-type (*1/*1,*1/*1 genotype: 244 cases, which accounted for 48.90%), heterozygous type (*1/*28-*1/*1, *1/*1-*1/*6, and *1/*28-*1/*6 genotype: 234 cases, which accounted for 46.89%), and heterozygous type (*28/*28-*1/*1, *1/*1-*1/*6, *1/*28-*1/*6, and *28/*28-*1/*6 genotype: 21 cases, which accounted for only 4.21%).

**Abbreviations:** mCRC, metastatic colorectal cancer; SNP, single-nucleotide polymorphism; UGT1A1, uridine diphosphate glucuronosyltransferase 1A1.
improve the therapeutic effect by dosage adjustment based on SN-38 pharmacokinetics. The MTD restricts dose escalation because of the factors such as the dose increase extent, escalation intervals, and patients’ compliance, leading to different subclinical doses administered to patients and distress in judging the outcomes of CPT-11. Accordingly, it is necessary to take SN-38 pharmacokinetics into account when the correlation between different genotypes and outcomes are evaluated, particularly for the heterozygous genotype, which accounts for a large proportion of patients and shows variable UGT1A1 activities.5,21

Previous studies of pharmacokinetics showed that the plasma CPT-11 levels reached a peak at 1.5 hours and decreased to minimum levels at 25.5 hours after intravenous CPT-11 infusion,22 and thus, the plasma SN-38 levels at 1.5 and 49 hours after CPT-11 infusion were evaluated to reflect the metabolism of CPT-11 in this study to examine CPT-11 dose individualization over a relatively short period. It is difficult to determine MTD by computing the AUC8,23 because of factors such as repeated blood sampling, high cost of the examination, long submission cycle, difficult promotion, and poor compliance of patients. Our results showed that the C_{SN-38 1.5h} and C_{SN-38 49h} of the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes were close to that of the *1/*1-*1/*1 genotype, indicating that the UGT1A1 activities of the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes were similar to that of the *1/*1-*1/*1 genotype. Thus, we

![Figure 2](image-url)

**Figure 2** C_{SN-38 1.5h} and C_{SN-38 49h} between UGT1A1*6 and *28 wild-type and UGT1A1*6 and *28 heterozygous genotype as well as DPD activities between C_{SN-38 49h} greater than 14.34 ng/mL and ≤14.34 ng/mL subgroups.

**Notes:** The C_{SN-38 1.5h} of *1/*1-*1/*1 genotype was 60.84±11.13 ng/mL, having no significant difference with those of *1/*28-*1/*1 and *1/*1-*1/*6 genotype (61.74±11.49 and 63.10±10.29 ng/mL, P=0.57 and 0.13, respectively), but with statistical difference being found in that of *1/*28-*1/*6 genotype (75.10±23.16 ng/mL, P<0.001, seen in A). Likewise in C_{SN-38 49h}, the C_{SN-38 49h} of *1/*28-*1/*1 and *1/*1-*1/*6 genotype were 11.49±5.06 and 10.29±3.70 ng/mL, respectively, which did not differ obviously from that of *1/*1-*1/*1 genotype (11.13±4.95 ng/mL, P=0.52 and 0.13), while being significantly different from that of *1/*28-*1/*6 genotype (23.16±6.95 ng/mL, P<0.001, shown in B).

In C, the DPD activity of C_{SN-38 49h} (14.34 ng/mL subgroup was 3.24±1.02, remarkably lower than that of C_{SN-38 49h} ≤14.34 ng/mL subgroup with obvious difference (4.93±2.08, F=11.20, P<0.001).

**Abbreviations:** CPT-11, irinotecan; C_{SN-38 1.5h}, plasma SN-38 level 1.5 hours after CPT-11 administration; C_{SN-38 49h}, plasma SN-38 level 49 hours after CPT-11 administration; DPD, dihydropyrimidine dehydrogenase; UGT1A1, uridine diphosphate glucuronosyltransferase 1A1.

### Table 2

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**Abbreviations:** CPT-11, irinotecan; C_{SN-38 1.5h}, plasma SN-38 level 1.5 hours after CPT-11 administration; C_{SN-38 49h}, plasma SN-38 level 49 hours after CPT-11 administration; DPD, dihydropyrimidine dehydrogenase; PFS, progression-free survival.
Figure 3 mPFSs of the corresponding Csn-38 1.5h and Csn-38 49h subgroups in *1/*28-*1/*1 and *1/*1-*1/*6 genotype and mPFS of DPD activities between >4.13 and ≤4.13 subsets in Csn-38 49h >14.34 ng/mL and ≤14.34 ng/mL subgroups, respectively, accordingly.

Notes: No statistical difference was observed about the mPFS among *1/*28-*1/*1, *1/*1-*1/*6, and *1/*28-*1/*6 genotypes (A) (6.73±0.13 months vs 6.73±0.18 months vs 6.80±0.32 months, \( \chi^2 = 1.11, \ P = 0.57 \)), but differences were displayed clearly between the mPFS of Csn-38 1.5h >51.82 ng/mL and that of ≤51.82 ng/mL subgroup in *1/*28-*1/*1 (B) and *1/*1-*1/*6 genotypes (C) (6.83±0.17 vs 4.87±0.13 months, \( P < 0.001 \); 6.93±0.34 vs 5.63±0.31 months, \( P < 0.001 \)), which were divided by the adjusted predictive values and stand errors of Csn-38 1.5h, while the mPFS did not differ between Csn-38 49h >14.34 ng/mL and ≤14.34 ng/mL subgroups grouped by the same way in *1/*28-*1/*1 (D) and *1/*1-*1/*6 genotypes (E) (6.83±0.48 vs 6.63±0.13 months, \( P = 0.80 \); 7.27±0.35 vs 6.70±0.21 months, \( P = 0.59 \)). The mPFS of DPD activities >4.13 and ≤4.13 subset divided based on the adjusted predictive values and stand errors did not differ obviously in Csn-38 49h >14.34 ng/mL and ≤14.34 ng/mL subgroups of *1/*28-*1/*1 (F) and *1/*1-*1/*6 genotypes (G) (6.83±0.33 vs 7.27±0.53 months, \( \chi^2 = 0.04, \ P = 0.85 \); 6.60±0.12 vs 6.73±0.22 months, \( \chi^2 = 0.07, \ P = 0.79 \)).

Abbreviations: CPT-11, irinotecan; Csn-38 1.5h, plasma SN-38 level 1.5 hours after CPT-11 administration; Csn-38 49h, plasma SN-38 level 49 hours after CPT-11 administration; DPD, dihydropyrimidine dehydrogenase; mPFS, median PFS; PFS, progression-free survival.
selected the *1/*28-*1/*1 and *1/*1-*1/*6 genotypes for retrospective analysis because of the relatively low risk of ADRs for dose personalized adjustment of CPT-11. Table 1 shows that the clinical characteristics of the two genotypes were comparable in combined analysis, and stepwise regression analysis revealed that C_{SN-38 1.5h} was relevant to PFS and C_{SN-38 49h} was associated with ADRs such as bone marrow hypocellularity. Further analysis indicated that the mPFS of the C_{SN-38 1.5h}>51.82 ng/mL subgroup was significantly longer than that of the ≤51.82 ng/mL subgroup, whereas no difference of mPFS was observed between the C_{SN-38 49h}>14.34 ng/mL subgroup and≤14.34 ng/mL subgroup, suggesting that the efficacy can be improved by increasing the CPT-11 dose while monitoring C_{SN-38 1.5h}>51.82 ng/mL subgroup. For ADRs, we found no significant difference between the C_{SN-38 1.5h}>51.82 and≤51.82 ng/mL subgroups; however, the ADRs incidence in the C_{SN-38 49h}>14.34 ng/mL subgroup was significantly higher than that in the ≤14.34 ng/mL subgroup. Although a previous study indicated that 5-FU did not change the metabolic process of CPT-11 within a regular dose range,24 the determination of CPT-11-associated ADRs can be affected because similar 5-FU-correlated ADRs are caused by decreased or inhibited DPD activities. To evaluate the high incidence of ADRs in the C_{SN-38 49h}>14.34 ng/mL subgroup, stepwise regression was conducted, which showed that when DPD activities were correlated with bone marrow hypocellularity and increased alanine aminotransferase, the DPD activities of C_{SN-38 49h}>14.34 ng/mL subset were remarkably lower than that of the ≤14.34 ng/mL subset; however, there was no significant difference in mPFS between the DPD activities >4.13 subset and ≤4.13 subset (Figure 2C), indicating that 5-FU-associated ADRs due to decreased activities of DPD were misclassified as CPT-11-related ADRs caused by reduced UGT1A1 activities. This leads to mistaken down-regulation of the CPT-11 dose, and thus, the activity or SNPs of DPD must be determined before adjusting the 5-FU dosage before CPT-11-based chemotherapy. In addition, reducing the doses of 5-FU did not affect the outcomes.

Subsequent dose individualization of CPT-11 and its effect on outcomes require further analysis via plasma 5-FU levels monitoring, improving the stability and repeatability of the method to detect the plasma SN-38 levels, and conducting prospective randomized controlled studies with larger samples. In addition, other biomarkers such as members of the ATP-Binding Cassette Subfamily C (ABCC),25–27 organic anion-transporting polypeptide 1B1,28,29 and other factors including obesity10 and human organ function11,12 require further analysis to identify better biomarkers or combinations of biomarkers for predicting the efficacy and/or ADRs of CPT-11-based chemotherapy.

**Conclusion**

According to our analyses, a dose increase of CPT-11 based on C_{SN-38 1.5h} may improve the efficacy in patients with lower C_{SN-38 1.5h} levels. For cases with relatively low DPD activity, advisable primary and subsequent dose adjustment of 5-FU based on the plasma 5-FU levels may be a practical strategy for reducing the incidence of 5-FU-associated ADRs for
individualized administration of CPT-11 to those with the UGT1A1*6 or *28 heterozygous type.

Ethics approval and consent to participate

The plan of the research has taken full consideration in the principles of safety and fairness and would be risk free to the patients. This article does not contain any studies with human participants or animals performed by any of the authors. The investigator would protect the patients’ rights and privacy to the maximum extent and make sure that there were no conflicts of interest between the contents and the results of the research. Although no formal consent is required for this type of retrospective research, to ensure the implementation of the project, the patients were admitted to the study providing that they came across this principle of “Ethics, consent and permissions”. Before the plasma specimen being collected, the patients were fully informed as follows: the purposes and methods of the study, the plasma specimen as part of the context, the project would not increase the extra medical costs and pain of patients, and the materials and results of the study were used for the purposes of scientific research without conflict of interest. Any report and publication of the individual patient data (in the form of images, videos, voice recordings, etc) needed the approval of the patients enrolled in the study.

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Author contributions

This work was completed with the cooperation of all authors. Xun Cai and Rongyuan Zhuang defined the research objective. Xun Cai, Chuan Tian, and Haifeng Ying designed methods and experiments, carried out the laboratory experiments, analyzed the data, interpreted the results, and wrote the manuscript. Rongyuan Zhuang, Haifeng Ying, Xiaowei Zhang, Hongmin Lu, Hui Wang, Qi Li, and Chungang Wang worked together on patient screening and associated data collection, and Shuowen Wang provided guidance on the pharmacokinetic tests. All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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