Dose-dependent association between UGT1A1*28 genotype and irinotecan-induced neutropenia: Low doses also increase risk

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Statement of Translational Relevance

In 2005, FDA made the recommendation that patients with a UGT1A1*28/*28 genotype should receive a lower starting dose of irinotecan. Then came conflicting results from a meta-analysis by Hoskins et al., suggesting UGT1A1*28 had no effect on irinotecan-induced neutropenia among patients treated with a low dose of irinotecan. In this study, we performed an updated and refined meta-analysis. We found UGT1A1*28/*28 genotype was associated with a twofold increased risk of neutropenia not only at medium doses but at low doses as well. We suggest genotype-based dosing may be necessary for patients treated with low doses of irinotecan.
Abstract

Purpose: A previous meta-analysis showed the association between UGT1A1*28 genotype and irinotecan-induced neutropenia was influenced by irinotecan dose and that the risk of neutropenia was similar at low doses for patients with all genotypes. However, the sample sizes for the low and high-dose group were small. Because additional studies have now been reported, an updated and refined meta-analysis is needed.

Experimental Design: Meta-analyses were performed to assess the relationship between UGT1A1*28 and neutropenia. The association between UGT1A1*28 and relative extent of glucuronidation (REG) of SN-38 was also examined. The studies included were stratified into different dose groups.

Results: 1998 patients were included for the analysis of neutropenia and 581 patients were included for REG. The risk of neutropenia at low doses was significantly higher among patients with a UGT1A1*28/*28 genotype than among those carrying UGT1A1*1 allele(s) (RR = 2.43, 95% confidence interval (CI) = 1.34–4.39; P = 0.003). In addition, the relative risk (RR) of neutropenia at low doses was comparable to that of medium doses (2.43 vs 2.00). RR of neutropenia at high doses was significantly higher than that of low and medium doses (7.22 vs 2.04). We found weighted mean difference (WMD) of REG (UGT1A1*1/*1 or UGT1A1*1/*28
vs UGT1A1*28/*28) increased with increasing dose of irinotecan.

Conclusions: In conclusion, UGT1A1*28/*28 genotype was associated with an increased risk of neutropenia not only at medium or high doses of irinotecan but also at low doses. The dose-dependent manner of SN-38 glucuronidation explained why the association between UGT1A1*28 and neutropenia was dose-dependent.
Introduction

Irinotecan is approved for use in combination with 5-fluorouracil and leucovorin for first-line treatment of metastatic colorectal cancer (1). Irinotecan efficacy is dependent on activation by carboxyesterases to form the active metabolite SN-38. The major route of SN-38 elimination is via glucuronidation by hepatic UGT1A enzymes (2).

UGT1A1*28 is a common allele with seven TA repeats in the TATA box of the UGT1A1 promoter compared with the wild-type allele, which has six TA repeats. UGT1A1*28 was demonstrated to be associated with decreased SN-38 glucuronidation in humans (3–11). The association between UGT1A1*28 genotype and irinotecan-induced neutropenia was extensively studied (3–5, 12–23). Seven of these studies found UGT1A1*28/*28 patients had an elevated risk of neutropenia compared with those carrying UGT1A1*1 allele(s) (4, 12, 13, 17, 19–21).

In 2005, the US Food and Drug Administration (FDA) recommended the Pfizer to amend the package insert of irinotecan to warn of the elevated risk of neutropenia for UGT1A1*28/*28 patients. A subsequent meta-analysis showed the association between UGT1A1*28 and neutropenia was influenced by the dose of irinotecan and that the risk of neutropenia was similar at lower doses for patients with all genotypes (1). It was noteworthy that the sample sizes for the low and high-dose group were small (229 patients and 81 patients). In addition, the studies included in this meta-analysis seemed to be weighted by sample size. This weighted method is not the
recommended method in the Cochrane Handbook for Systematic Reviews of Interventions version 5.0.2. Because additional studies have now been reported, an updated and refined meta-analysis is needed. What’s more, the pharmacokinetic mechanism for the dose-dependent association between UGT1A1*28 and neutropenia is currently unclear.

In this study, we performed an updated and refined meta-analysis to study the association between UGT1A1*28 genotype and irinotecan-induced neutropenia. To explore the pharmacokinetic mechanism for the dose-dependent association between UGT1A1*28 and neutropenia, the association between UGT1A1*28 and relative extent of glucuronidation (REG) of SN-38 was also examined.

Materials and methods

**Search strategy and selection Criteria.** Two investigators (ZYH, QY) independently searched Medline, PubMed and Embase (from 1980 until April 15, 2010) databases using the terms “irinotecan,” “UGT1A1,” and “neutropenia (or pharmacokinetics)”. Furthermore, we reviewed citations in the retrieved articles to search for additional relevant studies. Data derived from abstracts were also used.

A priori we defined strict criteria for inclusion of studies. Studies were included if 1) they could be defined as clinical trials; 2) the exposure of interest was UGT1A1*28 genotype; 3) the outcome of interest was irinotecan-induced neutropenia (grade III–IV) or relative extent of glucuronidation (REG) of SN-38; and 4) Numbers of patients
with and without neutropenia were provided (or can be calculated by relevant data).

We excluded studies that were not published in English; studies that included fewer
than twenty patients; studies that included less than four UGT1A1*28/*28 patients
(This criterion was not considered when we study the association between
UGT1A1*28 and REG); studies that reported hematological toxicity instead of more
specific neutropenia; studies that included children patients.

Data Extraction. The following information was abstracted from included
publications: study design, year of publication, race, irinotecan dose, number of
patients with and without neutropenia (grade III–IV) in each genotype group
(UGT1A1*1/*1, UGT1A1*1/*28 and UGT1A1*28/*28), REG values in each
genotype group (converted to the units of ng·h/mL versus ng·h/mL), mutation
detection method, plasma sampling scheme, analytical method and potential
confounders.

Two irinotecan-containing regimens were given to patients in the N9741 study (21),
and in our analyses, we analyzed the patients treated with each regimen as two
separate samples. Two irinotecan regimens (250 mg/m² and 350 mg/m²) were
administered to patients in another study (13). In this study, only three
UGT1A1*28/*28 patients were included in the 350-mg/m² dose group. Hence, this
group was excluded from our analysis according to the inclusion criterion. The
patients treated with different regimens were analyzed as one sample if separate data
was not available. If the patients in a trial received different irinotecan doses and only combined toxicity-related data (or REG data) was available, we calculated the average dose. In one study (16), the numbers of chemotherapy cycles with neutropenia in each genotype group were provided instead of the number of patients. We calculated the number of patients as \( C \times tN / tC \), where \( C \) is the number of chemotherapy cycles with neutropenia in each genotype group, \( tC \) is total number of chemotherapy cycles with neutropenia, \( tN \) is total number of patients with neutropenia. REG values were reported as medians and ranges instead of means and SDs in three studies (5, 6, 9), we imputed the means and SDs as described by Hozo et al (24). The REG values were obtained by visual measurement of the figures in two studies (10, 11). The REG values obtained from UGT1A1*1/*28 patients and UGT1A1*1/*1 patients were combined by the formula suggested in the Cochrane Handbook for Systematic Reviews of Interventions version 5.0.21.

**Assessment of study quality.** The use of quality scoring system in meta-analyses of observational studies is controversial. Methodological components of study designs, rather than aggregate scores themselves, may be important (25). Here, we did not assign a single grade or scores to represent the quality of a study. Instead, we focused on certain items that are reflective of methodological and reporting quality of the studies as delineated in the published guidelines for reporting of pharmacogenetic studies (26). In addition, we also pay attention to other quality criterions that are
specific to our study. These issues of concern are source of population, mutation
detection method, analytical method, plasma sampling scheme, type of cancer,
chemotherapy regimens and grade criteria for neutropenia (see Supplementary Tables
S1 and S2).

**Statistical analysis.** With regard to the association between UGT1A1*28 and
neutropenia, the effect measures of interest were relative risks (RRs). The effect
measures of interest were mean differences (MDs) for the association between
UGT1A1*28 and REG of SN-38. Statistical heterogeneity among studies was
evaluated using the $\chi^2$ test, $P$ values, and $I^2$ statistics (27). We considered both the
presence of significant heterogeneity at the 10% level of significance and values of $I^2$
exceeding 56% as an indicator of significant heterogeneity. A fixed effects model was
used to obtain summary RR (Mantel-Haenszel method) or weighted mean difference
(WMD) (Inverse variance method). We evaluated potential publication bias
statistically with the methods described by Begg and Mazumdar (28) and Egger (29).

The studies included were stratified into different dose groups. Irinotecan dose
levels were pooled into the following three subgroups: low (<150 mg/m$^2$), medium
(150–250 mg/m$^2$), and high (≥250 mg/m$^2$) doses on the basis of the three most
commonly used dosage regimens (1). We used meta-regression analyses to investigate
the effect of irinotecan dose on the association between UGT1A1*28 and neutropenia
(or REG). Hardy-Weinberg equilibrium (HWE) was tested using chi-square test. We
conducted a sensitivity analysis in which one or two studies were removed, and the
rest were analyzed to evaluate whether there was a statistically significant effect on
the results. All statistical tests were two-sided. Meta-analysis was done with Stata
(version 10.1; Stata Corp, College Station, TX).

Results

Study characteristics and methodological quality (Association between
UGT1A1*28 and neutropenia). 68 potentially relevant studies were evaluated (Fig. 1
shows the numbers of studies evaluated at each stage). Fifteen clinical trials including
1998 patients were identified. Table 1 details the studies’ characteristics. Three
included studies were published as abstracts (21–23). Patients were predominantly
Caucasians in eleven trials. Four trials did not clearly report the race of the
participants (16, 20, 22, 23). However, the patients in these four trials may be
Caucasians because these trials were conducted in Europe or America and the
reported frequency of UGT1A1*28 allele was similar to that of Caucasians. No
deviation from HWE was detected in any of the identified studies (P > 0.05;
chi-square test).

Quality-assessment tables are shown in full in Supplementary Table S1. Study
sample sizes were small (median 89; range 20–628). Four of the fifteen trials (27%)
described a sample size calculation and a priori defined the power to detect effect
sizes. Eleven trials (73%) were investigated prospectively. Genotyping procedures
were described by ten trials (67%). None of the trials described the mode of
inheritance or checked for the presence of population stratification. Six trials (10%) checked for HWE and none of the trials found deviation from HWE. Eight trials (53%) were multicentre trials. DNA sequencing method was used in five trials (33%). In thirteen trials (87%), the patients were suffering from the same type of cancer. Single chemotherapy regimen was employed in eleven trials (73%). Toxicities were evaluated on the basis of National Cancer Institute Common Toxicity Criteria in most of the included trials (80%).

The results of meta-analysis are summarized in Table 3.

Study characteristics and methodological quality (Association between *UGT1A1*<sup>28</sup> and REG). 72 potentially relevant studies were evaluated (Fig. 1 shows the numbers of studies evaluated at each stage). Nine clinical trials including 581 patients were identified. Table 2 details the studies’ characteristics.

Quality-assessment tables are shown in full in Supplementary Table S2. Study sample sizes were small (median 61; range 20–134). Two trials (22%) described a sample size calculation and a priori defined the power to detect effect sizes. Four trials (44%) were investigated prospectively and the other four trials did not report the study design (44%). Genotyping procedures were described by five trials (56%). Three trials (33%) checked for HWE and none of the trials found deviation from HWE. Four trials (44%) were multicentre trials. DNA sequencing method was used in five trials (56%). In one trial (11%), the patients were suffering from the same type of cancer.
cancer. Single chemotherapy regimen was employed in five trials (56%). Five trials (56%) determined the concentrations of SN-38 glucuronide (SN-38G) directly (without hydrolysis of SN-38G) and the other two trials (22%) did not report it clearly. In six trials (67%), the plasma samples from each patient appear enough for the accurate determination of the pharmacokinetic parameters.

The results of meta-analysis are summarized in Table 3.

**Association between UGT1A1*28 and neutropenia (UGT1A1*28/*28 vs UGT1A1*1/*1 or UGT1A1*1/*28).** Relevant data for the comparison of the risk of neutropenia between UGT1A1*28/*28 patients and those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype was available in fifteen included trials (3–5, 12–23). Considering the individual subgroup, there was no evidence of publication bias according to either the Begg’s test or Egger’s test (Table 3). However, when all the dose groups are combined, publication bias may exist (Egger’s test, P = 0.043) (Table 3). This may be caused by the three high-dose studies with high RR estimates.

Overall analyses suggest an increased risk of neutropenia among UGT1A1*28/*28 patients as compared with those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype (RR = 2.20, 95% confidence interval (CI) = 1.82–2.66; P < 0.001) (Fig. 2 A). Heterogeneity was not statistically significant across all studies (I² = 16.3%, P = 0.267). The value of I² suggested the existence of weak heterogeneity across all studies, though without statistical significance. Hence, we did meta-regression to...
determine whether irinotecan dose is a significant source of heterogeneity across studies. Not unexpectedly, meta-regression showed that irinotecan dose was a significant source of heterogeneity (P = 0.006) (Fig. 3 A1).

Analyses were further stratified by irinotecan doses. Unexpectedly, the risk of neutropenia at low doses was significantly higher among UGT1A1*28/*28 patients than among those with at least one UGT1A1*1 allele (RR = 2.43, 95% CI = 1.34–4.39; P = 0.003) (Fig. 2 A). The risk of neutropenia at medium doses was also higher among UGT1A1*28/*28 patients than among those with at least one UGT1A1*1 allele (RR = 2.00, 95% CI = 1.62–2.47; P < 0.001) (Fig. 2 A). With regard to the high-dose subgroup, the risk of neutropenia was much higher among UGT1A1*28/*28 patients than among those with at least one UGT1A1*1 allele (RR = 7.22, 95% CI = 3.10–16.78; P < 0.001) (Fig. 2 A). RR of neutropenia at high doses was significantly higher than that of low and medium doses (RR = 7.22, 95% CI = 3.10–16.78 vs RR = 2.04, 95% CI = 1.67–2.49) (Table 3).

Figure 3 (A1) showed the relationship between RRs of neutropenia and irinotecan doses. There was only slight increase in RRs of neutropenia when irinotecan doses increased from 80 to 250 mg/m². However, RRs of neutropenia increased dramatically when doses increased from 250 to 350 mg/m².

It should be pointed out that N9741 trial included only grade IV toxicity (21). The study by Roth et al. (22) was given the most weight (54%) in our meta-analysis. However, exclusion of both studies from the meta-analysis did not change the general
result. For example, the risk of neutropenia at low doses was still higher among UGT1A1*28/*28 patients than among those with at least one UGT1A1*1 allele (RR = 2.61, 95% CI = 1.39–4.91; P = 0.003). In addition, RR of neutropenia at low doses was comparable to that of medium doses (2.61 vs 2.14).

Association between UGT1A1*28 and neutropenia (UGT1A1*1/*28 vs UGT1A1*1/*1). Fourteen trials compared the risk of neutropenia between patients with a UGT1A1*1/*28 genotype and those with a wild-type genotype (3–5, 12–19, 21–23). One study was excluded from analysis because none of the patients suffered from neutropenia (3). The Egger’s test showed evidence of publication bias (Table 3). When we excluded the study by Roth et al. (22), the signs of publication bias disappeared (Egger’s test, P = 0.183 for low and medium-dose group; P = 0.980 for medium-dose group). It is noteworthy that the study by Roth et al. (22) was published as abstract.

The pooled RR was 1.43 (95% CI = 1.16–1.77; P = 0.001) for all studies (Table 3). No statistical heterogeneity was detected (I² = 0, P = 0.529). Meta-regression showed that irinotecan dose was not a significant source of heterogeneity (P = 0.626) (Fig. 3 B1). RR of neutropenia did not show significant difference among three subgroups (Table 3).

In a sensitivity analysis excluding two studies (N9741 trial (21) and the study by Roth et al. (22)), the results were unchanged. For example, RR of neutropenia still did
not show significant difference among three subgroups. Irinotecan doses had no influence on RR of neutropenia (P = 0.454).

**Association between UGT1A1*28 and REG (UGT1A1*1/*1 or UGT1A1*1/*28 vs UGT1A1*28/*28).** Nine included trials compared REG of SN-38 between patients carrying UGT1A1*1 allele(s) and those with a UGT1A1*28/*28 genotype (3–11). There was no evidence of publication bias according to either the Begg’s test or Egger’s test (Table 3).

Overall analyses suggest an increased REG of SN-38 among patients carrying UGT1A1*1 allele(s) as compared with those with a UGT1A1*28/*28 genotype (WMD = 2.44, 95% CI = 1.73–3.14; P < 0.001) (Fig. 2 B). No statistical heterogeneity was detected (I² = 0, P = 0.642). Analyses were further stratified by dose. WMD of REG at low and medium doses (WMD = 1.62, 95% CI = 0.57–2.68; P = 0.002) was lower than that of high doses (WMD = 3.08, 95% CI = 2.14–4.02; P < 0.001) (Fig. 2B). Heterogeneity between subgroups was significant (P = 0.043). Figure 3 (A2) showed a non-significant positive correlation between WMD of REG and irinotecan doses (P = 0.124).

**Association between UGT1A1*28 and REG (UGT1A1*1/*1 vs UGT1A1*1/*28).** Nine included trials compared REG of SN-38 between patients with a UGT1A1*1/*1
genotype and those with a UGT1A1*28/*28 genotype (3–11). There was no evidence of publication bias according to either the Begg’s test or Egger’s test (Table 3).

Analyses were stratified by dose. WMD of REG showed no significant difference between low/medium-dose group (WMD = 1.85, 95% CI = 1.00–2.70; P < 0.001) and high-dose group (WMD = 1.03, 95% CI = -0.09–2.16; P = 0.072) (Table 3). Heterogeneity between subgroups was not significant (P = 0.225). There was no correlation at all between WMD of REG and irinotecan doses (Figure 3 B2).

Discussions

The primary finding of this study is that UGT1A1*28/*28 genotype was associated with an increased risk of neutropenia not only at medium or high doses of irinotecan but at low doses as well. RRs of neutropenia among UGT1A1*28/*28 patients were comparable at low and medium doses. Secondary finding is that there was no correlation between RRs of neutropenia among patients heterozygous for UGT1A1*28 (as compared with wild-type genotype) and irinotecan doses. The last finding is that WMD of REG (UGT1A1*1/*1 or UGT1A1*1/*28 vs UGT1A1*28/*28) increased with increasing dose of irinotecan. In contrary, when comparing REG between patients with a UGT1A1*1/*1 genotype and those with a UGT1A1*1/*28 genotype, WMD of REG did not change with increasing dose of irinotecan. This finding shed light on the mechanism of the dose-dependent association between UGT1A1*28 and neutropenia.
On the basis of the findings of four initial studies (4, 15, 17, 30), FDA made the recommendation that patients with a UGT1A1*28/*28 genotype should receive a lower starting dose of irinotecan. Then came conflicting results from a meta-analysis (821 patients) by Hoskins et al. (1), suggesting the association between UGT1A1*28 and neutropenia was dose dependent; UGT1A1*28 had no effect in patients treated with a low dose of irinotecan (<150 mg/m²). Our current meta-analysis based on a large sample size (1999 patients) indicates that UGT1A1*28/*28 patients are at an increased risk of neutropenia not only if they are being treated with medium (RR = 2.00) and high doses (RR = 7.22) of irinotecan but also if they are being treated with low doses (RR = 2.43) (80 to 145 mg/m²).

Diarrhea is another important side effect related to irinotecan administration. Recently, we found UGT1A1*28/*28 patients were at an increased risk of diarrhea at medium (RR = 1.79, 95% CI = 1.08–2.97) or high doses (RR = 2.32, 95% CI = 1.25–4.28) of irinotecan, but not at low doses (RR = 0.65, 95% CI = 0.27–1.58) (31). As a result, when selecting the dose of irinotecan for UGT1A1*28/*28 patients, this information should be considered together with the results of the current meta-analysis.

Implications for clinical practice should be considered. When regimens with a high dose of irinotecan are being considered, dose reduction is advisable for UGT1A1*28/*28 patients (neutropenia RR = 7.22 and diarrhea RR = 2.32). When regimens with a medium dose of irinotecan are being considered, dose reduction is
also recommended for UGT1A1*28/*28 patients (neutropenia RR = 2.00 and diarrhea RR = 1.79). However, dose reduction is optional for UGT1A1*28/*28 patients (neutropenia RR = 2.43) treated at low irinotecan doses. In this regard, we suggest that UGT1A1*28/*28 patients with other predictors of irinotecan-induced toxicity should be given a reduced dose of irinotecan. Other predictors of irinotecan-induced toxicity could be nongenetic factors (neutrophil baseline levels or sex) or genetic factors (UGT1A1*93, ABCC1 IVS11 –48C>T or SLCO1B1*1b) (32).

Recently, Toffoli et al. (33) evidenced that the recommended dose of 180 mg/m² for irinotecan in FOLFIRI is considerably lower than the dose that can be tolerated by the non–UGT1A1*28/*28 patients. This result was supported by our data that incidence of neutropenia (%) was not increased with increasing dose of irinotecan in non–UGT1A1*28/*28 patients (Supplementary Figure S1).

Limitations of this meta-analysis need to be considered. Firstly, the possibility of information and selection biases cannot be completely excluded because some of the included studies were retrospective. Secondly, despite no statistical heterogeneity was observed among the analyzed studies, there were many sources of heterogeneity among the analyzed studies, such as study design, source of population, dose administered, chemotherapy regimens, mutation detection methods, toxicity grade criteria, sampling scheme, type of tumor and stage of disease. Thirdly, three included trials were published as abstracts. Fourthly, in our analysis, grade III–IV neutropenia data was available for most studies, whereas only grade IV neutropenia information
could be extracted from two studies (20, 21). Fifthly, for the study of the association between UGT1A1*28 and REG of SN-38, two included trials were conducted on Asian patients. Ethnic difference was not considered here.

To assess the potential for publication bias to have influenced the results of our meta-analysis, we calculated the fail-safe numbers using a weighted method (35). A fail-safe number can be defined as the number of non-significant, unpublished studies that would be needed to reduce a statistically significant observed result to non-significance (34, 35). The fail-safe number was 14 for the low-dose group, 148 for the medium-dose group, and 21 for the high-dose group (at $\alpha = 0.05$). It seems unlikely that such numbers of studies with null findings exist and have not been published.

In the present meta-analysis, we excluded two trials conducted on children according to the exclusion criterion (36, 37). The children in these two trials were administrated with very low doses of irinotecan (30 and 50 mg/m²). Pooled RR from these two trials (118 patients) showed the risk of neutropenia was similar between UGT1A1*28/*28 patients and those carrying UGT1A1*1 allele(s) (RR = 0.63, 95% CI = 0.14–2.72; $P = 0.532$). However, it is still too early to draw reliable conclusion based on two trials including only 118 patients. As a result, further studies are required in this area.

In conclusion, UGT1A1*28/*28 genotype was associated with an increased risk of neutropenia not only at medium or high doses of irinotecan but also at low doses.
The dose-dependent manner of SN-38 glucuronidation explained why the association between UGT1A1*28 and neutropenia was dose-dependent.

Footnotes

1 http://www.cochrane-handbook.org/

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Table 1 Characteristics of trials included in the meta-analysis of the association between UGT1A1*28 and neutropenia.

| Study               | Study design  | No. of patients | Age (median or mean) | Source of population | Mutation detection methods* | Races† | Type of tumors‡ | Chemotherapy regimens§ | Irinotecan dose (mg/m²)/schedule | Toxicity grade criteria |||
|---------------------|---------------|-----------------|----------------------|----------------------|----------------------------|--------|-----------------|--------------------------|---------------------------------|-----------------------------|
| Iyer 2002 (3)       | Prospective   | 20              | Unknown              | Single centre        | SPR                        | C      | Solid tumors    | IRI alone                | 300/every 3 week                | NCI                         |
| Innocenti 2004 (4)  | Prospective   | 59              | 60                   | Single centre        | SPR                        | Mainly C | Solid tumors    | IRI alone                | 350/every 3 week                | NCI                         |
| Toffoli 2006 (5)    | Prospective   | 250             | 61                   | Multicentre          | PYRS                       | C      | mCRC            | modified FOLFIRI or FOLFIRI | 180/biweekly                   | NCI                         |
| Cote 2007 (12)      | Prospective   | 89              | Unknown              | Multicentre          | SPR                        | C      | Stage III mCRC  | FOLFIRI                  | 180/biweekly                   | NCI                         |
| Kweekel 2008 (13)   | Retrospective | 138             | 62                   | Multicentre          | PYRS                       | C      | mCRC            | CAPIRI                   | 250/every 3 week                | NCI                         |
| Ferraldeschi 2009 (14)| Prospective     | 92              | 63                   | Single centre        | SPR                        | C      | mCRC            | FOLFIRI, CAPIRI, or IRI plus VEGF inhibitor | 180/biweekly                   | NCI                         |
| Marcuello 2004 (15) | Prospective   | 95              | 68                   | Unknown              | SPR                        | C      | mCRC            | IRI alone, IRI plus Tomudex, or IRI plus 5FU or LV | 80/weekly, 180/biweekly or 350/every 3 week | WHO                         |
| Massacesi 2006 (16) | Prospective   | 56              | 64                   | Multicentre          | Sequencing                 | Unknown | Advanced CRC   | IRI plus RAL             | 80/weekly                       | NCI                         |
| Rouits 2004 (17)    | Retrospective | 73              | 62                   | Single centre        | PYRS                       | C      | mCRC            | modified FOLFIRI or FOLFIRI | 85/weekly or 180 biweekly       | NCI                         |
| Carlini 2005 (18)   | Prospective   | 62              | 61                   | Multicentre          | SPR                        | Mainly C | mCRC            | CAPIRI                   | 100 or 125/weekly              | NCI                         |
| Glimelius 2010 (19) | Retrospective | 136             | 62                   | Multicentre          | SPR                        | C      | mCRC            | modified FOLFIRI or FOLFIRI | 180/biweekly                   | NCI                         |
| Pillot 2006 (20)    | Retrospective | 34              | 60                   | Single centre        | PYRS                       | Unknown | NSCLC          | IRI plus CAR             | 200/every 3 week               | NCI                         |
| McLeod 2006** (21)  | Prospective   | 103 or 109      | Unknown              | Multicentre          | Unknown                    | Mainly C | Advanced CRC   | IROX or FOLFIRI           | 200/every 3 week or 100/weekly | NCI                         |
| Roth 2008†† (22)    | Prospective   | 628             | Unknown              | Multicentre          | SPR                        | Unknown | Stage III CRC  | FOLFIRI                  | 180/biweekly                   | NCIC                        |
| Tan 2008†† (23)     | Prospective   | 54              | Unknown              | Unknown              | Unknown                    | Unknown | CRC             | Unknown weekly, biweekly or every 3 week | 200/every 3 week or 100/weekly | NCI                         |

* SPR, Sizing of PCR products (analysis of fragment size); PYRS, Pyrosequencing; Sequencing, other DNA sequencing methods.
† C, Caucasian.

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited. Copyright © 2010 American Association for Cancer Research
† Solid tumors, Multiple solid tumor types; NSCLC, non-small-cell lung cancer; mCRC, Metastatic colorectal
cancer.

§ IRI, irinotecan; CAP, capecitabine; CAR, carboplatin; OXA, oxaliplatin; 5FU, 5-fluorouracil; LV, leucovorin;
RAL, raltitrexed; CAPIRI, capecitabine plus irinotecan; FOLFIRI, irinotecan plus 5FU and leucovorin; IROX,
irinotecan plus OXA.

∥ NCI, National Cancer Institute common toxicity criteria; WHO, World Health Organization criteria; NCIC,
National Cancer Institute of Canada Common Toxicity criteria.

** Published as abstract. 103 patients were administrated with 200 mg/m² of irinotecan every 3 week; 109 patients
were administrated with 100 mg/m² of irinotecan weekly.

†† Published as abstract.
Table 2 Characteristics of trials included in the meta-analysis of the association between UGT1A1*28 and relative extent of glucuronidation (REG) of SN-38.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>No. of patients</th>
<th>Age (median or mean)</th>
<th>Races†</th>
<th>Type of tumors‡</th>
<th>Sampling scheme</th>
<th>Irinotecan dose (mg/m²)/schedule</th>
<th>Analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iyer 2002 (3)</td>
<td>Prospective</td>
<td>20</td>
<td>Unknown</td>
<td>C</td>
<td>Solid tumors</td>
<td>15 time points, up to 24 h</td>
<td>300/every 3 week</td>
<td>HPLC-Flu§</td>
</tr>
<tr>
<td>Innocenti 2004</td>
<td>Prospective</td>
<td>61</td>
<td>60</td>
<td>Mainly C</td>
<td>Solid tumors</td>
<td>14 time points, up to 25.5 h</td>
<td>350/every 3 week</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Toffoli 2006 (5)</td>
<td>Prospective</td>
<td>71</td>
<td>61</td>
<td>C</td>
<td>mCRC</td>
<td>16 time points, up to 50 h</td>
<td>180/biweekly</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>de Jong 2007 (6)</td>
<td>Retrospective</td>
<td>134</td>
<td>55</td>
<td>C</td>
<td>Solid tumors</td>
<td>Multiple time points, up to 20 time points</td>
<td>350/every 3 week</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Mathijssen 2003</td>
<td>Unknown</td>
<td>53</td>
<td>53</td>
<td>Mainly C</td>
<td>Solid tumors</td>
<td>14 time points, up to 48 h</td>
<td>200 to 350/every 3 week</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Mathijssen 2004</td>
<td>Prospective</td>
<td>30</td>
<td>55</td>
<td>C</td>
<td>Solid tumors</td>
<td>15 time points, up to 55 h</td>
<td>350/every 3 week*</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Paoluzzi 2004 (9)</td>
<td>Unknown</td>
<td>86</td>
<td>54</td>
<td>C</td>
<td>Solid tumors</td>
<td>Unknown</td>
<td>350/every 3 week*</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Minami 2007 (10)</td>
<td>Unknown</td>
<td>85</td>
<td>61</td>
<td>A</td>
<td>Solid tumors</td>
<td>7 time points, up to 24 h</td>
<td>60 to 150/weekly or biweekly</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Sai 2004 (11)</td>
<td>Unknown</td>
<td>41</td>
<td>62</td>
<td>A</td>
<td>Solid tumors</td>
<td>7 time points, up to 24 h</td>
<td>60 to 150/weekly or biweekly</td>
<td>HPLC-Flu</td>
</tr>
</tbody>
</table>

* Patients were treated with irinotecan once every 3 weeks at a fixed dose of 600 mg. A fixed dose of 600 mg was similar to a dose of 350 mg/m².
† A, Asian; C, Caucasian.
‡ Solid tumors, Multiple solid tumor types; mCRC, Metastatic colorectal cancer.
§ High performance liquid chromatography with fluorescence detection.
## Table 3 Summary of meta-analysis

<table>
<thead>
<tr>
<th>Comparison or outcome</th>
<th>No. of trials</th>
<th>No. of participants</th>
<th>Irinotecan doses</th>
<th>Statistical method</th>
<th>Effect size (95% confidence intervals)</th>
<th>Test for heterogeneity</th>
<th>Begg’s test</th>
<th>Egger’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia (*28/*28 vs *1/*1 or *1/*28)</td>
<td>4</td>
<td>300</td>
<td>Low</td>
<td>RR (fixed)</td>
<td>2.43 (1.34, 4.39)</td>
<td>0.003*</td>
<td>0.964</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1481</td>
<td>Medium</td>
<td>RR (fixed)</td>
<td>2.00 (1.62, 2.47)</td>
<td>&lt; 0.001*</td>
<td>0.325</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>1781</td>
<td>Low and medium</td>
<td>RR (fixed)</td>
<td>2.04 (1.67, 2.49)</td>
<td>&lt; 0.001*</td>
<td>0.612</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>217</td>
<td>High</td>
<td>RR (fixed)</td>
<td>7.22 (3.10, 16.78)</td>
<td>&lt; 0.001*</td>
<td>0.713</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1998</td>
<td>Total</td>
<td>RR (fixed)</td>
<td>2.20 (1.82, 2.66)</td>
<td>&lt; 0.001*</td>
<td>0.276</td>
<td>16.3</td>
</tr>
<tr>
<td>REG (*1/*1 or *1/*28 vs *28/*28)</td>
<td>3</td>
<td>197</td>
<td>Low and medium</td>
<td>WMD (fixed)</td>
<td>1.62 (0.57, 2.68)</td>
<td>0.002†</td>
<td>0.854</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>384</td>
<td>High</td>
<td>WMD (fixed)</td>
<td>3.08 (2.14, 4.02)</td>
<td>&lt; 0.001†</td>
<td>0.897</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>581</td>
<td>Total</td>
<td>WMD (fixed)</td>
<td>2.44 (1.73, 3.14)</td>
<td>&lt; 0.001†</td>
<td>0.642</td>
<td>0.175</td>
</tr>
<tr>
<td>Neutropenia (*1/*28 vs *1/*1)</td>
<td>4</td>
<td>270</td>
<td>Low</td>
<td>RR (fixed)</td>
<td>2.94 (1.36, 6.35)</td>
<td>0.006‡</td>
<td>0.747</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1288</td>
<td>Medium</td>
<td>RR (fixed)</td>
<td>1.29 (1.04, 1.62)</td>
<td>0.023‡</td>
<td>0.581</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1558</td>
<td>Low and medium</td>
<td>RR (fixed)</td>
<td>1.40 (1.14, 1.74)</td>
<td>0.002‡</td>
<td>0.462</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>180</td>
<td>High</td>
<td>RR (fixed)</td>
<td>2.65 (0.70, 9.94)</td>
<td>0.149</td>
<td>0.547</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1738</td>
<td>Total</td>
<td>RR (fixed)</td>
<td>1.43 (1.16, 1.77)</td>
<td>0.001‡</td>
<td>0.529</td>
<td>0</td>
</tr>
<tr>
<td>REG (*1/*1 vs *1/*28)</td>
<td>3</td>
<td>182</td>
<td>Low and medium</td>
<td>WMD (fixed)</td>
<td>1.85 (1.00, 2.70)</td>
<td>&lt; 0.001§</td>
<td>0.148</td>
<td>47.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>356</td>
<td>High</td>
<td>WMD (fixed)</td>
<td>1.03 (-0.09, 2.16)</td>
<td>0.072</td>
<td>0.591</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>538</td>
<td>Total</td>
<td>WMD (fixed)</td>
<td>1.55 (0.87, 2.23)</td>
<td>&lt; 0.001§</td>
<td>0.357</td>
<td>9.4</td>
</tr>
</tbody>
</table>

* The risk of toxicity was significantly higher among patients with a UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype.

† The relative extent of glucuronidation (REG) was significantly higher among patients with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype than among those with a UGT1A1*28/*28 genotype.

‡ The risk of toxicity was significantly higher among patients with a UGT1A1*1/*28 genotype than among those with a UGT1A1*1/*1 genotype.

§ REG was significantly higher among patients with a UGT1A1*1/*1 genotype than among those with a UGT1A1*1/*28 genotype.

|| Publication bias may exist.

ne, Begg’s and Egger’s test were not done if less than eight studies were included in the analyzed subgroup.
Figure legends

Fig. 1 Studies evaluated at each stage of the meta-analysis (*Supplementary search includes: reference lists, contacting authors).

Fig. 2 A, Summary relative risk (RR) of irinotecan-induced neutropenia for UGT1A1*28/*28 vs UGT1A1*1/*1 or UGT1A1*1/*28; B, Weighted mean difference (WMD) of relative extent of glucuronidation (REG) for UGT1A1*1/*1 or UGT1A1*1/*28 vs UGT1A1*28/*28. A fixed-effects model was used for all analyses. Squares represent study-specific estimates (size of the square reflects the study-specific statistical weight); Horizontal lines represent 95% confidence intervals (CIs); Diamonds represent summary estimates with corresponding 95% CIs.

Fig. 3 A1, Relative risk (RR) of irinotecan-induced neutropenia (UGT1A1*28/*28 vs UGT1A1*1/*1 or UGT1A1*1/*28) against dose of irinotecan; A2, Weighted mean difference (WMD) of relative extent of glucuronidation (REG) (UGT1A1*1/*1 or UGT1A1*1/*28 vs UGT1A1*28/*28) against dose of irinotecan; B1, Relative risk (RR) of irinotecan-induced neutropenia (UGT1A1*1/*28 vs UGT1A1*1/*1) against dose of irinotecan; B2, Weighted mean difference (WMD) of relative extent of glucuronidation (REG) (UGT1A1*1/*1 vs UGT1A1*1/*28) against dose of irinotecan. Size of circle is proportional to the study-specific statistical weight. Marked “excluded” indicates the excluded study by Iyer et al.³ (zero incidence of neutropenia).
### Figure 2

#### A: Risk Ratio (RR) and Weight (%)

<table>
<thead>
<tr>
<th>Study</th>
<th>RR (95% CI)</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carlini et al. (2005)</td>
<td>1.93 (1.0, 3.57)</td>
<td>0.69</td>
</tr>
<tr>
<td>McLeod et al. (2006)</td>
<td>1.98 (0.49, 8.03)</td>
<td>2.69</td>
</tr>
<tr>
<td>Massacesi et al. (2006)</td>
<td>2.33 (0.28, 19.44)</td>
<td>1.11</td>
</tr>
<tr>
<td>Rouilla et al. (2004)</td>
<td>2.77 (1.49, 5.17)</td>
<td>4.83</td>
</tr>
<tr>
<td><strong>Subtotal</strong> (I-squared = 0.0%, p = 0.964)</td>
<td>2.43 (1.34, 4.39)</td>
<td>9.33</td>
</tr>
<tr>
<td><strong>Medium dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toffoli et al. (2006)</td>
<td>1.34 (0.52, 3.44)</td>
<td>8.09</td>
</tr>
<tr>
<td>Ferraldessi et al. (2009)</td>
<td>1.50 (0.41, 5.46)</td>
<td>3.61</td>
</tr>
<tr>
<td>Roth et al. (2008)</td>
<td>1.76 (1.33, 2.32)</td>
<td>54.19</td>
</tr>
<tr>
<td>Marcuello et al. (2004)</td>
<td>1.89 (0.80, 4.48)</td>
<td>5.62</td>
</tr>
<tr>
<td>Tan et al. (2008)</td>
<td>2.45 (0.70, 8.52)</td>
<td>2.20</td>
</tr>
<tr>
<td>Cote et al. (2007)</td>
<td>2.70 (1.18, 6.19)</td>
<td>4.00</td>
</tr>
<tr>
<td>Pillot et al. (2006)</td>
<td>2.81 (1.24, 6.39)</td>
<td>2.79</td>
</tr>
<tr>
<td>Gimelius et al. (2010)</td>
<td>3.64 (1.54, 8.59)</td>
<td>3.68</td>
</tr>
<tr>
<td>McLeod et al. (2006)</td>
<td>4.56 (2.10, 9.89)</td>
<td>3.48</td>
</tr>
<tr>
<td><strong>Subtotal</strong> (I-squared = 13.1%, p = 0.325)</td>
<td>2.00 (1.62, 2.47)</td>
<td>87.64</td>
</tr>
<tr>
<td><strong>High dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kweekel et al. (2008)</td>
<td>4.62 (1.01, 21.11)</td>
<td>1.18</td>
</tr>
<tr>
<td>Innocenti et al. (2004)</td>
<td>7.07 (2.58, 19.38)</td>
<td>1.51</td>
</tr>
<tr>
<td>Iyer et al. (2002)</td>
<td>17.50 (0.97, 299.22)</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Subtotal</strong> (I-squared = 0.0%, p = 0.713)</td>
<td>7.22 (3.10, 16.78)</td>
<td>3.03</td>
</tr>
<tr>
<td><strong>Overall</strong> (I-squared = 16.3%, p = 0.287)</td>
<td>2.20 (1.82, 2.66)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

#### B: Weighted Mean Difference (WMD) and Weight (%)

<table>
<thead>
<tr>
<th>Study</th>
<th>WMD (95% CI)</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low and medium dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toffoli (2006)</td>
<td>1.14 (-0.92, 3.20)</td>
<td>11.56</td>
</tr>
<tr>
<td>Sai (2004)</td>
<td>1.70 (0.05, 3.35)</td>
<td>17.95</td>
</tr>
<tr>
<td>Minami (2007)</td>
<td>1.91 (0.09, 3.73)</td>
<td>14.89</td>
</tr>
<tr>
<td><strong>Subtotal</strong> (I-squared = 0.0%, p = 0.854)</td>
<td>1.62 (0.57, 2.68)</td>
<td>44.40</td>
</tr>
<tr>
<td><strong>High dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innocenti (2004)</td>
<td>2.49 (0.40, 4.58)</td>
<td>11.25</td>
</tr>
<tr>
<td>Jong (2007)</td>
<td>2.57 (0.88, 4.26)</td>
<td>17.12</td>
</tr>
<tr>
<td>Paoluzzi (2004)</td>
<td>3.55 (1.43, 5.27)</td>
<td>13.35</td>
</tr>
<tr>
<td>Mathijssen (2003)</td>
<td>3.71 (0.58, 6.84)</td>
<td>5.02</td>
</tr>
<tr>
<td>Mathijssen (2004)</td>
<td>3.86 (1.08, 6.64)</td>
<td>6.34</td>
</tr>
<tr>
<td>Iyer (2002)</td>
<td>4.58 (0.16, 9.00)</td>
<td>2.52</td>
</tr>
<tr>
<td><strong>Subtotal</strong> (I-squared = 0.0%, p = 0.897)</td>
<td>3.08 (2.14, 4.02)</td>
<td>55.60</td>
</tr>
</tbody>
</table>

Heterogeneity between groups: p = 0.043
Overall (I-squared = 0.0%, p = 0.642) 2.44 (1.73, 3.14) 100.00
Dose-Dependent Association Between UGT1A1*28 Genotype and Irinotecan-Induced Neutropenia: Low Doses Also Increase Risk

Zhe-Yi Hu, Qi Yu, Qi Pei, et al.

Clin Cancer Res Published OnlineFirst June 18, 2010.

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