UGT1A1 genotyping: a predictor of irinotecan-associated side effects and drug efficacy?

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Irinotecan [Camptosar (CPT-11), Pfizer Pharmaceuticals, New York, USA] is one of the most effective chemotherapeutic agents in the treatment of metastatic colorectal cancer. *In vivo*, the prodrug CPT-11 is biotransformed by carboxylesterase into its active metabolite SN-38. SN-38 is inactivated by uridine disphosphate glucuronosyl transferase 1 (UGT1A1) into the inactive compound SN-38G, which is excreted with the bile.

This review concentrates on a critical evaluation of UGT1A1 gene polymorphism as a predictor of toxicity and treatment efficacy in patients who received irinotecan for metastatic colorectal cancer. Irinotecan is explained with its main toxicities as well as the underlying mechanisms. The enzyme UGT1A1 is shown in the context of other metabolic pathways and different UGT enzymes involved. We will review in detail the controversy of the current literature with regard to the significance of identifying patients carrying the homozygous genotype *UGT1A1*28*. Racial differences concerning UGT enzymes have to be considered when discussing a pragmatic approach to determine gene polymorphisms as a predictor of treatment efficacy and outcome in patients receiving irinotecan-based chemotherapy. Dose dependency of toxicity and the clinical relevance of various UGT1 enzymes and single nucleotide polymorphisms in different alternative metabolic pathways are clarified to put UGT1A1 genotyping in a broad context with additional and competing strategies of patient-tailored therapy. *Anti-Cancer Drugs* 20:867–879 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

With the increasing understanding of molecular mechanisms, drug metabolizing enzymes have been put into researchers' focus of interest. Data from the 1950s could build a basis for the concept of individual drug response because of genetic polymorphism, when Alving *et al.* [1] reported a primaquine-induced haemolysis because of a deficiency of the glucose-6-phosphatase dehydrogenase. The scientific approach to elucidate the relationship of inherited genetic polymorphism and drug response was called pharmacogenetics. Through the rapid gain of information within the frame of the Human Genome Project this activity even extended to pharmacogenomics [2]. The number of 1.42 million polymorphisms in the human genome has been exceeded by far with one estimated single nucleotide polymorphism (SNP) every 1.000–3.000 base pairs in the human genome [3].

As proposed by Innocenti *et al.* [4], genetic polymorphism in drug metabolism is of interest when (i) the metabolic pathway is the main factor in drug clearance, (ii) the drug has a narrow therapeutic range and (iii) the pharmacokinetics of the drug correlates with its activity or toxicity. Data from 1994 revealed more than 2 million patients suffering from an adverse drug reaction (ADR) leading to 100 000 deaths, making ADR the fourth to sixth leading cause of death in the US [5]. Owing to the narrow therapeutic range and life-threatening side effects of chemotherapeutic agents, identification of patient groups who are at high risk for such ADRs is greatly essential.

In 2005, the US Food and Drug Administration (FDA) have decreed actions to save patients with metastatic colorectal cancer (mCRC) receiving irinotecan-based chemotherapy from potentially life-threatening side effects. These side effects may arise from a genetic polymorphism in the enzyme that metabolizes SN-38, the active metabolite of irinotecan. Therefore, the FDA approved a genetic test to identify patients with a homozygous gene polymorphism of the uridine disphosphate glucuronosyl transferase 1 (UGT1A1) gene and a consecutive lower detoxification capacity for irinotecan. Furthermore, an addendum was added to the package insert, warning for an increased risk of side effects and advising a lower dose in patients bearing the homozygous allele [6–8].

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This review concentrates on a critical evaluation of UGT1A1 gene polymorphism in the promoter region of the gene (*UGT1A1*28*) as a predictor of toxicity and treatment efficacy in irinotecan-based chemotherapy.

Methods

The MEDLINE database was searched from 1980 to 2009 using variations in the search terms: 'Irinotecan, colorectal cancer, UGT1A1, gene polymorphism, toxicity, efficacy, delayed diarrhoea, neutropenia, racial variability, drug metabolism'. Moreover, the 'American Society of Clinical Oncology Annual Meeting Proceedings' was searched from 2000 to 2009 for reports of new or ongoing trials. A search was also conducted for published practice guidelines, meta-analyses and systematic reviews.

Relevant articles and abstracts were selected and the reference lists from these sources were searched for additional trials.

Articles were selected for inclusion in this review of the evidence, if they were fully published reports or published abstracts of clinical trials or meta-analyses of clinical trials. Articles were required to report on any of the following specified outcomes of interest: adverse events, response rate (RR), progression-free survival (PFS), overall survival (OS) or quality of life. Trials published in a language other than English or German were excluded because of limited translation resources.

Most of the data in this topic have the limitation of being retrospective and comparability within the trials was hampered by various doses of irinotecan.

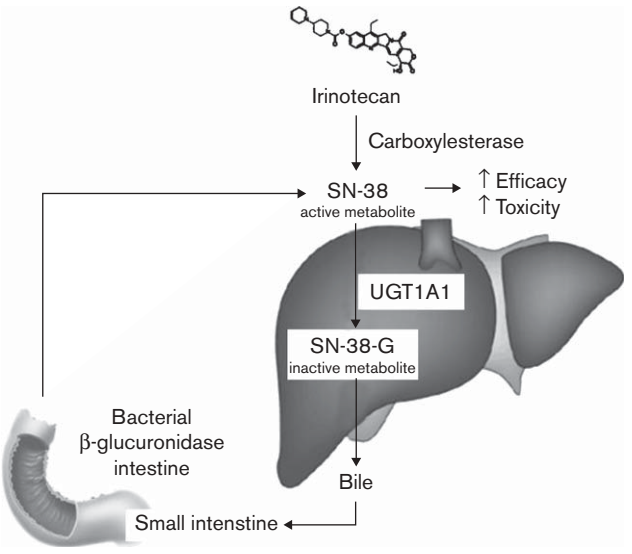
Irinotecan

Irinotecan is one of the most effective chemotherapeutic agents in the treatment of mCRC [9,10]. Data from phase III studies indicate an improved clinical response of patients receiving irinotecan-based regimen when compared with patients receiving 5-FU/LV (5-fluorouracil, leucovorin) alone. As reported by Saltz *et al.* [11], the introduction of irinotecan not only improved RR (39 vs. 21%; $P < 0.001$), but also PFS (7.0 vs. 4.3 months; $P = 0.004$) and OS (14.8 vs. 12.6 months; $P = 0.04$). Data provided by Douillard *et al.* [12] showed a prolonged OS of 3.3 months by the addition of irinotecan to 5-FU/LV ($P = 0.031$). Besides FOLFOX4 (5-FU, LV and oxaliplatin), FOLFIRI (5-FU, LV and irinotecan) represents an efficient first-line regime of mCRC [13]. Colucci *et al.* [14] presented data from patients treated with the FOLFIRI or the FOLFOX4 regimen showing no differences for OR, time to progression and OS. Integration of irinotecan or oxaliplatin to 5-FU/LV into the treatment of patients with mCRC has shown an improved OS with a preferable sequence of FOLFIRI followed by FOLFOX [15,16]. In trials investigating predictive factors of survival, the use of irinotecan within the course of treatment was associated with a better survival in patients with mCRC [17,18].

Almost 50 years ago, the plant alkaloid camptothecin was isolated from *Camptotheca acuminata* [19,20]. Irinotecan is derived from camptothecin and acts as an inhibitor of intracellular topoisomerase I [21]. Antitumour activity is dependent on absorption and metabolic transformation [22]. An active lactone ring form and an inactive carboxylate form are in a pH-dependent equilibrium [23]. *In vivo*, the prodrug CPT-11 is biotransformed by carboxylesterase (CE) into its active metabolite SN-38 [24]. In-vitro studies have shown that the conversion rate of the lactone form was twice as high as the carboxylate form [25]. SN-38 is inactivated by UGT1A1 into the inactive compound SN-38G, which is excreted with the bile (Fig. 1) [26,27]. Recent data suggest different metabolic pathways apart from glucuronidation: 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxy-camptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecine (NPC) are products of a cytochrome P450 isoform 3A (CYP3A)-mediated biotransformation. Both APC and NPC have limited cytotoxic activity compared with SN-38 [28,29].

The most common side effects of irinotecan are haematotoxicity (neutropenia, febrile neutropenia) and nonhaematological toxicities, such as nausea, alopoecia and delayed diarrhoea [30]. The latter has often been described as the main toxicity of irinotecan [31]. It is caused by the fact that β-glucuronidase of the bowel reactivates SN38-G into the active metabolite SN-38 (Fig. 1) [32]. Severity of

Fig. 1



Metabolism of irinotecan: the pro-drug irinotecan (CPT-11) is bio-transformed by carboxylesterase (CE) into its active metabolite SN-38 which is inactivated by UGT1A1 into the inactive compound SN-38G. SN-38G is excreted with the bile. In the small bowel bacterial β-glucuronidase converts SN-38G back into the active form SN-38.

delayed diarrhoea directly correlates with SN-38 area under the curve (AUC) and the local intestinal concentration [33]. Treatment of delayed diarrhoea consists of loperamide and compensation of fluid loss. However, in case of delayed diarrhoea accompanied by fever, neutropenia or extensive fluid loss, an admission to the hospital becomes inevitable. Therefore, as an attempt to predict life-threatening toxicity dose, route and regimen of irinotecan have been studied [34].

For almost 10 years, UGT1A1 has been in the focus of interest when investigating irinotecan metabolism and its associated side effects. There is an ongoing controversial debate concerning the clinical value of UGT1A1 gene polymorphism as a predictor of irinotecan-associated toxicity and treatment efficacy.

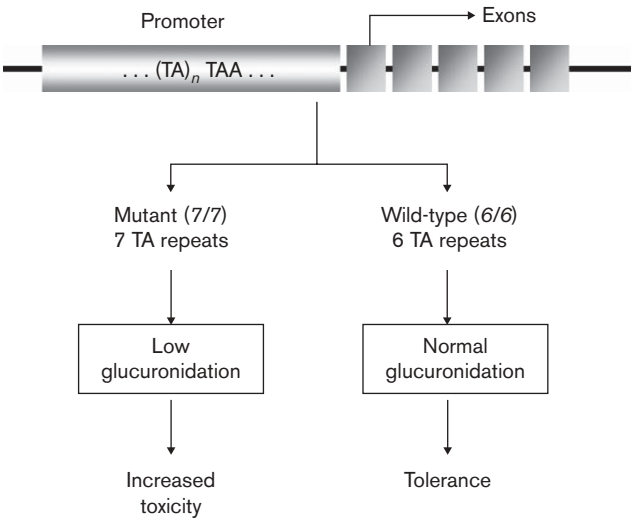
UGT1A1

The human UGTs are classified into the UGT1 and UGT2 families [35]. The UGT1 gene consists of 13 unique isoforms with variable exon 1 and common exons 2–5. Each exon 1 has its own promotor and is spliced to the different common exons. The UGT1A1 gene therefore belongs to family of at least 12 UGT-glucuronosyl transferase enzymes encoded by the UGT1 locus on chromosome 2 (2q37) [4]. Each isoform has its own substrate specificity [36]. UGT1A1 is responsible for the glucuronidation of bilirubin and is integrated in the metabolism of irinotecan. UGT1A1 has been reported to be expressed in the human liver as well as in the intestine [37,38].

Variances in the metabolism of bilirubin lead to several clinical conditions and disorders ranging from the harmless condition of mild jaundice of Gilbert's syndrome to the potentially deadly Crigler–Najjar syndrome [39]. The clinical features of Gilbert's syndrome are a mild unconjugated hyperbilirubinaemia without any structural liver disease or haemolysis. It has been shown that the activity of UGT1A1 is decreased in patients suffering from Gilbert's syndrome compared with a healthy population [40,41]. Several mutations in the UGT1A1 gene have been described for the Crigler–Najjar syndrome [42]. The genetic lesions resulting in Gilbert's syndrome are dependent on racial affiliations [43,44].

By molecular analysis, it has been shown that in the Caucasian population Gilbert's syndrome is most commonly caused by a polymorphism in the UGT1A1 gene [45]. Depending on the number of TA insertions in the TATAA element of the 5′-promotor region, the wild-type genotype is named *(6/6)*, the heterozygous *(6/7)* and the homozygous genotype *(7/7)* or *UGT1A1*28*. Therefore, patients with the wild-type genotype *(6/6)* (33% in the Caucasian population) are homozygous with six repeats of the TA insertion. Patients with the *(7/7)* genotype are homozygous with seven TA repeats, whereas the heterozygous genotype *(6/7)* consists of one allele

Fig. 2



Polymorphisms of UGT1A1: the presence of seven TA repeats in the promoter region of the UGT1A1 gene is associated with reduced UGT1A1 activity which leads to a lower glucuronidation rate of SN-38 to the inactive compound SN-38G. As a result SN-38 accumulates leading to increased toxicity.

with six TA repeats and of one with seven TA repeats (Fig. 2). Apart from that, there are rare genotypes with fewer than five or more than seven TA repeats leading to variable enzyme levels.

As reported by Wasserman *et al.* [46], patients with Gilbert's syndrome experienced severe toxicity during irinotecan-based chemotherapy. In-vitro experiments have shown that UGT1A1 is responsible for the glucuronidation of SN-38 [27]. Patients carrying the heterozygous or homozygous genotype experienced a decreased expression of the UGT1A1 enzyme resulting in lower rates of bilirubin and SN-38 glucuronidation (Fig. 2) [47,48].

A number of clinical trials have shown an association between UGT1A1 gene polymorphism and an increased toxicity in patients who received irinotecan-based chemotherapy. Owing to the importance of irinotecan in the treatment of mCRC and the considerable risk of side effects, many efforts have been undertaken to define subgroups of patients carrying mutations of the UGT1A1 gene being therefore susceptible to irinotecan-associated toxicities.

UGT1A1 genotyping and irinotecan-based chemotherapy: clinical data

Table 1 shows a synopsis of clinical studies that investigated an association of UGT1A1 genotype and toxicity and/or clinical outcome.

The prospective studies by Iyer *et al.* [49] ($n = 20$) and Innocenti *et al.* [50] ($n = 65$) provide data from single-agent irinotecan given to patients with various

Table 1 UGT1A1 status correlation with irinotecan-associated toxicity and treatment efficacy

Author	*n*	Tumour	CTX schedule	UGT1A1 distribution	%	Haematotoxicity grade 3/4 %	Delayed diarrhoea grade 3/4 %	Overall survival
Iyer *et al.* [49]	20	Various solid tumours	CPT-11 300 mg/m^2 q3w	WT *(6/7)* *(7/7)*	45 35 20	Only in *(6/7)* and *(7/7)*	Only in *(6/7)* and *(7/7)*	–
Innocenti *et al.* [50]	65	CRC, lung, other GI tumours	CPT-11 350 mg/m^2 q3w	WT *(6/7)* *(7/7)*	49 41 10	0 12.5 50 *P*=0.001 (neutropenia grade 4)	0 8.3 16 (only grade 3)	–
Ando *et al.* [51]	118	Various solid tumours	CPT-11 q1w/q2w/q3w/q4w ± platinum salts/others	WT *(6/7)* *(7/7)*	79 15 6	15 44 57 *P*<0.001	– – –	–
Cote *et al.* [52]	89	CRC	CPT-11 180 mg/m^2 q2w + 5-FU/LV	WT *(6/7)* *(7/7)*	42 50 8	16 25 50 *P*=0.06	16 27 25 *P*=0.31	52% 42% 87% 3-year DFS; *P*=0.06
Toffoli *et al.* [53]	250	CRC	CPT-11 180 mg/m^2 q2w + 5-FU/LV	WT *(6/7)* *(7/7)*	46 46 8	2 6 (odds ratio 3.5) 14 (odds ratio 8.6)	4 3 (odds ratio 0.6) 14 (odds ratio 4.1)	20.4 months 22.3 months (odds ratio 0.84) 22.9 months (odds ratio 0.81)
Rouits *et al.* [54]	75	CRC	CPT-11 85 mg/m^2 q1w, 180 mg/m^2 q2w + 5-FU/LV	WT *(6/7)* *(7/7)*	41 47 9	10 40 71 *P*=0.001	13 20 29 NS	–
Marcuello *et al.* [55]	95	CRC	CPT-11 + 5-FU/tomudex 80 mg/m^2 q1w, 180 mg/m^2 q2w, 350 mg/m^2 q3w	WT *(6/7)* *(7/7)*	42 47 11	15 27 40 *P*=0.2	17 33 70 *P*=0.005	33 months 21 months *P*=0.09
Font *et al.* [56]	47	NSCLC	CPT-11 70 mg/m^2 q1w + docetaxel	WT *(6/7)* *(7/7)*	49 36 15	–	26 29 14 *P*=0.84	8 months 11 months *P*=0.27
McLeod *et al.* [57]	520	CRC	IFL (CPT-11 : 125 mg/m^2 d 1, 8, 15, and 22 every 6 weeks), reduced dose 100 mg/m^2, IROX (CPT-11: 200 mg/m^2 q3w), FOLFOX	WT *(6/7)* *(7/7)*	46 44 9	14.8 18.2 36.2 *P*=0.007 (neutropenia grade 4)	NS	NS
Seymour *et al.* [58] Braun *et al.* [59]	915	CRC	5-FU/LV or 5-FU/LV + CPT-11 (CPT-11: 180 mg/m^2 q2w) or 5-FU/LV + oxaliplatin	WT (6/7) (7/7)	52 38 11	*(7/7)* not associated with CPT-11 toxicity	*(7/7)* not associated with CPT-11 toxicity	–
Schulz *et al.* (in preparation)	105	CRC	mFOLFIRI (CPT-11: 80 mg/m^2 d 1, 8, 15, 22, 29, 36 q50d), mIROX (CPT-11: 80 mg mg/m^2 d 1, 15, 29 q50d)	WT *(6/7)* *(7/7)*	40 50 10	1 3.1 *P*=0.27	6.2 13.0 *P*=0.08	21.2 months 18.9 months *P*=0.725
Roth *et al.* [60]	628	CRC	5-FU/LV or 5-FU/LV + CPT-11 (CPT-11: 180 mg/m^2 q2w)	WT *(6/7)* *(7/7)*	44 43 13	25.5 25.9 44.8 *P*=0.006 (*6/6*>*6/7*>*7/7*)	16.0 10.7 8.0 *P*=0.02 (*6/6*>*6/7*>*7/7*)	–
Liu *et al.* [61]	128	mCRC	CPT-11 180 mg/m^2 q2w + 5-FU/LV	WT *(6/7)* *(7/7)*	79.7 15.6 4.7	4.9 53.8 *P*<0.01	5.9 26.9 *P*<0.01	18 months 19 months *P*=0.84
Kweekel *et al.* [62]	80	mCRC	CPT-11 350 mg/m^2 q3w (second line)	WT *(6/7)* *(7/7)*	58 39 4	2.2 19.4 0 Febrile neutropenia *P*=0.015	15.2 22.6 66.7 *P*=0.09	–
Kweekel *et al.* [62]	138	mCRC	CAPIRI: CPT-11 250 mg/m^2 q3w + capecitabine 1000 mg/m^2 b.i.d. day 1 to day 14 q3w (first line)	WT *(6/7)* *(7/7)*	50 48 8.5	1.5 6.5 18.2 Febrile neutropenia *P*=0.031	21.5 22.6 36.4 *P*=0.43	–

b.i.d., twice daily; DFS, disease-free survival; mCRC, metastatic colorectal cancer; NSCLC, non-small-cell lung cancer; q1w, weekly; q2w, given every 2 weeks; q3w, given every 3 weeks; q4w, given every 4 weeks; WT, wild type.

cancer types. Irinotecan was applied at a 3 weekly dose of 300 and 350 mg/m^2, respectively. Delayed diarrhoea was rarely observed in these trials and was solely observed among patients with the *(6/7)* and *(7/7)* genotype. Patients with those genotypes experienced more frequently neutropenia when compared with patients carrying the wild-type *(6/6)* genotype. Restrictively, a statistically significant difference was detected only in the trial by Innocenti *et al.* [50].

Ando *et al.* [51] reported on a significantly increased incidence of severe haematological toxicity in patients carrying the homozygous *(7/7)* or heterozygous *(6/7)* allele in a retrospective study including 118 Japanese patients suffering from various malignancies ($P < 0.001$). Several chemotherapeutic agents, among them platinum salts, were applied in combination with predominantly weekly scheduled irinotecan. Combination chemotherapy was considered to be an additional risk factor for toxicity [51].

Cote *et al.* [52] showed an association of the UGT1A1 polymorphism and the incidence of severe toxicity during chemotherapy with 5-FU/LV plus irinotecan. Patients carrying the genotypes *(6/7)* and *(7/7)* experienced more frequently haematological toxicity (grade 3 and 4) ($P = 0.06$) but not gastrointestinal toxicity ($P = 0.31$). In patients carrying the *UGT1A1*28* genotype, 3-year disease-free survival (DFS) was better by trend compared with patients carrying the wild-type genotype [*(6/6)*: 52% vs. *(6/7)*: 42% vs. *(7/7)*: 87%; $P = 0.06$] [52].

With a comparable regimen [5-FU/LV and irinotecan 180 mg/m^2 q2w (given every 2 weeks, schedule is repeated on day 14)] Toffoli *et al.* [53] have shown, in a prospective study including 250 patients suffering from mCRC, that the *(7/7)* genotype was associated with a higher risk of experiencing haematotoxicity at grades 3 and 4 [odds ratio (OR): 8.63; 95% confidence interval (95% CI): 1.31–56.55]. Restrictively, the higher risk was relevant only for the first cycle, and was not consistently observed throughout the subsequent treatment period for patients with both variant alleles. A nonsignificant survival advantage was observed for the *(7/7)* genotype when compared with *(6/6)* genotype-carrying patients [hazard ratio (HR): 0.81; 95% CI: 0.45–1.44]. This was explained by the finding of a higher RR because of a different pharmacokinetics with a higher biliary index [irinotecan AUC × (SN38 AUC/SN38G AUC)] and a lower glucuronidation ratio (SN38G AUC/SN38 AUC) associated with the *(7/7)* genotype [53].

A further analysis by Rouits *et al.* [54] included 75 patients who had received IRIFUFOL or FOLFIRI for mCRC. Patients carrying the homozygous *(7/7)* or heterozygous *(6/7)* genotype experienced a significantly increased risk for developing severe neutropenia ($P = 0.001$), whereas the risk for delayed diarrhoea was not significantly enhanced ($P > 0.05$) [54].

In contrast to the findings of Rouits *et al.* [54], who reported on a predominantly haematological increased toxicity in *(6/7)* and *(7/7)* patients, Marcuello *et al.* [55] have observed a significantly higher incidence of delayed diarrhoea in patients with the mutant genotypes ($P = 0.005$) and a nonsignificantly increased risk of haematotoxicity ($P = 0.2$). A trend towards an improved survival of patients carrying the wild type was explained by the toxicity-related dose reduction in patients with the mutant genotypes [55].

In contrast, Font *et al.* [56] reported a study including 47 patients suffering from non-small-cell lung cancer (NSCLC) who received combination chemotherapy consisting of irinotecan and docetaxel. Patients who carried the mutant genotypes *(6/7)* or *(7/7)* have shown a trend towards an improved survival [OS *(6/6)* 8 months vs. *(6/7)* and *(7/7)* 11 months; $P = 0.27$] [56]. One can argue that patients carrying the mutant UGT1A1 genotype experienced less detoxification of SN-38 because of a smaller amount of enzyme. A consecutively higher blood level of the active compound finally leads to an increased antitumour effect.

McLeod *et al.* [57] reported on a multicentre trial including more than 200 genotyped patients who had received an irinotecan-based chemotherapy for advanced CRC. They found a statistically significant association between the *(7/7)* genotype and the frequency of grade 4 neutropenia in patients ($P = 0.007$). Furthermore, UGT1A1 genotype has not significantly influenced efficacy parameters in terms of RR, time to progression or OS [57].

Moreover, data from a large, prospective, randomized trial by Seymour *et al.* [58] ($n = 1.188$ patients, FOCUS trial) did not confirm an association between UGT1A1 genotype and any irinotecan-associated toxicity [58,59].

Own results from a retrospective analysis including 105 patients suffering from mCRC have shown that the UGT1A1 genotype of patients who had received a modified IROX or FOLFIRI did not significantly influence the frequencies of toxicity or any parameters of treatment efficacy (Schulz *et al.*, in preparation).

At the ASCO GI meeting 2008, Roth *et al.* [60] presented the results of a large multicentre trial of patients with mCRC treated with FOLFIRI. The authors concluded that the incidence of grade 3–4 neutropenia and febrile neutropenia was increased in patients who carried the homozygous UGT1A1 genotype *(7/7)*. Interestingly, the rate of severe diarrhoea was decreased in such patients [60].

Finally, the results of a retrospective analysis of 128 Chinese patients with mCRC who received biweekly irinotecan indicate that the heterozygous and homozygous genotypes UGT1A1 *(6/7)* and *(7/7)* predict severe neutropenia and diarrhoea, but not treatment efficacy [61].

Necessity for dose reduction was significantly associated with the *(6/7)* or *(7/7)* genotype (42.3 vs. 12.7%; $P < 0.01$).

Another recent study reported on prospectively genotyped patients suffering from mCRC who had received either first-line irinotecan/capecitabine or second-line single-agent irinotecan. RRs, number of dose reductions and applied chemotherapy cycles were similar within the different genotypes [62].

Critical discussion of present data

A number of publications during the last years support the idea of a relationship between the homozygous *(7/7)* and heterozygous *(6/7)* genotypes of the UGT1A1 gene locus and the risk of experiencing severe side effects during irinotecan-based chemotherapy. Drawing a general conclusion is difficult for the large differences of the origin of the data:

Some of the trials are carried out retrospectively and may therefore serve only for generating hypothesis [51,54]. Implementation of UGT genotyping in prospective phase III studies is therefore advisable to further evaluate the necessity of genotyping.

Most studies included a rather small number of analysed patients [49,50,52,54,56]. In general, drawing conclusions from a small number of patients is problematic, and moreover the value of analysing subgroups [e.g. the minor frequency *(7/7)* genotype] remains debatable.

Some analysed study populations suffered from various solid tumours [49–51]. On account of a different response to chemotherapy, a stratification according to tumour type, comedication and particularly pretreatment seems to be inevitable. Thus, subgroups can be defined and the association of genotype and toxicity would be more precise.

A major limitation consists of the use of additional chemotherapeutic agents, pretreatment surgery or radiotherapy, which has to be largely considered as additional risk factor for the development of any toxicity independent of the UGT1A1 genotype [54,55]. The coadministration of another chemotherapeutic agent might enhance the toxicity of irinotecan-based chemotherapy as well [12,63]. Emphasizing the impact of UGT1A1 genetic polymorphism on toxicity or efficacy, the data from single-agent irinotecan or alternatively of comparable regimen with identical dosage are of greater value [49,50,52,53].

Influence of pretreatment serum bilirubin levels

When treating patients with an irinotecan-based schedule, hypersensitivity towards the medication and an inadequate liver function or jaundice must be excluded. Certainly, the latter leads to a bias when excluding patients who may potentially carry the heterozygous or homozygous UGT1A1 genotype or have known Gilbert's syndrome. In contrast, a Japanese group presented data from a UGT1A1 tailored phase I study of irinotecan at the ASCO 2006. The authors recommended the following genotype-adapted doses of irinotecan for phase II and III studies: 150 mg/m^2 q2w for genotype *(6/6)* and 70 mg/m^2 q2w for genotype *(6/7)* in combination with doxifluridine [64].

Innocenti *et al.* [50] defined pretreatment bilirubin levels as a predictor of severe neutropenia. Marcuello *et al.* [55] reported on 95 patients with mCRC who received single-agent irinotecan or an irinotecan-based chemotherapy. As a result they found that the median bilirubin levels were significantly increased in patients with the *(6/7)* and *(7/7)* genotypes during treatment. In a study that investigated the relationship of baseline bilirubin levels to efficacy and toxicity in patients suffering from mCRC, an elevated bilirubin level was found to be a predictor of neutropenia of higher grades, but solely when irinotecan was administered on a weekly basis [65]. Elevation of serum bilirubin seemed not predictive with regard to treatment efficacy. Ramchandani *et al.* [66] showed that the *(7/7)* genotype and elevated baseline bilirubin levels were significantly associated with a lower absolute neutrophil count nadir. In a study with 127 patients with lung cancer who underwent irinotecan/cisplatin-based chemotherapy, the pretreatment bilirubin levels were associated with severity of neutropenia as well [67]. In cancer patients with hepatic dysfunction, baseline bilirubin levels can be useful in the determination of the appropriate irinotecan dose [68]. Other authors even recommend the assessment of bilirubin before each cycle of chemotherapy [69].

In summary, bilirubin seems to be a useful predicator of toxicity in patients treated with irinotecan, when interpreted in combination with UGT1A1 genotyping.

UGT1A1 genotyping and irinotecan dose

On 21 July 2005, the FDA of the USA and Pfizer Pharmaceuticals changed the package insert information for irinotecan: a patient's *UGT1A1*28* genotype was included as a risk factor for the development of severe neutropenia. This change was a result of the findings of four different pharmacogenetic studies that identified a 2.5-fold to 17-fold increased risk of toxicity in homozygous *UGT1A1*28* patients receiving an irinotecan-based chemotherapy. Notably, a minority of the patients in these studies carried the homozygous genotype *(7/7)* ($n = 34$). Subsequent trials on larger patient populations treated with other regimens have failed to consistently replicate the strong *UGT1A1*28* genotype–toxicity associations. Thus, the development of dosing recommendations was frustrating until now. Hoskins *et al.* [70] reviewed the data of 10 pharmacogenetic studies using irinotecan (825 patients) and estimated the correlations between the incidence of irinotecan-induced haematological toxicities of higher grades in patients carrying the

Table 2 Irinotecan-associated neutropenia with regard to UGT1A1 status and irinotecan dose (presented by Hoskins *et al.* [70])

Author	Irinotecan dose (mg/m^2)	Incidence of grade 3 and 4 haematological toxicity in all patients (%)	Incidence of grade 3 and 4 haematological toxicity in *UGT1A1*28 (7/7)* patients (%)
Innocenti *et al.* [50]	350	18	83
Iyer *et al.* [49]	300	10	50
McLeod *et al.* [57]	200	17	55
Rouits *et al.* [54]	180	33	60
Chiara *et al.* [71]	180	28	57
Marcuello *et al.* [55]	180	25	60
Toffoli *et al.* [53]	180	15	18
Carlini *et al.* [72]	125	5	0
McLeod *et al.* [57]	100	10	18
Massacesi *et al.* [73]	80	7	14

homozygotous genotype *(7/7)*, irinotecan dosage and the overall toxicity. The incidence of grade 3 and 4 haematotoxicity in patients with *(7/7)* genotype correlated with both the irinotecan dosage (Spearman's rank correlation $r_S = 0.68$; $P = 0.04$; $n = 10$ studies) and the incidence of toxicity of a chemotherapeutic regimen ($r_S = 0.88$; $P = 0.002$; $n = 10$ studies). When analysing all genotypes, the incidence of severe haematological toxicity was not related to irinotecan dosage ($r_S = 0.44$; $P = 0.20$; $n = 10$ studies) [70]. These data suggest that the risk of experiencing irinotecan-induced haematological toxicity for patients carrying the *(7/7)* genotype is a function of the dose of irinotecan administered (Table 2). The authors recommend a genotype-based dosing of irinotecan for high doses of irinotecan, but they believe that it may not be useful for lower doses of irinotecan [74]. This recommendation is supported by data achieved in a paediatric study population. Stewart *et al.* [75] observed that severe toxicity was not increased in paediatric patients receiving low dose irinotecan.

UGT1A1 genotyping and ethnic differences

Similar to most gene polymorphisms, UGT1A1 is subject to interracial variability. In a study by Lampe *et al.* *UGT1A1*28 (7/7)* was found in 11% of the Caucasian patients, but in none of the Asians, leading to the conclusion that the frequency of the TA *(7/7)* genotype is much lower among an Asian population [76–78]. Liu *et al.* [79] showed the predominance of the *UGT1A1 (6/6)* genotype in Asians with 76% compared with 46% in Caucasians. These findings were confirmed by the results of a large study comparing the frequency of the allele TA_6 of the TATAA box polymorphism of the UGT1A1 gene between African–Americans (0.45), Caucasians (0.59) and Japanese (0.9) [80]. Innocenti *et al.* [81] have described a linkage disequilibrium between various functional polymorphisms of UGT1A1 and a different haplotype structure of the promotor between Caucasian and African–Americans. It remains debatable whether UGT1A1 genotyping in general and the determination of the *UGT1A1*28* allele in particular is sufficient in non-Caucasians or whether it should be combined and expanded with additional pharmacogenetic tests. Data from Japanese patients disclosed an increasing susceptibility to toxicity during irinotecan-based chemotherapy in patients carrying both the *UGT1A1*28* and the *UGT1A1*6* genotype even when being heterozygous [82]. In another study of 45 patients from Singapore, the presence of *UGT1A*6* allele was associated with a three-fold increased risk for grade 4 neutropenia compared with patients carrying the wild-type genotype [83]. With a high variability of the incidence of *UGT1A1*28* within different Asian subpopulations, a combined genotyping of *UGT1A1*28* and, at least, of *UGT1A1*6* seems reasonable in Japanese and probably other Asian patients [84–86].

Complexity of irinotecan metabolism

The role of UGT1A1

The clinical relevance of functional polymorphisms of the UGT1A1 gene has been addressed in several publications. In-vitro data suggested that polymorphisms in the coding region of UGT1A1 (*G71R*, *P229Q*) lead to a reduction of SN-38 glucuronidation either alone or in combination with other SNPs in the exon or in the promotor region [87].

In a study of genetic variants in a Chinese population, Zhang *et al.* [88] have genotyped the functional polymorphisms *–3279T > G* (*UGT1A1*60*) and *3156G > A* in the enhancer region, $(TA)_{6>7}$ in TATAA box (*UGT1A1*28*) and *211G > A* (*G71R*, *UGT1A1*6*) and *686C > A* (*P229Q*, *UGT1A1*27*) in the exon 1 region of the UGT1A1 gene. Within patients being homozygous for the *–3279G* allele in the enhancer region of the UGT1A1 gene those with the UGT1A1 *(7/7)* genotype have increased bilirubin levels compared with patients carrying the heterozygous or wildtype genotype. The distribution of the various polymorphisms differed greatly among distinct Chinese subpopulations. An analysis among 195 Japanese patients who had received an irinotecan-based chemotherapy identified UGT1A1 haplotypes associated with increased bilirubin levels and reduced AUC ratios (SN-38G/SN-38). Other functional variants of UGT1A1 in patients of Japanese origin were *–3279T > G* (*UGT1A1*60*) and *211G > A* (*UGT1A1*6*) and contribute as well to the enzyme function [89]. The authors suggest an additive effect of the *UGT1A1*28* and the *UGT1A1*6* haplotypes on irinotecan toxicicty. As a result of a highly significant linkage disequilibrium between the *–3279T > G* and the *UGT1A1*28* polymorphism, the determination of both genotypes is recommended by some authors [90]. It can be expected that in the near future even more functional relevant SNPs of the UGT1A1 gene locus will be characterized.

The role of other UGTs

Apart from UGT1A1, UGT1A7 and UGT1A9 are also involved in the glucuronidation of SN-38 [91–93].

A minor role is suggested for UGT1A6, UGT1A8 and UGT1A10. Numerous functional polymorphisms have been characterized and their relationships with bilirubin and irinotecan metabolism have been described.

A *−57T > G* SNP of the TATA box in the promoter region of the UGT1A7 gene reduces the promoter activity to 30% and may lead to an altered SN-38 metabolism in association with other variants of UGT1 [94]. In a study by Carlini *et al.* [72], 67 patients with CRC who had received irinotecan and capecitabine were analysed to clarify the impact of UGT1A7 and UGT1A9 gene polymorphisms on treatment efficacy and toxicity. Both UGT1A7 and UGT1A9 genetic variants were predictive for tumour response and the development of diarrhoea. Interestingly, *UGT1A1*28 (7/7)* was not found to have an impact on the incidence of diarrhoea. Irinotecan-associated haematotoxicity was observed more frequently among patients carrying the *UGT1A1*28* allele in combination with the *UGT1A7 N129K/R131 K* and *UGT1A7 −57T > G* SNPs [95]. A Taiwanese study supported these data indicating an increased risk for Gilbert's syndrome because of a combination of UGT1A1 and UGT1A7 genotpyes [96]. In-vitro data scaling glucuronidation of SN-38 indicate residual activity and a reduced SN-38 glucuronidation capacity for UGT1A1 and UGT1A7 variants compared with the wild-type genotype [97].

Ando *et al.* [98] reported the distribution of *UGT1A7*1–3* genotypes to be different between Japanese and Caucasian patients. The authors concluded that UGT1A7 genotyping is not useful for predicting irinotecan-associated toxicity. Although the clinical relevance is not yet well defined, there are further indications for an interracial variability of the UGT1A7 alleles [99]. The results indicate a strong association of the *UGT1A1 211G > A* (*G71R*) genotype with the presence of *UGT1A7*3*.

In-vitro studies of UGT1A9 polymorphisms identified *I399C > T* and $-118(dT)_{(9/10)}$ as possible candidates for additional genotyping in combination with UGT1A1 genotyping to better predict SN-38 metabolism [100]. In an analysis of 67 patients who had received irinotecan/capecitabine for CRC, patients carrying the *UGTA9* $-118(dT)_{(9/9)}$ genotype experienced less toxicity ($P = 0.002$), but interestingly, an increased RR ($P = 0.047$) [72]. As the *UGT1A9* $-118(dT)_{(9/9)}$ genotype predicted improved efficacy and low toxicity, the *UGT1A9* $-118(dT)_{(10/10)}$ genotype was found to be predictive for poor response.

However, data from a Korean study including 81 patients with NSCLC treated with irinotecan and cisplatin indicated contrarily that patients who carried the *UGT1A9* $-118(dT)_{(9/9)}$ genotype had a nonsignificant trend towards an increased rate of severe irinotecan-associated toxicity, but not tumour response [101]. The *G71R* polymorphism of the UGT1A1 gene was identified as another potential predictor of tumour response and survival. The authors accentuated a close linkage and the interaction of UGT1A1, UGT1A7 and UGT1A9 with their specific gene polymorphisms. Lacking conclusive data, an association of rare genetic variants in UGT1A9 and irinotecan-associated toxicity cannot be finally evaluated without further studies [102].

Alternative metabolic pathways

Cytochrome isoforms

In-vitro data show the involvement of cytochrome P450 3A4 in the metabolism of irinotecan [103]. With regard to other isoforms, further characterization of this metabolic way and its role in the determination of toxicity and treatment outcome is essential [104,105]. Results from a retrospective analysis are encouraging with a statistically significant correlation of CYP3A4 phenotype and irinotecan and SN-38 pharmacokinetics [106]. According to the authors, *UGT1A1*28* genotyping might be combined with the assessment of CYP3A4 phenotype. In a Japanese study, an impaired metabolism of irinotecan to the inactive APC was detected which had no impact on total clearance or toxicity [107].

On the basis of the available data, there is currently no indication for routine CYP3A4 genotyping in combination or instead of UGT1A1 genotyping [108].

Carboxylesterase

Preclinical data refer to an association of irinotecan efficacy and the concentration of CE that catalyses the inactive prodrug CPT-11 to the active form SN-38 [109]. Alternatively, NPC can be transformed to SN-38 by human carboxylesterase 2 (hCE2). In a study with 65 patients, there was no significant relationship between gene polymorphism of CES1 and CES2 with irinotecan metabolism, probably because of low allele frequency [110]. Probably, the presence of hCE in the human gut partly contributes to an increased gastrointestinal toxicity of irinotecan [111].

Drug pumps

Elimination of SN-38 is partly mediated by membrane-localized, energy-dependent and outward-directed drug pumps. ABCB1 (MDR1/P glycoprotein), ABCC1 (multidrug resistance-associated protein 1), ABCC2 (multidrug resistance-associated protein 2) and ABCG2 (breast cancer resistance protein) belong to the super family of ABC transporters [112–115]. The homozygous *ABCB1 1236C > T* polymorphism significantly increased the exposure to both irinotecan and SN-38 in cancer patients treated with irinotecan [110]. The presence of the *ABCC2*2* haplotype was associated with a decreased rate of irinotecan-related diarrhoea, potentially as a result of reduced hepatobiliary secretion in Caucasian patients [116]. However, this effect was solely observed in patients carrying the *UGT1A1*28 (6/6)* wild-type genotype. Data provided from a Korean study including 107

patients who received irinotecan and cisplatin for NSCLC indicated that the ABCB1 genotypes *3435TT* and *2677TT* were associated with higher efflux activity. The *2677GG* genotype led to more grade 4 neutropenia, patients with the *3435TT* genotype experienced significantly more grade 3 diarrhoea ($P = 0.047$). However, tumour response in patients with the *ABCC2 –24TT* and *3972TT* genotypes was better [117]. Genetic polymorphisms in the ABCG2 gene might be important for irinotecan pharmacokinetics as well [118]. Results from clinical studies of polymorphic variants of the organic transporting peptide OATPB1 hinted at a decreased clearance by certain genotypes and therefore needs further investigation [119].

Topoisomerase-1

With over 1600 patients assessed for biomarkers within the UK MRC FOCUS trial, high topoisomerase-1 (Topo1) was associated with a survival benefit with first-line combination chemotherapy, whereas patients with low or moderate Topo1 did not benefit [59]. Elevated levels of Topo1 were identified as a poor prognostic factor. In a study with 107 patients with advanced CRC undergoing irinotecan-based chemotherapy by Hoskins *et al.* [120], TOP1 haplotype tagging SNP (htSNP) was related to grade 3/4 neutropenia ($P = 0.04$) and response ($P = 0.04$). However, TOP1 polymorphism was not an independent predictive marker of neutropenia by logistic regression. Still, the predictive value of Topo1 needs to be further investigated [121].

Other factors contributing to toxicity and outcome

Classical risk factors

Freyer *et al.* [122] reported on a phase II study including 455 patients who had received second-line irinotecan for mCRC. They verified the clinical usefulness of baseline bilirubin and haemoglobin levels, number of organs involved and the time from diagnosis to metastasis as valuable predictors for neutropenia, whereas performance status (PS), serum creatinine, leukocyte count and prior irradiation may serve as independent risk factors for the development of delayed diarrhoea. Data from a retrospective analysis of 200 Canadian patients who had received FOLFOX or FOLRIRI for mCRC indicated that severe diarrhoea was associated with an impaired performance status ($PS \geq 3$), severe comorbidity, baseline diarrhoea, elevated baseline bilirubin, primary tumour resection, higher stage, chemotherapy beyond first line, FOLFOX chemotherapy or toxicity in the previous cycle [123].

Interactions

The metabolism of irinotecan is highly complex with numerous enzymes involved. In addition, drug interactions, as described earlier for a variety of agents, must be considered in case of treatment failure or increased toxicity. Reviewing the literature, interactions of irinotecan and its metabolites are described; for example, for antimycotic agents such as ketoconazole, green tea, milk thistle, St. John's Wort, valproic acid and numerous other agents [124–129]. Even cigarette smoking has an impact on irinotecan metabolism and lowers the exposure of irinotecan and the risk of treatment-induced neutropenia [130]. Mathijssen *et al.* [131] showed that cotreatment with St. John's Wort lowered SN-38 serum levels to an extent endangering treatment outcome.

Conclusion

Owing to the severity of irinotecan-associated side effects, a safe and patient-tailored chemotherapy is an aspiring goal in modern antitumour therapy, particularly in the palliative setting. Advances have been made with the accelerating progress of molecular science so that by panels multiple SNPs in genes involved in drug metabolism, transport, drug targets, DNA repair, cell cycle and apoptosis can be genotyped [132].

The data from trials analysing the impact of UGT1A1 gene polymorphism are somehow contrary and a general conclusive recommendation is impossible. Nevertheless, the awareness of the FDA and the possibility of genetic testing is a step towards patient's safety and cost reduction in public health. At the same time, the unreflective use of UGT1A1 genotyping must be avoided. The effect of dosage on the occurrence of side effects is striking and should be the physician's first choice to decree the best regimen for his patient and to decide whether testing is reasonable. Regarding cost-effectiveness of UGT1A1 genotyping, Obradovic *et al.* [133] analysed mCRC patients who received second-line irinotecan for mCRC. The authors recommended UGT1A1 genotyping restrictively to those patients with subsequent dose reductions during high-dose irinotecan. Moreover, UGT1A1 genotyping remains cost-effective solely in African or Caucasian population, but not in an Asian population. Therefore, factors such as ethnic differences, environmental factors, diet and co-medication have to be carefully considered. As suggested by some authors, the additional genetic testing of other UGT1 isoforms and specific gene polymorphisms should be done depending on ethnicity. PS, organ functions, resistance mechanisms and tumour sensitivity all contribute to the actual outcome. For the future analysis, it is desirable to gain information from prospective genotype-guided phase III studies with a proper stratification.

As irinotecan metabolism is complex, numerous genes beside UGT1A1 have to be analysed. Neither an overall genetic testing with DNA microarray technique nor the limitation to UGT1A1 alone will open Pandora's box in the prediction of treatment efficacy or toxicity. Its reasonable use and the focus on consolidated findings and their improvement surely turn out to be more fruitful. Thus, it might be possible to define subgroups with therapeutic consequences instead of withholding irinotecan from patients who could benefit from its considerate use.

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