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Original Research

Clinical and pharmacogenetic determinants of 5-fluorouracyl/leucovorin/irinotecan toxicity: Results of the PETACC-3 trial☆

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Abstract ***Purpose:*** Irinotecan (CPT-11) in combination with 5-fluorouracil (5FU) is widely used in the treatment of colorectal cancer. We assessed potential clinical variables that may predict toxicity and more specifically the role of UGT1A1 polymorphisms associated with irinotecan toxicity. We used data from the PETACC3 trial, which randomised patients in adjuvant setting to 6 months of leucovorin (LV) and 5FU (LV5/FU2) or LV5/FU2 + irinotecan.
Patients and methods: Clinical and toxicity data were available for 2982 patients, DNA was available for 1200 (40%) of these patients. We genotyped the polymorphisms UGT1A1*28 and UGT1A1-3156G > A. Risk factors for neutropenia and diarrhoea were assessed by univariable and multivariable analyses.
Results: In univariable analysis, UGT1A*28 genotype was associated with an increased incidence of grade III–IV neutropenia (incidence: 44% versus 26%; odds ratio [OR]: 2.3; 95%

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confidence interval [CI]: 1.4–3.7). In multivariable analysis, the most important predictors (ordered in terms of contribution to R^2) were baseline neutrophil count (OR for 1-unit (10^9/l) decrease: 1.8, 95% CI: 1.3–1.7), female sex (OR: 1.8, 95% CI: 1.1–3.0), body surface area (OR for 0.1-unit increase: 0.8, 95% CI: 0.7–1.0), UGT1A1 (OR: 2.8, 95% CI: 1.6–5.0), age (OR per 10 years: 1.3, 95% CI: 1.1–1.6) and poor performance status (OR: 1.6, 95% CI: 1.0–2.6). The main predictors for grade IV neutropenia were sex, age, performance score and UGT1A1. The main predictors for diarrhoea were sex and age.

Conclusions: We found that a complex of risk factors is involved in the development of toxicity, including UGT1A1. Parameters that are readily available in clinical practice, notably sex, age and performance status, are stronger predictors than the UGT1A1*28 genotype. Further studies beyond the UGT1A1*28 genotype are needed to fully understand the determinants of toxicity risk, notably in females.

1. Introduction

Irinotecan is a mildly active prodrug which is metabolised into to the topoisomerase I inhibitory metabolite (SN-38). SN-38 is subsequently detoxified by uridine diphosphate-glycosyltransferase 1, encoded by the *UGT1A1* gene. Polymorphisms in the *UGT1A1* gene have been investigated in the context of hyperbilirubinemia syndromes, and they are common at position −53 of the TATA box, where between 5 and 8 TA repeats are found of which the T6 allele is the most frequent and T7 allele (*UGT1A1*28*) the second most. *UGT1A1* 7/7 promoter variant is associated with reduced transcriptional activity and overall lower bilirubin glucuronidating capacity. Increased irinotecan toxicity due to decreased glucurono-conjugation was first shown in patients with Gilbert syndrome [1]. Associations between irinotecan toxicity and UGT1A1 polymorphisms, including SNP3156G > A, have been found in cancer patients [2–4]. Hence, the Food and Drug Administration recommended the use of *UGT1A1* 7/7 polymorphism testing before the administration of irinotecan, to reduce the risk of undue and sometimes fatal toxicity [5,6]. However, recent studies on the capacity of these tests to predict irinotecan toxicity have shown mitigated results [7,8]. Several studies identified sex, age and treatment schedule as important predictors, limiting the relevance of *UGT1A1* polymorphisms [9,10]. Tsunedomi *et al.* proposed an algorithm based on a combination of UGT1A genotypes, sex and age for predicting irinotecan toxicity [11]. Other gene polymorphisms, including those in *UGT1A6, UGT1A7* and *UGT1A9*, SNP 3156G > A and enzymes, such as CYP3A, CES2 and DPYD, have been found predictive for irinotecan toxicity [10–16].

Against this background we revisited the role of the *UGT1A1* 7/7 polymorphism as predictor of irinotecan toxicity, in a large clinical trial population treated with the 5-fluorouracyl/leucovorin/irinotecan (FOLFIRI) regimen, taking other clinical and genetic markers into consideration.

2. Patients and methods

2.1. PETACC trial

In the PETACC-3 trial (NCT00026273), 3241 patients with stage II and stage III colon cancer were randomly allocated to receive leucovorin5 and fluorouracil (LV5/FU2) (LV 200 mg/m^2 as a 2-h infusion, followed by 5FU as a 400 mg/m^2 bolus and then a 600 mg/m^2 continuous infusion over 22 h, days 1 and 2, every two weeks for 12 cycles) with (FOLFIRI) or without (LV5/FU) irinotecan (180 mg/m^2 as a 30–90 min infusion, day 1, every two weeks) after curative surgery [17]. Alternatively, centres could use a high-dose FU schedule (LV 500 mg/m^2 as a 2-h infusion, followed by FU 2000 mg/m^2 as a 24-h infusion). Dose capping at a body surface area (BSA) of 2 m^2 was foreseen in the protocol. During the study, at 6 week intervals, medical history since the previous infusion was recorded, and a physical examination was performed. Haematological and biochemical assessments were performed every 2 and 6 weeks, respectively. All patients gave informed consent as to the planned translational studies.

Treatment-emergent adverse events were assessed according to the National Cancer Institute Common Toxicity Criteria Grading System (Version 2.0). Baseline neutrophil counts (BNCs) were defined as the last value before any treatment. For subsequent analyses, adverse events were classified as occurring after the first treatment (i.e. recorded before day 15 of cycle 1) or as the worst toxicity over the whole treatment period. The time to occurrence of an adverse event was also recorded. Any grade III or IV toxic event resulted, as per protocol, in a 20% dose reduction for subsequent cycles after toxicity resolution or treatment was postponed. Dose reduction was used as a global measure of toxicity.

2.2. Genotyping methodology

Germline DNA was extracted from normal tissue areas from 5 μm sections in formalin-fixed paraffin embedded tumour tissue blocks. To determine the length of the alleles, present status of the TA repeat (6/6, 6/7 or 7/7) in

the TATA box of the *UGT1A1* promoter region was identified by polymerase chain reaction amplification of the region and gel electrophoresis. For the SNP 3156G > A (rs10929302) in *UGT1A1*, alleles were determined by the use of TaqMan probes (Applied Biosystems). Detailed methodology is available in addendum 1.

2.3. Statistical analysis

2.3.1. List of predictors

As predictors we used age (continuous), sex, BSA (continuous), body mass index (BMI), World Health Organisation (WHO) performance status (0,1), UGT1A1 (7/7 versus other or 6/6 versus 6/7 versus 7/7), *UGT1A1* SNP 3156 (AA versus other or GG versus GA versus AA), total bilirubin (continuous or—for tables—low versus high [cut-off: 0.5 × upper limit of normal]), BNC (10^9/L) (last value before any treatment, continuous) and neutrophil count after one administration (i.e. on day 15 of cycle 1)(continuous) and treatment arm.

2.3.2. List of outcomes

As a primary measure of outcome, we used worst neutropenia grade III or IV occurring at any time during the whole treatment period or occurring after day 15; other outcomes were worst diarrhoea grade III or IV, neutrophil count after first administration (i.e. on day 15 of cycle 1), worst febrile neutropenia, total number of serious adverse events (SAEs), dose reductions at any time and time to neutropenia grade III or IV.

2.3.3. Analyses

We assessed associations between the predictors by non-parametric methods (Pearson's chi-square test, Wilcoxon rank-sum test, Spearman correlation and Kappa measure of agreement) followed by univariable analyses with UGT1A1 status as predictor and toxicity outcomes. Standard techniques for contingency tables were used (chi-square tests). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. For the total number of SAEs, negative binomial regression was performed. Kaplan–Meier curves for time to toxicity were drawn, and Gray-Tsiatis tests [18] were used. Treatment stops were censored if they were not due to the toxic event under consideration; time points of absolute dose reductions were also censored (in case of low dose 5FU, absolute dose reduction consisted of 5FU bolus < 800 mg, in case of 5FU infusion, this was <1200 mg and in case of CPT11, this was <180 mg).

Univariable contingency table or logistic regression analyses for other potential predictors were performed on the full population. BMI and BSA were highly correlated. BSA turned out to be a stronger predictor than BMI and was hence used in subsequent analyses. To find the stronger of two competing predictors, bivariate regression models were calculated, i.e. for the two UGT1A1 variants, for UGT1A1 and bilirubin, for sex and BSA. Then multiple logistic regression analyses were performed with predictors such as age, sex, BSA, performance status, UGT1A1, baseline bilirubin and BNC or neutrophil count after first administration for worst neutropenia after day 15. All analyses were done by treatment arm. Statistical computing was performed using the following software packages: SAS 9.1 (SAS Institute Inc., Cary, NC, USA), S-PLUS 7.0 (Insightful Corporation, Seattle, WA, USA), StatXact 5.0 (Cytel Software Corporation, Cambridge, MA, USA).

3. Results

3.1. Patient populations

In total, 3241 patients were randomised in the PETACC-3 trial. For this toxicity analysis, we excluded all patients treated with the high-dose FU regimen (n = 259). Of the remaining 2982 patients, 1192 (40.0%) were evaluable, 598 patients in the FOLFIRI arm and 594 in the LV5FU arm (Table 1). Few multivariables of these patients had missing values in one of the two UGT1A1 genotypes, in laboratory values or in patient characteristics. The distribution of the main predictor variables among the various patient groups is presented in Table 1.

3.2. The UGT1A1 7/7 and SNP 3156 genotype

The incidence of both UGT1A1 genotypes is given in Table 2. The frequencies are in accordance with those expected in a Caucasian population [19]. There was a strong association between the two UGT1A1 genotypes, in the 3-level and the 2-level arrangements (7/7 versus all others and AA versus all others; $p < 0.0001$, kappa measure of agreement: 0.83 for the 3-level and 0.82 for the 2-level); consequently only one of the two was used in a multivariable model. In bivariate logistic regression with UGT1A1 7/7 versus other and UGT1A1 SNP 3156 (AA versus other) as predictors for bilirubin >0.5 versus ≤ 0.5 × upper limit of normal, only the 7/7 version remained significant. This approach also confirmed the predictive value of UGT1A1 for grade III or grade IV neutropenia (data not shown). For these reasons, further analysis was limited to UGT1A1 6/6, 6/7 and 7/7 genotypes.

3.3. Associations between other predictors

There were no meaningful two-way associations between baseline predictors, except for baseline bilirubin and sex (Wilcoxon rank-sum test, $p < 0.001$; median 0.40 [range: 0.06–1.26] and 0.45 [0.01–2.2] in females and males, respectively).

When considering the neutrophil count at day 15, we found a relevant association with BNC (Spearman correlation: 0.481: $p < 0.001$) and with sex (Wilcoxon rank-sum test, $p < 0.0001$; median 2.60 [range: 0.10–10.3] and 2.86 [0–13.3] in females and males, respectively).

Table 1
Baseline and on-treatment characteristics of the trial population.

Patient characteristics	Population treated with standard dose 5FU				Population treated with high dose FU	
	Population with biomarker data		Population without biomarker data		Excluded from biomarker analysis	
	FOLFIRI arm	LV5FU arm	FOLFIRI arm	LV5FU arm	With irinotecan	Without irinotecan
	N = 598	N = 594	N = 887	N = 903	N = 135	N = 124
Baseline characteristic						
Age median (range)	60 (21–76)	60 (25–76)	59 (18–76)	61 (21–75)	60 (30–74)	60 (26–75)
Female sex no (%)	247 (41)	254 (43)	412 (46)	409 (45)	72 (53)	53 (43)
BSA median (range)	1.80 (1.31–2.39)	1.79 (1.31–2.36)	1.77 (1.08–2.62)	1.77 (1.30–2.40)	1.76 (1.34–2.37	1.79 (1.36–2.20)
PSWHO no (%)	482 (81)	472 (80)	746 (85)	750 (83)	114 (84)	91 (74)
Use of hormone replacement therapy	6	4	7	1	1	2
Baseline serum bilirubin (mg/dl) median (range)	0.44 (0.08–1.24)	0.42 (0.06–1.23)	0.40 (0.01–1.25)	0.43 (0.01–2.20)	0.38 (0.08–1.00)	0.36 (0.09–0.90)
Baseline neutrophil count (10^9/l) median (range)	3.90 (1.72–10.80)	3.84 (1.70–9.53)	3.81 (1.37–13.40)	3.80 (1.42–9.97)	3.90 (1.80–10.30)	4.01 (1.84–8.92)
UGT1A1 data available	574	572	–	–	58	50
SNP 3156G > A	587	582			58	51
On treatment characteristics						
Neutrophil count on day 15 (10^9/l) median (range)	2.40 (0.08–9.96)	3.00 (0.30–13.30)	2.50 (0.00–8.73)	3.10 (0.45–8.70)	2.90 (0.10–6.06)	3.07 (1.08–9.59)
Toxicity outcomes						
Worst neutropenia no (%)						
Grade III	115 (19.2)	23 (3.9)	195 (22.0)	40 (4.4)	13 (9.6)	4 (3.2)
Grade IV	54 (9.0)	15 (2.5)	56 (6.3)	11 (1.2)	5 (3.7)	4 (3.2)
Worst diarrhoea no (%)						
Grade III	47 (7.9)	26 (4.4)	85 (9.6)	37 (4.1)	23 (17.0)	22 (17.7)
Grade IV	15 (2.5)	4 (0.7)	30 (3.4)	16 (1.8)	15 (11.1)	11 (8.9)
Worst febrile neutropenia no (%)						
Grade III	3 (0.5)	2 (0.3)	4 (0.5)	1 (0.1)	–	–
Grade IV	–	2 (0.3)	4 (0.5)	3 (0.3)	2 (1.5)	2 (1.6)
Total number of SAEs	258	143	300	173	104	77
Patients with SAE no (%)	127 (21.2)	84 (14.1)	165 (18.6)	107 (11.8)	47 (34.8)	35 (28.2)
Dose reduction no (%)	185 (30.9)	92 (15.5)	247 (27.9)	98 (10.9)	64 (47.4)	46 (37.1)
Efficacy outcomes						
5-year RFS	70%	69%	65%	61%	65%	68%
5-year RFS stage ≤ II	83%	85%	84%	78%	83%	92%
5-year RFS stage ≥ III	65%	61%	58%	55%	60%	62%
5-year OS	79%	79%	77%	75%	75%	80%
5-year OS stage ≤ II	89%	91%	91%	87%	90%	96%
5-year OS stage ≥ III	75%	72%	72%	70%	71%	76%
UICC stage ≤ II No (%)	182 (30.4)	196 (33.0)	255 (28.7)	249 (27.6)	30 (22.2)	24 (19.4)

BSA, body surface area; PSWHO, WHO performance status; FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; SAE, serious adverse event; OS, overall survival; RFS, relapse-free survival.

3.4. Univariable analysis: incidence of toxicities according to various predictors

3.4.1. Neutropenia grade III–IV

3.4.1.1. UGT1A1. In univariable analyses, grade III–IV neutropenia and UGT1A1 status were associated in FOLFIRI but not in LV/5FU patients (Tables 3 and 4). The OR for 7/7 against other types was 2.3 (95% CI: 1.4–3.7, p < 0.001) in the FOLFIRI arm and 1.7 (95% CI: 0.7–4.0, p = 0.30) in the LV/5FU arm.

Table 2
Frequencies of the UGT1A1 genotypes and SNP 3156 alleles.

Frequency \ allele	SNP 3156 GG	SNP 3156 GA	SNP 3156 AA	Total
6/6	468	15	2	485
6/7	56	422	5	483
7/7	1	34	113	148
Total	525	471	120	1116

In patients treated with FOLFIRI, Kaplan–Meier curve analysis (Fig. 1) showed UGT1A1 7/7 genotype to be significantly associated with more frequent and earlier neutropenia grade III–IV than the other genotypes (Gray-Tsiatis; p < 0.001).

3.4.1.2. Bilirubin, age, performance status and BSA. The univariable analysis results for bilirubin, age and performance status are summarised in Table 3. Of note, only in the FOLFIRI arm we found a weak but significant association of bilirubin and age with grade III–IV neutropenia. In both arms, patients with higher BSA showed a significantly lower risk of grade

Table 3
Univariable results for neutropenia grade III–IV.

Predictor	FOLFIRI		LV5FU	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Age, 10 year increase	1.23 (1.10–1.38)	<0.001	1.08 (0.87–1.33)	0.493
Sex, F versus M	2.21 (1.76–2.79)	<0.001	1.50 (0.98–2.31)	0.063
BSA, 0.1-unit increase	0.84 (0.80–0.90)	<0.001	0.83 (0.74–0.93)	0.001
Subpopulation F	0.99 (0.90–1.10)	0.978	0.78 (0.64–0.96)	0.018
Subpopulation M	0.85 (0.76–0.94)	0.002	0.87 (0.72–1.06)	0.174
BMI, 1-unit increase	0.90 (0.86–0.95)	<0.001	0.84 (0.76–0.94)	0.001
Subpopulation F	0.99 (0.92–1.06)	0.734	0.88 (0.76–1.01)	0.067
Subpopulation M	0.87 (0.80–0.95)	0.003	0.83 (0.70–0.98)	0.032
PSWHO, 1 versus 0	1.30 (0.97–1.75)	0.077	0.83 (0.46–1.49)	0.530
UGT1A1, 7/7 versus other	2.29 (1.41–3.71)	<0.001	1.68 (0.71–3.99)	0.301
Subpopulation other, 6/7 versus 6/6	0.98 (0.65–1.47)	0.918	1.17 (0.56–2.42)	0.676
Bilirubin, $> 0.5 \times$ LUN versus $\leq 0.5 \times$ LUN	1.41 (1.11–1.79)	0.004	0.98 (0.62–1.54)	0.916
Baseline neutrophils, 1-unit decrease	1.46 (1.33–1.61)	<0.001	1.56 (1.28–1.92)	<0.001

BSA, body surface area; PSWHO, WHO performance status; FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; BMI, body mass index; CI, confidence interval.
UGT1A1: biomarker population; other predictors: full population.

Table 4
Multivariable results for neutropenia grade III–IV.

Predictor	FOLFIRI			LV5FU		
	Odds ratio (95% CI)	p-value	R^{2}*	Odds ratio (95% CI)	p-value	R^{2}*
Age, 10 year increase	1.29 (1.05–1.58)	0.016	92%	1.06 (0.76–1.47)	0.738	99%
Sex, F versus M	1.82 (1.11–2.98)	0.018	72%	1.06 (0.44–2.57)	0.899	32%
BSA in F, 0.1-unit increase	0.95 (0.79–1.13)	0.541		0.78 (0.57–1.06)	0.112	
BSA in M, 0.1-unit increase	0.83 (0.69–1.00)	0.045		0.75 (0.54–1.06)	0.101	
PSWHO, 1 versus 0	1.63 (1.02–2.61)	0.041	95%	0.69 (0.28–1.74)	0.434	95%
UGT1A1, 7/7 versus other	2.89 (1.65–5.07)	<0.001	82%	2.05 (0.79–5.29)	0.139	84%
Bilirubin, $> 0.5 \times$ LUN versus $\leq 0.5 \times$ LUN	0.97 (0.62–1.50)	0.874	100%	0.73 (0.33–1.63)	0.449	95%
Baseline neutrophils, 1-unit decrease	1.47 (1.25–1.73)	<0.001	67%	1.12 (0.86–1.46)	0.398	94%

BSA, body surface area; PSWHO, WHO performance status; FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; CI, confidence interval.
*$\mathbf{R^2}$ if predictor dropped from the full model compared to full model $\mathbf{R^2}$ (=100%).
UGT1A1: biomarker population; other predictors: full population.

III/IV neutropenia. In the FOLFIRI arm after stratifying BSA for sex, a significant BSA effect was found only in males but not in females. In the LV/5FU arm, the reverse was true.

3.4.1.3. Sex. Women were significantly more at risk for grade III–IV neutropenia in the FOLFIRI arm (OR for women was 2.2 (95% CI: 1.8–2.8; see Tables 3 and 4) but not in the LV/5FU arm.

The impact of sex on time to neutropenia grade III–IV in patients treated with FOLFIRI was evaluated by Kaplan–Meier curves (Fig. 2A). When stratified by genotype, we found grade III–IV neutropenia in women significantly more frequently and earlier than in males, both in the UGT1A1 7/7 and in the other genotypes (Fig. 2B and C).

3.4.1.4. Baseline neutrophil count. Of the patients, 14% had a neutrophil count on day 1, 69% had a neutrophil count up to 1 week and 99% up to 2 weeks before the start of the treatment. There was a strong association between BNC and grade III–IV neutropenia in both arms. In the FOLFIRI arm, the OR for a 1-unit (10^9/l) decrease was 1.46 (95% CI: 1.33–1.61; $p < 0.001$). In the LV/5FU arm, this was 1.56 (95% CI: 1.28–1.92; $p < 0.001$).

3.4.2. Neutropenia grade IV and febrile neutropenia
Febrile neutropenia and grade IV neutropenia were rare (Table 1). The univariable results for neutropenia grade III/IV and neutropenia grade IV were similar in the two arms. For neutropenia grade IV, there were fewer events than for neutropenia grade III/IV, which explains the lesser significance. The results are summarised in addendum 2.

3.4.2.1. Diarrhoea. Univariable predictors of diarrhoea grade III or more are reported in the table in addendum 3. There was a moderate protective effect of the 7/7 genotype in both arms. The trend became significant when we modelled both arms together with treatment

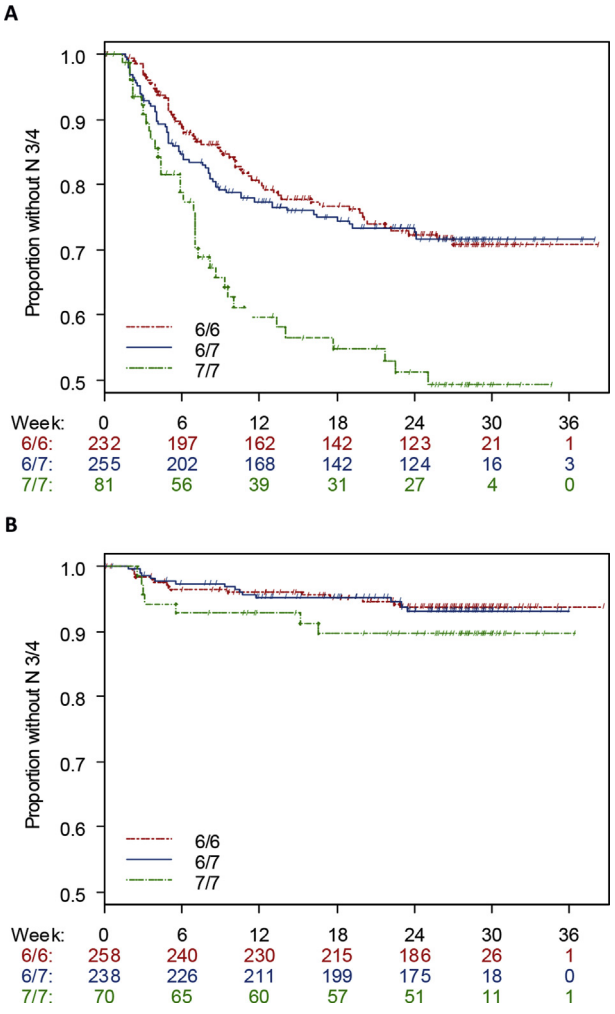

Fig. 1. Grade III/IV neutropenia-free survival curves estimated by the Kaplan–Meier method for patients in the FOLFIRI arm (A) or the LV5FU arm (B) according to the UGT1A1 status. The proportions of patients without neutropenia grade III/IV, i.e. the proportions at which the curves stabilise are 71% (95% CI: 65%–77%), 72% (95% CI: 66%–78%) and 49% (95% CI: 39%–63%) for the 6/6, 6/7 and 7/7 genotypes respectively in the FOLFIRI arm (Gray-Tsiatis; $p < 0.001$) and 94% (95% CI: 91%–97%), 93% (95% CI: 90%–97%) and 90% (95% CI: 83%–97%) in the LV5FU arm (Gray-Tsiatis; $p = 0.469$).

arm as cofactor ($p = 0.015$), suggesting that the 7/7 genotype protects from diarrhoea in both treatment arms.

3.4.2.2. Total serious adverse effects. In the FOLFIRI arm, the mean SAE rates per patient were 0.68 in the 7/7 and 0.40 in the other genotypes ($p < 0.01$; negative binomial regression, data not shown). In the LV/5FU arm, the rates were 0.22 and 0.23 for the two genotypes, respectively. There were further tendencies: older people in the FOLFIRI arm and in both arms, people with the worse performance had more SAE (data not shown).

3.4.2.3. Dose reduction. Dose reductions are most often induced by toxicity and can be taken as an integrated way of assessing toxicity and the subsequent physician's

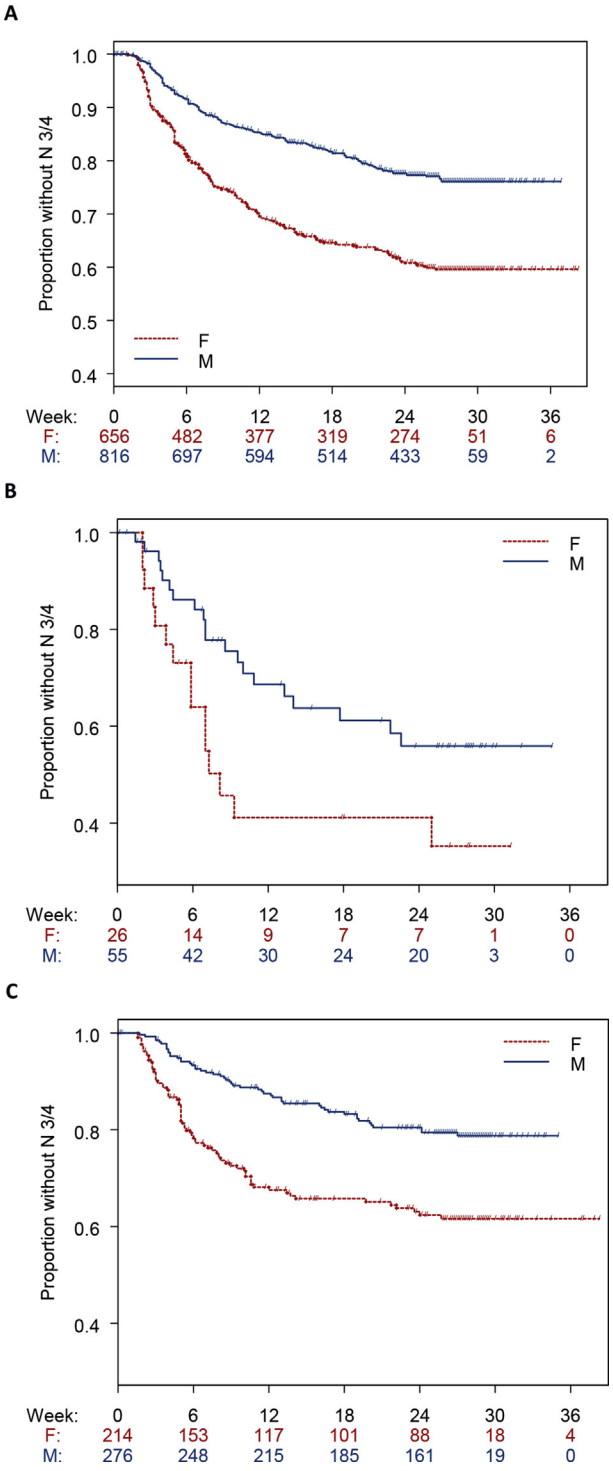

Fig. 2. The impact of sex on time to neutropenia grade III/IV in patients treated with FOLFIRI was evaluated by Kaplan–Meier curves. Patients were censored at the time of any dose reduction for reasons not related to neutropenia or at the end of the treatment. (A) The whole FOLFIRI population, (B) the UGT1A1 7/7 subgroup and (C) the other UGT1A1 genotypes. The proportions of patients without neutropenia grade III/IV are 35% (95% CI: 20–63%) and 56% (95% CI: 43–73%) for the females and males respectively in the 7/7 genotype (Gray-Tsiatis; $p = 0.08$) and 61% (95% CI: 55–69%) and 79% (95% CI: 74–84%) in the other genotypes (Gray-Tsiatis; $p < 0.001$).

response to drug-induced toxicity. In univariable analysis in the FOLFIRI arm, sex, BNC, UGT1A1 status and neutrophil count at day 15 were significantly associated with a dose reduction decision at any time point. In univariable analysis in the LV/5FU arm, sex, age, performance status and neutrophil count at day 15 were significantly associated with a dose reduction decision at any time point (addendum 4).

3.5. Multivariable analysis: combination of predictors

3.5.1. Neutropenia grade III–IV and grade IV

The results on the predictors such as age, sex, BSA per sex, performance status, UGT1A1 genotype, baseline bilirubin and BNC in the biomarker population are summarised in Table 4.

In the FOLFIRI arm, the most important predictors were in decreasing order: BNC, sex and BSA combination, UGT1A1, age and performance status, as evaluated by the incremental decrease of R^2 when the covariates were sequentially retrieved from the model. In the LV/5FU arm, the significance of BSA and sex combination disappeared.

Most of the results for neutropenia grade III/IV and for neutropenia grade IV were similar by multivariable analysis. In the FOLFIRI arm, there was one exception: BNC's were significant predictors for neutropenia grade III/IV but not for neutropenia grade IV (addendum 2). Results for diarrhoea and dose reduction are given in addendum 3 and 4.

3.5.2. Neutrophil count on day 15 predicts subsequent neutropenia

From this analysis, we excluded patients with grade III–IV neutropenia before or on day 15 (n = 37 for biomarker population and n = 96 for full population) and patients with missing values on day 15 (n = 13 and n = 36, respectively) or all values missing after day 15 (n = 9 and n = 28, respectively).

First, we identified the significant predictors of the neutrophil count on day 15: sex ($p = 0.002$), UGT1A1 7/7 ($p = 0.020$) and BNC ($p < 0.001$). We then evaluated the impact of neutrophil count on day 15 to predict subsequent grade III–IV neutropenia and found a close association between both variables (OR for a 1 unit decrease in neutrophil count on day 15: 2.4 [95% CI: 1.8–3.2], $p < 0.001$) (Fig. 3). The same effect was found in the whole population: OR for a 1 unit decrease in neutrophil count on day 15: 2.7 (95% CI: 2.3–3.3; $p < 0.001$). There was no difference for the effect of day 15 neutrophil count in predicting subsequent neutropenia between males and females.

In a multivariable model to predict worse neutropenia after day 15, with neutrophil count on day 15 and all baseline variables as covariates, only neutrophil count on day 15 was retained as a significant predictor.

3.6. Relapse-free survival of UICC stage III patients according to UGT1A1 genotype

Fig. 4A shows a survival advantage approaching significance for UGT1A1 7/7 genotypes in the FOLFIRI arm; whereas for the non-7/7 genotypes (Panel B), we found no difference between the two arms. An explanation might be that due to slower inactivation of CPT11, patients with the 7/7 genotype have a higher exposure to CPT11 and SN38.

4. Discussion

On the basis of the findings of four initial studies, the US Food and Drug Administration (FDA) made the recommendation that patients with a UGT1A1 7/7 genotype should receive a lower starting dose of irinotecan. Subsequent studies found rather moderate effects, and further questions were raised in the meta-analysis by Hoskins *et al.* [20], which suggested that the association between UGT1A1 7/7 and neutropenia is dose dependent. UGT1A1 7/7 had no effect in patients treated with a low dose of irinotecan (<150 mg/m^2).

We used the PETACC3 trial data [17] to study clinical and pharmacogenetic predictors of toxicity of the FOLFIRI regimen. While this is a large and uniformly treated cohort, some inherent limitations are the retrospective nature of the analysis, potential selection bias, because of only 40% of patients tissue was available for evaluation of biomarkers such as UGT1A1, and potential predictors were not addressed (e.g. CES2).

Our study patients were treated with irinotecan 180 mg/m^2, which resulted in an unadjusted OR of 2.3 (95% CI: 1.4–3.7) for grade III–IV neutropenia in UGT1A1 7/7 patients treated with FOLFIRI. This

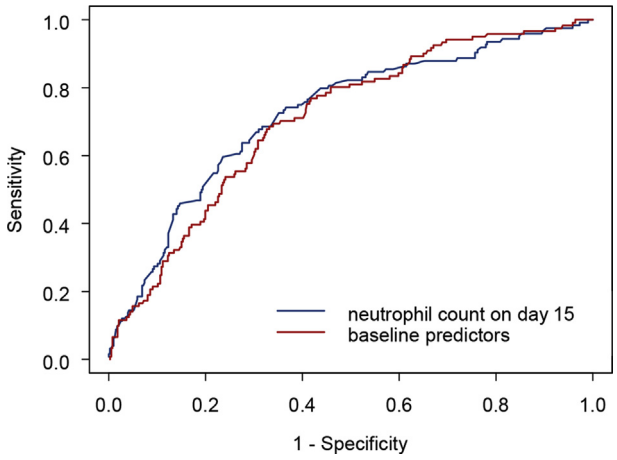

Fig. 3. Receiver operating characteristics curves of neutrophil count at day 15 (blue line) or a multivariable model combining several baseline variables in predicting the occurrence of grade III/IV neutropenia after day 15. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

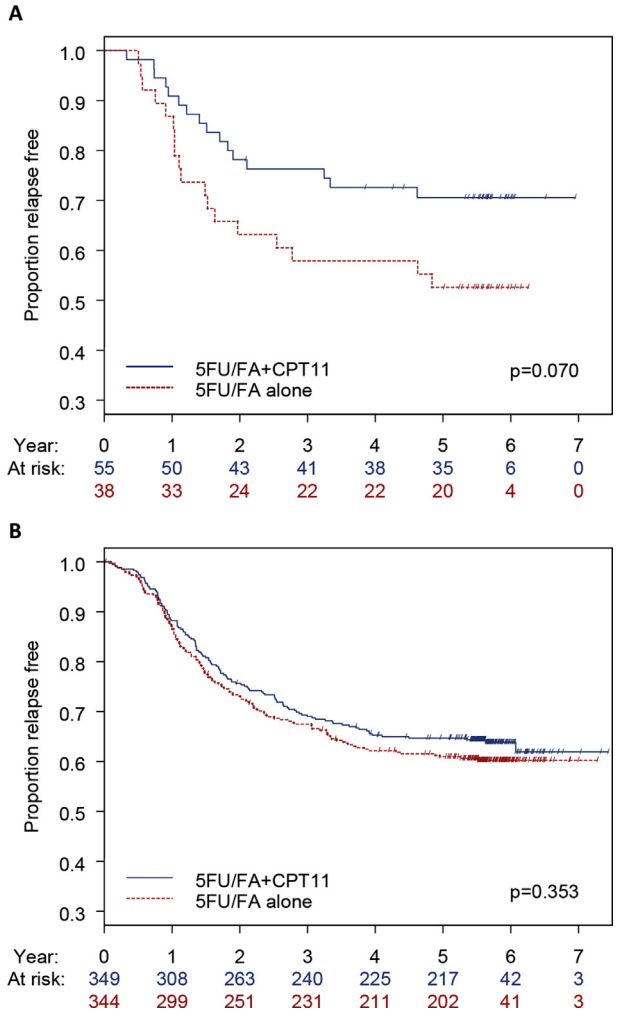

Fig. 4. Relapse-free survival of UICC stage III patients stratified per UGT1A1 genotype when treated with FOLFIRI compared to LV5FU (A: 7/7 genotype; B: non-7/7 genotypes). FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; CPT11, irinotecan.

confirms an increased risk at what is considered a moderate dose level, but much lower than that was reported when the FDA recommendation was issued. We confirm that UGT1A1 status predicts neutropenia during FOLFIRI treatment but not the occurrence of diarrhoea. Multivariable assessment revealed female sex and BNC to be stronger and independent predictors of neutropenia at this dose level, underlining the need to combine them to get optimal prediction (e.g. a non-UGT1A1 7/7 woman runs a higher risk than a UGT1A1 7/7 male).

Women appear to be more at risk of toxicity than men, but while we excluded BSA and bilirubin levels as potential explanatory variables in our study, the responsible mechanism is unclear [21]. The higher toxicity we observed in women contrasts with data from Miya *et al.* [22], suggesting that both maximum plasma concentration and area under the plasma concentration versus time curve (AUC) of irinotecan and SN38 are lower in women. Also, a recent study in mice showed decreased toxicity in UGT1A1 7/7 bearing females [23]. However, female sex was recently reported as a predictor of severe haematological toxicity of FOLFIRI [8]. We found OR for neutropenia significantly higher for women treated with 5FU only, but this became highly significant when they were treated with FOLFIRI in univariable analysis (OR 2.2 [95% CI: 1.8–2.8] and OR 1.8 [95% CI: 1.1–3.0]), suggesting some interaction between irinotecan and sex. The recently observed influence of CYP3A4 genotypes on irinotecan toxicity [24,25] may have been confounded by a sex effect because CYP3 gene expression is strongly sex dependent [26,27]. In the FOLFIRI arm, increased toxicity in women translated into significantly lower dose intensities compared to males, both for 5FU (88% versus 92%; $p < 0.0001$) and irinotecan (87% versus 94%; $p < 0.0001$). We furthermore found strong predictive value of BNC for subsequent neutropenia in both arms of the study. Blood cell counts may indicate disease extent, comorbid conditions or impact of previous chemotherapy, but these factors were controlled for in our study population [17]. Pretreatment white blood cell counts were shown to be predictive of febrile neutropenia in breast cancer patients [28].

Neutrophil counts were also significant when we used the first-cycle nadir in leucocyte counts to predict subsequent neutropenia, as has been done for other drugs [29–33]. We found neutrophil count at day 15 (i.e. before the second dose) to be the only significant predictor of subsequent occurrence of neutropenia. This suggests that once treatment is initiated, neutrophil counts can be used efficiently as a monitoring tool in a so-called 'adaptive control' fashion. For the 96 patients who developed neutropenia grade III or IV at first administration, this approach could not have prevented this toxicity.

The predictive value of UGT1A1 7/7 for severe diarrhoea is not clear because some studies do and others do not show an association with the homozygous genotype. In a recent meta-analysis, diarrhoea risk was not associated with irinotecan dose for patients with the TA7/7 genotype [34], and our data showed only a weak association. Factors predicting diarrhoea in our study included age, as reported by others [35,36]. PETACC3 allowed us to study the role of UGT1A1 status in predicting toxicity also in LV/5FU treated patients, as our borderline observations in the non-irinotecan arm are in line with those of Goldberg [37] for patients treated with FOLFOX (5-FU, leucovorin, oxaliplatin), which questions the specificity of this marker. More studies leading to better pharmacogenomic markers and clinical and laboratory predictors are needed.

In summary, we found main predictors for grade IV neutropenia as sex, age, performance score and to a lesser extent UGT1A1 status. In future prospective studies of predictors of FOLFIRI toxicity, all these variables should be integrated in multivariable models.

Conflict of interest statement

Sabine Tejpar
Honoraria: Merck Serono, Sanofi Regeneron.
Consulting or Advisory Role: Merck Serono, Sanofi Regeneron.
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Dirk Klingbiel
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Addendum

Addendum 1

Ten ng of genomic DNA from whole blood was used for genotyping of the UGT1A1*28 promoter by polymerase chain reaction (PCR) amplification followed by fragment size analysis. In brief, PCR primers (Forward 5′FAM-GTCACGTGACACAGTCAAAC−3′ and Reverse 5′-GTTTCTTTTGCTCCTGCCAGAGGTT−3′) were used to amplify the region surrounding the TA repeat under the following conditions: initial denaturation at 95°C for 5 min followed by 35 cycles of (95°C for 30 s; 60°C for 30 s; 72°C for 30 s) with a final extension at 72°C for 10 min. Amplification product (1 μL) was subjected to capillary electrophoresis using an ABI 3730XL genetic analyzer (ABI). All data were analysed using ABI GeneMapper 4.0 software (ABI). Genotypes were denoted as 6/6, 6/7, 7/7, 5/6 or 5/7, depending on the number of TA repeats found in each allele. Genotyping of the UGT1A1 SNP 3156 was performed by TaqMan probes (Applied Biosystems).

Addendum 2

A: Univariable results for neutropenia 4.

Predictor	FOLFIRI		LV5FU	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Age, 10 year increase	1.31 (1.07−1.61)	0.010	1.21 (0.81−1.81)	0.345
Sex, F versus M	2.32 (1.55−3.47)	<0.001	1.47 (0.67−3.20)	0.330
BSA, 0.1-unit increase	0.90 (0.82−1.00)	0.041	0.78 (0.64−0.97)	0.022
Subpopulation F	1.09 (0.93−1.27)	0.294	0.65 (0.44−0.96)	0.031
Subpopulation M	0.94 (0.78−1.14)	0.539	0.86 (0.60−1.23)	0.405
PSWHO, 1 versus 0	1.96 (1.26−3.05)	0.003	0.81 (0.28−2.38)	0.999
UGT1A1, 7/7 versus other	2.07 (1.03−4.15)	0.037	0.50 (0.07−3.89)	0.999
Subpopulation other, 6/7 versus 6/6	1.62 (0.82−3.21)	0.165	1.99 (0.66−6.02)	0.216
Bilirubin, $> 0.5 \times$ LUN versus $\leq 0.5 \times$ LUN	1.35 (0.90−2.01)	0.146	0.46 (0.17−1.23)	0.112
Baseline neutrophils, 1-unit decrease	1.20 (1.03−1.40)	0.021	1.02 (0.77−1.34)	0.896

BSA, body surface area; PSWHO, WHO performance status; FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; CI, confidence interval.
UGT1A1: biomarker population; other predictors: full population.

B: Multivariable results for neutropenia 4.

Predictor	FOLFIRI			LV5FU		
	Odds ratio (95% CI)	p-value	R^2*	Odds ratio (95% CI)	p-value	R^2*
Age, 10 year increase	1.72 (1.19−2.49)	0.004	75%	0.96 (0.59−1.56)	0.863	100%
Sex, F versus M	3.19 (1.50−6.79)	0.003	63%	0.35 (0.06−1.98)	0.236	26%
BSA in F, 0.1-unit increase	1.07 (0.84−1.37)	0.584		0.58 (0.33−1.04)	0.067	
BSA in M, 0.1-unit increase	0.87 (0.62−1.20)	0.387		0.72 (0.45−1.17)	0.185	
PSWHO, 1 versus 0	2.01 (1.05−3.88)	0.036	89%	0.58 (0.13−2.66)	0.485	93%
UGT1A1, 7/7 versus other	2.33 (1.03−5.24)	0.041	90%	0.65 (0.08−5.35)	0.685	98%
Bilirubin, $> 0.5 \times$ LUN versus $\leq 0.5 \times$ LUN	1.20 (0.60−2.40)	0.609	99%	0.56 (0.15−2.12)	0.393	90%
Baseline neutrophils, 1-unit decrease	1.11 (0.88−1.40)	0.375	98%	0.96 (0.66−1.39)	0.828	99%

BSA, body surface area; PSWHO, WHO performance status; FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; CI, confidence interval.
*R^2 if predictor dropped from the full model compared to full model R^2 (=100%).

Addendum 3

A: Univariable results for diarrhoea grade III/IV.

Predictor	FOLFIRI		LV5FU	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Age, 10 year increase	1.18 (1.01–1.38)	0.043	1.51 (1.18–1.94)	0.001
Sex, F versus M	1.38 (1.01–1.89)	0.045	1.18 (0.76–1.84)	0.461
BSA, 0.1-unit increase	1.00 (0.93–1.08)	0.978	0.96 (0.86–1.08)	0.498
Subpopulation F	0.98 (0.85–1.13)	0.790	1.02 (0.83–1.25)	0.868
Subpopulation M	1.16 (1.02–1.33)	0.024	0.94 (0.78–1.14)	0.555
PSWHO, 1 versus 0	0.97 (0.63–1.47)	0.869	2.29 (1.42–3.71)	0.001
UGT1A1, trend 6/6 versus 6/7 versus 7/7*	0.69 (0.46–1.04)	0.072	0.61 (0.33–1.10)	0.097
Bilirubin, $> 0.5 \times$ LUN versus $\leq 0.5 \times$ LUN	1.06 (0.75–1.48)	0.752	1.17 (0.74–1.86)	0.494

BSA, body surface area; PSWHO, WHO performance status; FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; CI, confidence interval.
*odds ratio per 7 allele.
UGT1A1: biomarker population; other predictors: full population.

B: Multivariable results for diarrhoea grade III/IV.

Predictor	FOLFIRI			LV5FU		
	Odds ratio (95% CI)	p-value	R^2*	Odds ratio (95% CI)	p-value	R^2*
Age, 10 year increase	1.38 (1.02–1.85)	0.036	77%	1.62 (1.06–2.48)	0.026	59%
Sex, F versus M	1.80 (0.88–3.65)	0.104	44%	1.73 (0.66–4.53)	0.263	76%
BSA in F, 0.1-unit increase	0.86 (0.68–1.10)	0.227		0.91 (0.66–1.24)	0.541	
BSA in M, 0.1-unit increase	0.93 (0.72–1.21)	0.582		1.06 (0.74–1.51)	0.757	
PSWHO, 1 versus 0	0.80 (0.39–1.63)	0.542	98%	1.58 (0.69–3.62)	0.279	92%
UGT1A1, trend 6/6 versus 6/7 versus 7/7**	0.67 (0.44–1.03)	0.068	83%	0.63 (0.34–1.16)	0.136	83%
Bilirubin, $> 0.5 \times$ LUN versus $\leq 0.5 \times$ LUN	1.17 (0.65–2.14)	0.599	99%	1.17 (0.52–2.66)	0.702	99%

BSA, body surface area; PSWHO, WHO performance status; FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; CI, confidence interval.
*R^2 if predictor dropped from the full model compared to full model R-square (=100%).
**odds ratio per 7 allele.
UGT1A1: biomarker population; other predictors: full population.

Addendum 4

A: Univariable results for dose reduction.

Predictor	FOLFIRI		LV5FU	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Age, 10 year increase	1.08 (0.97–1.21)	0.150	1.17 (1.00–1.36)	0.046
Sex, F versus M	2.00 (1.59–2.50)	<0.001	1.43 (1.06–1.94)	0.020
BSA, 0.1-unit increase	0.92 (0.87–0.98)	0.005	0.91 (0.84–0.98)	0.015
Subpopulation F	1.06 (0.96–1.17)	0.292	0.91 (0.79–1.05)	0.182
Subpopulation M	1.00 (0.91–1.11)	0.952	0.96 (0.84–1.10)	0.540
PSWHO, 1 versus 0	1.23 (0.92–1.65)	0.165	1.92 (1.35–2.72)	<0.001
UGT1A1, trend 6/6 versus 6/7 versus 7/7*	1.32 (1.02–1.71)	0.031	0.91 (0.66–1.28)	0.596
Bilirubin, $> 0.5 \times$ LUN versus $\leq 0.5 \times$ LUN	1.32 (1.04–1.67)	0.021	1.20 (0.88–1.65)	0.254
Baseline neutrophils, 1-unit decrease	1.21 (1.11–1.31)	<0.001	1.11 (0.99–1.24)	0.072

BSA, body surface area; PSWHO, WHO performance status; FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; CI, confidence interval.
*odds ratio per 7 allele.
UGT1A1: biomarker population; other predictors: full population

B: Multivariable results for dose reduction.

Predictor	FOLFIRI			LV5FU		
	Odds ratio (95% CI)	p-value	R^2*	Odds ratio (95% CI)	p-value	R^2*
Age, 10 year increase	1.18 (0.97–1.43)	0.094	94%	1.18 (0.94–1.49)	0.157	93%
Sex, F versus M	2.27 (1.43–3.62)	<0.001	75%	2.09 (1.18–3.69)	0.012	77%
BSA, 0.1-unit increase	0.92 (0.81–1.03)	0.153	96%	0.93 (0.81–1.08)	0.371	97%
PSWHO, 1 versus 0	1.32 (0.84–2.09)	0.234	97%	2.20 (1.32–3.67)	0.002	68%
UGT1A1, trend 6/6 versus 6/7 versus 7/7**	1.35 (1.01–1.79)	0.040	91%	0.97 (0.68–1.38)	0.853	100%
Bilirubin, $> 0.5 \times$ LUN versus $\leq 0.5 \times$ LUN	1.18 (0.78–1.79)	0.428	99%	0.86 (0.51–1.46)	0.576	99%
Baseline neutrophils, 1-unit decrease	1.16 (1.01–1.33)	0.038	91%	0.98 (0.83–1.16)	0.841	100%

BSA, body surface area; PSWHO, WHO performance status; FOLFIRI, 5-fluorouracyl/leucovorin/irinotecan; FU, fluorouracil; LV, leucovorin; CI, confidence interval.
*R^2 if predictor dropped from the full model compared to full model R^2 (=100%).
**odds ratio per 7 allele.
UGT1A1: biomarker population; other predictors: full population.

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