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ORIGINAL ARTICLE

Gender-specific elimination of continuous-infusional 5-fluorouracil in patients with gastrointestinal malignancies: results from a prospective population pharmacokinetic study

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Abstract
Background This study was initiated to assess the quantitative impact of patient anthropometrics and dihydropyrimidine dehydrogenase (*DPYD*) mutations on the pharmacokinetics (PK) of 5-fluorouracil (5FU) and to explore limited sampling strategies of 5FU.
Patients and methods We included 32 patients with gastrointestinal malignancies, receiving 46-h continuous-infusional 5FU and performed PK-sampling at baseline, 15, 30, 45 min, 1 and 2 h after the start of infusion and at the end of infusion, for 2 subsequent cycles. Plasma concentrations of 5FU, 5-fluorodihydrouracil (5FUH2), uracil (U) and 5,6-dihydrouracil (UH2) were determined using LC–MS/MS and submitted to population PK analysis using nonlinear mixed-effects modeling. Broad genotyping of *DPYD* was performed, and the potential impact of the DPYD genotype on the elimination of 5FU was assessed. Limited sampling strategies were evaluated for their accuracy to predict steady-state concentrations of 5FU (CSS_{5FU}), using data simulations based on the final PK-model.
Results The area-under-the concentration–time curve of 5FU (AUC_{5FU}) was found to be <20 mg h/L in 33 occasions (58 %), between 20 and 30 mg h/L in 17 occasions (30 %) and >30 mg h/L in 7 occasions (12 %). Men had a 26 % higher elimination of 5FU and a 18 % higher apparent elimination of 5FUH2. Accordingly, women had a higher AUC_{5FU} compared to men (22 vs. 18 mg h/L, $p = 0.04$). No *DPYD* risk variants were found, and the DPYD variants detected (c.496A>G, c.1601G>A, c.1627A>G) were not significantly associated with the elimination of 5FU. Individual baseline UH_2/U ratio was significantly associated with AUC_{5FU} ($R = -0.49$, $p < 0.001$). Limited sampling strategies with time-points <3 h after the start of infusion were not adequate to predict CSS_{5FU}. Female gender was the only predictor of nausea/emesis in the multivariate model.
Conclusions Gender-specific elimination of 5FU is supported by the present data and may partly explain the gender-specific association between DPYD risk variants and 5FU-specific toxicity.

Keywords 5-fluorouracil · Dihydropyrimidine dehydrogenase · Polymorphism · Drug monitoring · Pharmacokinetics

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Introduction

Continuous intravenous 5-fluorouracil (5FU) is the backbone of the treatment of gastrointestinal malignancies.

Regardless of the regimen in which it is used (e.g. 5FU/leucovorin (LV)/oxaliplatin (FOLFOX), 5FU/LV/irinotecan (FOLFIRI) with or without bevacizumab or cetuximab), 5FU is dosed based on body surface area (BSA). However, BSA does not account for covariates with a substantial impact on 5FU clearance, including drug pathway-associated gene polymorphisms [1, 35], age [26], gender [26], drug–drug interactions, organ function or comorbidities. As a result, the substantial interindividual variability in 5FU clearance results in large differences in patient exposure to 5FU as calculated by the area-under-the concentration–time curve (AUC) (mg h/L) in constant infusional regimens [17]. Only 1–3 % of the administered drug is activated to cytotoxic metabolites such as fluorodeoxy-uridine monophosphate (FdUMP) [25], and more than 80 % of 5FU undergoes rapid degradation to 5-fluorodihydrouracil (5FUH2) [11] by the dihydropyrimidine dehydrogenase enzyme (DPD). This gives the catabolic pathway a particularly important role in determining a patient's response to 5FU, since a reduced activity of DPD results in a substantially increased half-life of the drug and an increased risk of severe toxicity [14]. The activity of DPD is highly variable in the population, with an estimated proportion of 3–5 % of individuals showing low or deficient DPD activity [12, 23]. There are mainly four large prospective screening studies assessing the predictive value of *DPYD* mutations on fluoropyrimidine-related toxicity [1, 10, 28, 31]. Morel et al. [28] detected deleterious *DPYD* mutations (c.1905+1G>A, c.1679T>G and c.2846A>T) in 31 % of patients with severe 5FU-related toxicity, whereas the fraction of toxicities explained by dysfunctional *DPYD* variants in the study by Schwab et al. (c.1905+1G>A and c.2846A>T) was only 8 % [31]. The Swiss FloxTox study used a comprehensive mutation screening of *DPYD* encompassing all exons and larger flanking intronic regions [1]. The two haplotypes B3 (comprising 4 variants: c.483+18G>A; c.680+139G>A; c.959-51T>G; c.1236G>A) and B6 (containing the intronic variant: c.234-123G>C) were found to be strongly associated with severe fluoropyrimidine-related toxicity [1]. Haplotype B3 was later found by van Kuilenburg et al. [36] to be linked with a deep intronic splice site mutation c.1129-5923C>G that leads to aberrant splicing and impaired enzymatic function. A recent Dutch study assessed the relationship between *DPYD* mutations and clinical outcome in 568 colorectal cancer patients receiving capecitabine treatment [10]. The known *DPYD* c.1905+1G>A and 2846A>T variants were found to be significant predictors of severe toxicity following the administration of capecitabine [10].

An alternative or complementary approach to improve the safety and efficacy of 5FU treatment is therapeutic drug monitoring (TDM) followed by personalized dosing to achieve a prespecified drug exposure, calculated by the area-under-the concentration–time curve (AUC). Clinical studies have suggested a proportion of up to 70 % of patients to be underdosed with BSA-based dosing of 5FU [17]. By applying AUC-targeted dosing of 5FU in a randomized fashion in 208 patients with colorectal cancer, Gamelin et al. [17] showed improved clinical outcome and a substantial reduction of 5FU-related diarrhea (18 vs. 4 %). Despite the promising data for TDM of 5FU, there are some important practical aspects for pharmacokinetic (PK) blood sampling that have to be considered [5], and limited sampling procedures are not yet established.

This study was initiated to assess the quantitative impact of patient anthropometrics and *DPYD* mutations on the PK of 5FU and to build a population pharmacokinetic (PopPK) model of 5FU to estimate the adequacy of limited sampling strategies for the implementation of TDM in the clinical routine.

Methods

Patient population and study treatment

This prospective study was carried out at the Cantonal Hospital St. Gallen (Switzerland). Main eligibility criteria included patients with cytologically or histologically confirmed colorectal, pancreatic or cholangiocellular cancer, receiving 46-h continuous-infusional 5FU (deGramont) alone or as part of combination chemotherapy. Patients had adequate kidney function (creatinine clearance $\geq$50 ml/min according to the Cockroft-Gault formula), liver function (total bilirubin <40 μmol/L, AST/ALT < 2xULN) and an ECOG performance status of $\leq$2. All patients provided written informed consent. The study was performed according to the declaration of Helsinki (5th amendment) and was approved by the local Ethics Committee (*EKSG 08/088*). Pretreatment evaluation included a medical and physical examination, baseline radiological tumor measurement and blood sampling. Treatment-related toxicity was graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0. Treatment-related toxicity was considered clinically relevant if it was CTC grade $\geq$3, plus 5FU-specific toxicity CTC grade $\geq$2, including diarrhea, mucositis, dehydration and hand-foot-syndrome. All patients received a continuous 46-h infusion of 5FU via an ambulatory infusion pump (AutoMed®, ACE Medical, Seoul, Korea). Treatment was repeated every 2 weeks, and the number of treatment cycles was at the discretion of the treating investigator. The starting dose remained fixed for all cycles, unless toxicity necessitated any dose reduction. Basic hematology assessments were performed at weekly intervals.

Pharmacokinetic blood sampling and bioanalysis

Blood sampling for 5FU, 5FUH2, uracil (U) and dihydrouracil (UH2) plasma concentrations was performed on day 1 and 3 of two of the first 4 treatment cycles via a separate venipuncture. The samples for 5FU were collected in heparin tubes prior to the start of 5FU, at 15, 30, 45 min, 1 and 2 h after the start, and at the end of infusion. Patients underwent PK-sampling for 2 subsequent administrations of continuous intravenous 5FU, allowing to calculate interoccasion variability of the pharmacology of 5FU. After sampling, heparin blood was immediately put on ice, centrifuged for 5 min at 3,000*g*, and plasma was stored at −20 °C until analysis. Plasma concentrations of U, UH2, 5FU and 5FUH2 were determined using liquid chromatography–tandem mass spectrometry (LC–MS/MS) as described previously [6]. Subset of samples were analyzed applying a similar method to another LC–MS/MS system consisting of a 5500 QTRAP® linear ion trap quadrupole mass spectrometer (AB SCIEX, Darmstadt, Germany) connected to an UltiMate 3000 RSLC system (Dionex, Olten, Switzerland). Further MS/MS parameters can be requested from the authors. The lower limits of quantification (LLOQ) concentrations for U, UH2, 5FU and 5FUH2 were 0.01, 0.1, 0.1 and 0.75 µmol/L, respectively [6]. Variation for both accuracy (%bias) and precision (%CV) were within the accepted range of ±20 % for all analytes at the LLOQ level [6].

Genotyping of the DPYD gene

The investigator performing genetic analyses (T.K.F.) was blinded to patient characteristics and clinical outcome. A 5-ml aliquot of ethylenediaminetetraacetic acid (EDTA) blood was collected from each individual, and genomic DNA was extracted from EDTA blood samples using the EZ1 BioRobot DNA Blood 350 µl Kit (Qiagen, Hilden, Germany). Genotyping of the 23 exons and flanking intronic regions of *DPYD* was performed as described previously [1]. The obtained sequences were compared to the NCBI reference sequences NG_008807.1 and NM_000110.3 using the software Sequencher 4.10.1 (Gene Codes Corp., Ann Arbor, US). Hardy–Weinberg equilibrium was evaluated using the χ^2-test.

Population pharmacokinetic and pharmacogenetic model

Analysis and reporting of the PK-data of 5FU and 5FUH2 was prespecified according to published guidelines [34, 39]. Population PK analysis (PopPK) of the concentration–time data of 5FU and 5FUH2 was performed using the nonlinear mixed-effect modeling program (NONMEM) version VII (ICON Development Solutions, Hanover, US). NONMEM uses a maximum likelihood criterion to simultaneously estimate population values of fixed-effects parameters (e.g. clearance of 5FU) and values of the random-effects parameters (e.g. interindividual and residual unexplained variability). Log-transformed plasma concentrations of 5FU and 5FUH2 were used together with NONMEM's first-order conditional estimation method with interaction (FOCE-I). Standard errors for all parameters were calculated using NONMEM's COVARIANCE option, and individual Bayesian PK parameters were obtained with the POSTHOC option. Concentration–time data of 5FU and 5FUH2 were described by using a linear two-compartment model, producing parameter estimates for the volume of the central 5FU compartment (V_1 in L), the respective compartment for 5FUH2 (V_2 in L), clearance of 5FU (CL_{5FU}) and apparent clearance of 5FUH2 (CL_{5FUH2}). Interindividual and residual (unexplained) variability was calculated for all PK parameters; interoccasion variability was additionally calculated for CL_{5FU} and CL_{5FUH2}. Interindividual variability was estimated using a proportional error model on the log-transformed data, as exemplified for V_1: $V_{1i} = V_{1POP} * (1 + \eta_i\ V_{1POP})$. V_{1i} represents the V_1 of the ith individual, V_{1POP} is the typical population value of V_1 and $\eta_i\ V_1$ is the interindividual random effect with mean zero and variance ω^2. For the intraindividual or residual variability, a proportional error model was used, with a different ε for 5FU and 5FUH2, respectively.

In a second step, anthropometric (gender, age, BSA), biochemical (creatinine clearance, AST, ALT, albumin, total bilirubin) and the *DPYD* genotype were all tested for their quantitative impact on CL_{5FU}, CL_{5FUH2} and volume of distribution. Continuous covariates, such as patient age, were centered to their median values: $CL_{5FU} = \theta_1 * (AGE/63)^{\theta 2}$, where θ_1 represents the CL_{5FU} of a (median) patient of age 63, and θ_2 is the exponential factor for patient age to describe the correlation with CL_{5FU}. Binary covariates were coded as follows: $CL_{5FU} = \theta_1 * \theta_2^{GEN}$, where θ_1 represents the CL_{5FU} in females (GEN = 0), and θ_2 is the change in CL_{5FU} for males (GEN = 1). For genetic polymorphisms of the *DPYD* gene, the influence on CL_{5FU} and CL_{5FUH2} was determined by estimating a separate fixed effect for the different genotypes (wild-type, heterozygous, homozygous mutant). For the sequential introduction of covariates into the model, the difference in the minimum value of the objective function was evaluated (forward inclusion), and the significance level was set at $p < 0.01$ that corresponds to a decrease of minimum value of objective function of >6.7. All significant covariates were included into the final covariate model. Throughout the analysis, model selection was based on the minimum value of objective function (OFV) as calculated by NONMEM,

the precision of parameter estimates (i.e. standard errors of parameter estimates), goodness-of-fit plots of the final covariate model and bootstrap analysis with 200 bootstrap runs of the final model. If the parameter estimates fell into the 95 % confidence interval obtained from the bootstrap analysis, the model was considered unbiased.

Limited sampling strategy of 5FU

Limited sampling strategies were evaluated for their accuracy to predict individual CSS_{5FU} at the end of drug infusion. For this purpose, data simulations based on the final covariate model were used to generate datasets of 1,000 patients with the following specifications: 5FU bolus dose 400 mg/m^2 over 1 min followed by a 46-h continuous infusion of 5FU at a dose of 2,400 mg/m^2, random BSA (normal distribution, median 1.9 m^2), age (normal distribution, median 63 years) and patient gender (males:females 66:33). In a first run, extensive blood sampling was simulated (at 20 and 45 min, 1, 1 ½, 2, 2 ½, 3, 5, 6, 24 and 46 h after the start of the 5FU bolus infusion), and the generated individual CSS_{5FU} values were used as reference (*strategy 1*). Subsequently, the adequacy of Bayesian estimation using NONMEM's POSTHOC function to predict individual CSS_{5FU} from extensive PK-sampling was evaluated when applying 4 limited sampling strategies, based on the mean prediction error (MPE) and the root-squared mean error (RSME). The 4 limited sampling strategies tested were as follows: Sampling at 20 min, 1 and 2 h (*strategy S1*); at 20 min, 1 and 3 h (*strategy S2*); 20 min and 1 h (*strategy S3*); 20 min and 2 h (*strategy S4*); and single PK-sampling at 20 min (*strategy S5*), 1 h (*strategy S6*), 2 h (*strategy S7*) and 3 h (*strategy S8*).

Statistical analysis

The area-under-the concentration–time curve of 5FU (AUC_{5FU}) was calculated from steady-state plasma concentrations of 5FU (CSS_{5FU}) and the duration of continuous 5FU drug infusion ("Time" in hours) as follows: $AUC_{5FU} = CSS_{5FU}/Time$. The distribution of AUC_{5FU} with regard to the recommended therapeutic range between 20 and 30 mg h/L was assessed and compared with historical controls [20]. Additionally, individual CSS_{5FU} was compared with the *DPYD* genotype (wild-type vs. mutant genotypes) using the Student's *T* test or the Wilcoxon rank-sum test for genotypes with more than two categories (wild-type/heterozygous-/homozygous mutant). The frequency of 5FU-related toxicity CTC grade $\geq$2 (i.e. diarrhea, mucositis, hand-foot syndrome, myelosuppression) was compared with the *DPYD* genotype using Fisher's exact test for homogeneity between wild-type and mutant genotypes, and the Wilcoxon rank-sum test for trend statistics in genotypes with more than two categories (wild-type/heterozygous-/homozygous mutant). Similarly, individual CSS_{5FU} was compared with the baseline UH_2/U ratio using pairwise correlation analysis, and the frequency of 5FU-related toxicity was compared with the individual baseline UH_2/U ratio using the Wilcoxon rank-sum test. Finally, potential predictors of 5FU-related toxicity (i.e. patient age, gender, AUC_{5FU}, ECOG performance status) were analyzed using multivariate logistic regression analysis. All tests of significance were two-sided; $p < 0.05$ was considered significant. All conventional statistical analyses were performed using STATA 11.0 software (STATA Corp, College Station, Texas, US).

Results

Patient population and study treatment

Thirty-two patients were included in the study between February 2009 and March 2011. One patient was not eligible because no chemotherapy was given following rapid deterioration of the patient's performance status. Accordingly, PK-data were available from 31 patients. EDTA blood for genotyping was available from 30 patients. Patient characteristics are outlined in Table 1. Metastatic tumor disease was found in 48 % of all patients. Twenty out of 31 patients (65 %) received FOLFOX chemotherapy, with or without the addition of bevacizumab. Fourteen patients (45 %) were 65 years of age or older. At the time of final analysis (July 2011), 22 patients experienced disease progression, 15 patients had deceased. One patient received continuous 5FU over 46 h without a bolus 5FU infusion; all other patients received a 5FU bolus followed by a 5FU infusion over 46 h (deGramont regimen).

Genotyping of the DPYD gene

No patient was found to be carrier of a risk variant c.1129-5923C>G, c.1905+1G>A, c.1679T>G, c.2846A>T or the potential risk variant c.234-123G>C. Minor allele frequencies for c.496A>G (exon 6), c.1601G>A (exon 13) and c.1627A>G (exon 13) were 26.9, 1.7 and 12.1 %, respectively (Supplementary Table). Hardy–Weinberg equilibrium was fulfilled for all variants.

Basic and covariate pharmacokinetic model

Twenty-six patients had PK-sampling from 2 subsequent cycles of 5FU; five patients had PK-data from a single PK-cycle. In eight out of 57 treatment cycles, 5FU end-of-infusion plasma concentrations >50 μmol/L clearly suggested accidental PK-sampling at the site of the 5FU

Table 1 Patient demographics and clinical characteristics

Clinical characteristic	No.	%
Sex		
Male	21	32
Female	10	68
Age (years)		
Median	62.7	
Range	31–81	
≥65	14	45
Malignant cancer		
Colorectal	26	84
Bilio-pancreatic	5	16
ECOG-PS		
0	12	39
1	17	55
2	2	6
Disease stage		
I–II	3	10
III	13	42
IV	15	48
Chemotherapy regimen		
FOLFOX[a]	20	65
FOLFIRI[a]	6	19
5FU[a]	4	13
FOLFIRINOX	1	3
Add. bevacizumab	9	29
Treatment intention		
Adjuvant	8	26
Neoadjuvant	1	3
1st-line	13	42
2nd or 3rd-line	9	29
Weight		
Median	76	
Range	58–98	
BSA		
Median	1.9	
Range	1.6–2.2	
Creatinine clearance		
Median	99	
Range	56–140[b]	
Liver metastases		
No	19	61
Yes	12	39
Objective response		
Partial response	10	32
Stable disease	3	10
Progressive disease	9	29
Not assessable[c]	9	29

ECOG eastern cooperative oncology group, *PS* performance status, *add* additional, *SA* body surface area

[a] With or without the addition of bevacizumab

[b] Calculated creatinine clearance >140 ml/min was set at 140 ml/min to account for overestimation due to low lean body mass

[c] Patients receiving adjuvant chemotherapy

drug infusion (central venous port). These samples were discarded and replaced by Bayesian estimations for the 5FU steady-state plasma concentrations from the final NONMEM PK-model (see below). Out of the 57 treatment cycles, 33 patients (58 %) were found to have an AUC_{5FU} <20 mg h/L, 17 patients (30 %) had values within the therapeutic target of 20–30 mg h/L, 7 patients (12 %) had AUC_{5FU} values >30 mg h/L (Fig. 1a).

Results of the final covariate model using NONMEM are outlined in Table 2. The final estimate for CL_{5FU} was 158 L/h or 83 L/h/m^2. The estimate for CL_{5FUH2} was moderately lower at 126 L/h or 65 L/h/m^2. Interindividual variability for CL_{5FU} was 21.9 %; residual variability for 5FU was 20.1 %. The present 5FU PK-data did not support a model implementing saturable (Michaelis–Menten) elimination or distribution from 5FU to 5FUH2. For reasons of identifiability, the fraction of 5FU metabolized to 5FUH2 was fixed to 0.85 or 85 % [14]. Covariate testing indicated a significant ($p < 0.01$) correlation between patient gender and the clearance of 5FU and 5FUH2, with men having a 26 % higher CL_{5FU} and an 18 % higher CL_{5FUH2} (Table 2). Accordingly, women were found to have a significantly higher AUC_{5FU} as compared to men (22 vs. 18 mg h/L, $p = 0.04$; Fig. 1b). Creatinine clearance was a significant covariate on CL_{5FUH2}, but not on CL_{5FU}. According to the final covariate model, CL_{5FUH2} was dependent on patient gender and creatinine clearance, according to the following equation:

$$CL_{5FUH2}[\text{L/min}] = 120 \times 1.18^{\text{SEX}}(\text{Creatinine clearance}/100)^{0.345} \quad (1)$$

With SEX being 0 for female and 1 for male patients. Tested variant genotypes of *DPYD* were not significantly associated with CL_{5FU} or CL_{5FUH2}. Importantly, BSA was not a significant covariate on CL_{5FU} according to the final PK-model. No covariate had a significant impact on 5FU and 5FUH2 volume of distribution. Goodness-of-fit plots of model-predicted and observed 5FU concentrations supported the adequacy of the model (Supplementary Figure). All PK parameter estimates fell within the 95 % CI of 200 bootstrap runs (Table 2). A majority of 156 out of the 200 bootstrap runs (78 %) minimized successfully, with 41 runs terminated due to problems with minimization and 3 runs terminated for boundary problems.

Limited sampling strategy of 5FU

Limited sampling strategies (without the inclusion of steady-state samples) were not adequate to predict CSS_{5FU} with the exception of 3 PK-samples done at 20 min, 1 and 3 h (Table 3; Fig. 2a). Single PK-sampling strategies up to 2 h after the start of 5FU infusion were poor predictors of

CSS_{5FU} (*strategies S5–S7*, Table 3). Because most patients exhibited steady-state conditions 3 h after the start of infusion, a single sample at 3 h is suggested to be adequate to predict CSS_{5FU} (*strategy S8* in Table 3).

Predictors of 5FU-related toxicity

Treatment-associated toxicity was generally mild. One patient experienced grade 3 skin infection in the second treatment cycle. Women experienced significantly more nausea of any grade (80 vs. 33 %, $p = 0.015$) and vomiting of any grade (60 vs. 24 %, $p = 0.049$) compared to men. Similarly, women experienced more diarrhea of any grade compared to men (80 vs. 48 %), but this was not statistically significant ($p = 0.088$). Female gender was associated with significantly more overall nausea (nausea of any grade) (OR = 7.7, $p = 0.04$) and overall emesis (emesis of any grade (OR = 6.0, $p = 0.05$), when adjusted for ECOG-PS, stage of disease, presence of liver metastases and AUC_{5FU}. No other covariates were significant predictors of 5FU-associated toxicity when submitted to multivariate logistic regression analysis. None of the patients was carrier of a risk variant, due to the small sample size and the low population frequency of these variants. None of the found *DPYD* mutations (c.496A>G, c.1601G>A, c.1627A>G) were significantly associated with CL_{5FU}, CL_{5FUH2} or with toxicity from chemotherapy. Similarly, baseline UH_2/U ratio was not significantly associated with 5FU-associated toxicity. However, there was a significant negative association between the baseline UH_2/U ratio and AUC_{5FU} ($R = -0.49$, $p < 0.001$; Fig. 3).

Discussion

As to our knowledge, this is the first study to quantitatively assess the gender-specific elimination of 5FU when given as a continuous infusion over 46 h in patients with gastrointestinal malignancy using PopPK. Women were found to have a moderately lower clearance of both 5FU and 5FUH2, and this was independent of anthropometric covariates such as patient weight or BSA. At the same time, broad genotyping of *DPYD* did not reveal a clinically important impact on the PK of the drug in this small group of patients. These data suggest that patient gender has to be taken into account when studying the effects of genetic variability in *DPYD* enzyme activity on the elimination of 5FU. Similarly, Etienne and colleagues analyzed *DPYD* activity using a radioenzymatic assay in 152 men and 33 women receiving continuous infusions of 5FU and found *DPYD* activity to be 15 % lower in women as compared to men [13]. However, there was only a moderate association between *DPYD* activity and 5FU clearance in the study by Etienne and colleagues ($R = 0.31$, $p = 0.002$). On the contrary, *DPYD* activity as measured in peripheral blood mononuclear cells (PBMC) using a ^{14}C-FU assay did not show any significant differences between men and women [8]. However, the latter study was smaller than the present one (18 men and 9 women).

5FU has been used for nearly 50 years, and there is a consistent association between 5FU plasma concentrations and the biological effects of 5FU treatment, both in terms of clinical efficacy and toxicity [3, 18, 21, 33, 41]. Some clinical studies suggested an improved treatment response in patients with a mean AUC_{5FU} of approximately 30 mg h/L during a 5-day continuous intravenous infusion [18, 27], but other did not find such a relationship [19, 32]. As a consequence, recent studies have evaluated the clinical benefit of TDM with subsequent target concentration intervention (TCI) to achieve a target AUC_{5FU} between 20 and 30 mg h/L [7, 17]. In the randomized study by Gamelin and colleagues, TCI of 5FU according to a predefined dosing algorithm significantly improved treatment response and reduced severe toxicity in 208 patients with metastatic colorectal cancer receiving once-weekly 5FU over 8 h [17]. In the newer study by the same group, 118

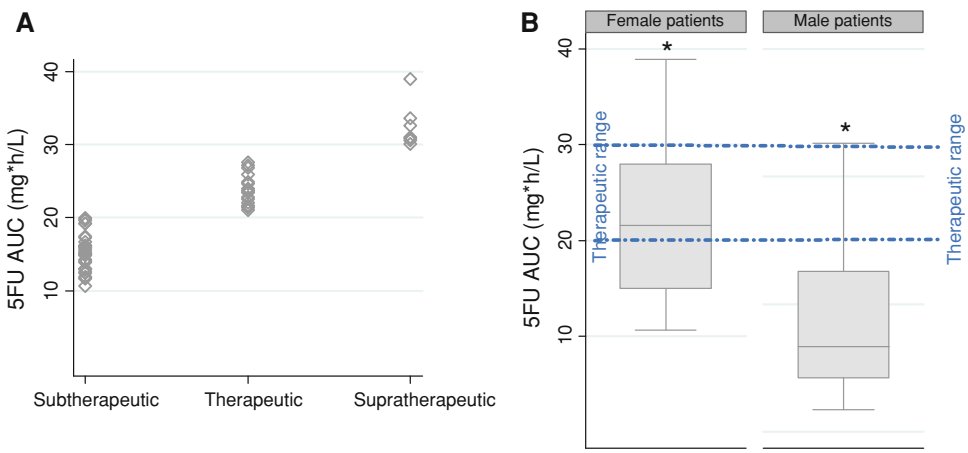

Fig. 1 Distribution of 5-fluorouracil area-under-the concentration–time curve (5FU AUC) (mg h/L) in all patients and treatment cycles (**a**) and compared between female and male patients (**b**). Women have a significantly higher AUC_{5FU} as compared to men (22 vs. 18 mg h/L, $p = 0.04$ for Student's *T* test). The recommended therapeutic range of 5FU AUC is between 20 and 30 mg h/L [20]

Table 2 5FU pharmacokinetic parameter estimates from population analysis

Parameter	Units	Final covariate model						Bootstrap on final model			Covariate effect on CL5FU			
											Male gender		Creatinine clearance	
		Estimate	RSE (%)	S (%)	IIV (%)	RSE on IIV (%)	IOV (%)	Median	2.5 P	97.5 P	Estimate (% CL)	S (%)	Estimate[a]	S (%)
CL_{5FU}	L/h	158	5.7	8.3	21.9	12.7	7.2	159	139	178	+26 % (±11.5)	12.5		
CL_{5FUH2}	L/h	126	5.4	25.6	20.1	14.8		124	103	143	+18 % (±16.4)	23.9	0.345 (±21.3)	25.4
V_{5FU}	L	54.9	7.9	21.7	18.5	4.3		54.9	49	63				
$V5_{FUH2}$	L	120	6.8	15.8	29.1	12.1		121	99	135				
RV_{5FU}	%	25.5	9.7	9.0				24.9	20.9	28.9				
RV_{5FUH2}	%	33.5	10.7	6.6			33.6	29.7	36.6					

RSE relative standard error, *IIV* interindividual variability, *IOV* interoccasion variability, *RV* residual variability, *V1* volume of distribution for 5FU, *V2* volume of distribution for 5FUH2, CL_{5FU} clearance of 5-fluorouracil, CL_{5FUH2} apparent clearance of 5-fluorodihydrouracil, *na* not available, *p* percentile, *S* shrinkage

[a] Estimate for exponential function, according to $CL_{5FUH2} = 120$ L/h × (Creatinine clearance/100) × EXP(0.345)

Table 3 Results of 8 limited sampling strategies for deGramont-like 5FU (R1 to R8) (1,000 runs each)

Sampling schedule			S1	S2	S3	S4	S5	S6	S7	S8
Sampling times	Units	Extensive	20 min, 1,2 h	20 min, 1,3 h	20 min, 1 h	20 min, 2 h	20 min	1 h	2 h	3 h
Median CSS_{5FU}	µmol/L	2.55	2.65	2.53	2.52	2.57	6.39	6.35	4.32	2.43
5 %	µmol/L	1.89	2.06	2.05	2.36	2.24	3.72	3.49	3.75	2.32
95 %	µmol/L	3.58	4.01	3.3	3.54	3.69	11.45	11.49	5.46	3.04
R^2			0.98	0.97	0.97	0.88	0.76	0.79	0.81	0.85
MPE	%		−4	0	+1	−1	−149	−149	−69	+5
RSME	%		3.9	4.3	4.2	9.1	14.4	13.3	11.6	5.1

CSS_{5FU} steady-state plasma concentration of 5FU at the end of infusion (µmol/L), *min* minutes, *hrs* hours, R^2 pairwise correlation coefficient, *MPE* mean percentage error (bias), *RSME* root-squared mean error (imprecision), extensive sampling: 20 and 45 min, 1, 1½, 2, 2½, 3, 4, 6, 24 and 46 h

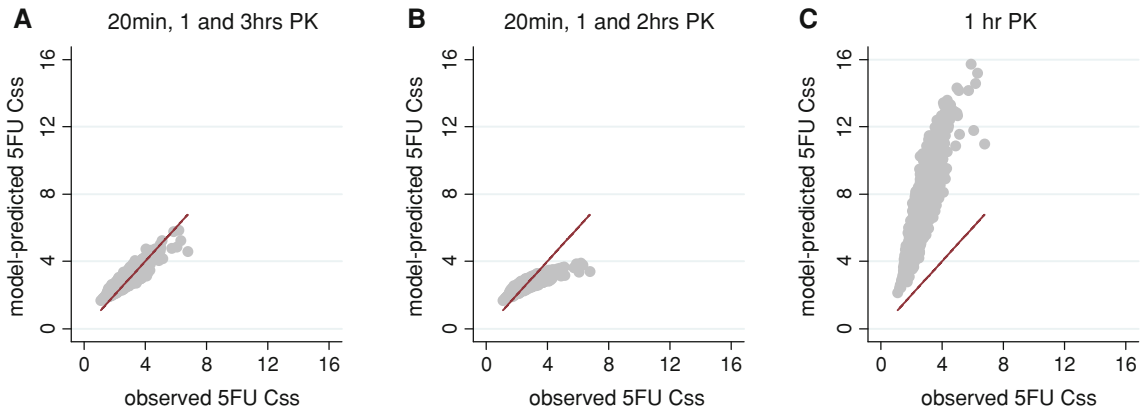

Fig. 2 Goodness-of-fit plots of observed versus model-predicted plasma concentration of 5-fluorouracil for 3 different sparse sampling strategies (**a** PK-sampling 20 min, 1 and 3 h after the start of infusion, **b** PK-sampling after 20 min, 1 and 2 h, **c** PK-sampling after 1 h). All concentrations of 5-fluorouracil are log-transformed

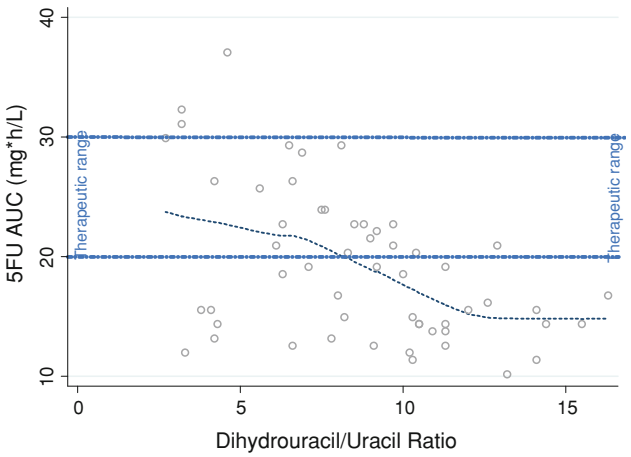

Fig. 3 5-fluorouracil area-under-the concentration–time curve (5FU AUC) (mg h/L) plotted against dihydrouracil/uracil ratio (pairwise correlation coefficient −0.49, $p < 0.001$). The recommended therapeutic range of 5FU AUC is between 20 and 30 mg h/L [20]

patients with metastatic colorectal cancer receiving FOLFOX chemotherapy were randomized to receive conventional BSA-based dosing as compared to TCI of 5FU [7]. In patients receiving 5FU TCI according to the same dosing algorithm as described in 2008 [17], overall survival was improved by a non-significant 6 months (from 22 to 28 months), and severe diarrhea was reduced from 12 to 1.7 % [7]. While it is well known that BSA-based dosing of 5FU results in extensive interindividual variability in the exposure to 5FU in colorectal cancer patients receiving FOLFOX chemotherapy [30], it is also known that TDM of 5FU causes some logistical and methodological challenges, including the optimal timing of 5FU plasma sampling, the application of 5FU dosing algorithms and the impact of different 5FU pumps (mechanical vs. electronic pumps) [5]. Nevertheless, current data suggest TDM to be more promising for the individualization of 5FU treatment as compared to dosing according to the patient's *DPYD* genotype or UH_2/U plasmatic ratio as a surrogate of plasmatic *DPYD* activity. With regard to the *DPYD* genotype, a point mutation in the splice site of intron 14 (c.1905+1G>A, previously named IVS14+1G>A or *DPYD*2A*) has been shown to result in the skipping of exon 14 and a non-functional enzyme [38, 40]. This mutation is by far the most frequently studied *DPYD* variant in the context of 5FU toxicity, and previous studies suggested that c.1905+1G>A accounts for up to 29 % of all grade ≥3 toxicities in patients receiving 5FU treatment [37]. However, subsequent studies suggested c.1905+1G>A to be responsible for only a minority of toxicity cases [9, 24]. Larger studies observed the c.1905+1G>A mutation in 14 % [28] and 5.5 % [31] of cases of 5FU toxicity, respectively, again questioning the clinical importance of the c.1905+G>A mutation in clinical practice. These rather controversial results together with the low proportion of c.1905+1G>A carriers (approximately 1.5 % in Caucasians [2]) made *DPYD* genotyping rather unattractive for implementation into clinical practice. It is only by extensive sequencing of *DPYD* (including flanking intron regions) that enabled the detection of the predictive B3 haplotype [1], and the linked deep intronic variant c.1129-5923C>G [36]. Recently, the hapB3/c.1129-5923C>G was found in roughly 5 % of Swiss patients, and the presence of at least one of 4 risk alleles (hapB3/c.1129-5923C>G, c.1679T>G, c.1905+1G>A, and c2846A>T) resulted in an absolute risk of 55 % for severe fluoropyrimidine-related toxicity [15], potentially reviving the interest in *DPYD*-guided dosing of 5FU or capecitabine.

Another point of controversy is the potential interaction between the patient's risk allele carrier status and gender. In the large prospective study by Schwab and colleagues, the effect of c.1905+1G>A strongly depended on patient gender, in that heterozygosity for c.1905+1G>A was associated with increased 5FU toxicity only in men (OR, 39.9, $p < 0.001$), but not in women (OR = 0.62, $p = 1.0$) [31]. On the contrary, the study by Loganayagam identified the c.1905+1G>A risk allele in 4 patients with severe 5FU toxicity, all of whom were women, questioning the proposed gender-specific effect of c.1905+1G>A [22]. In the large Swiss cohort of patients receiving fluoropyrimidine-based chemotherapy, 33 % of male patients with toxicity from 5FU-based chemotherapy were found to be risk allele carriers compared to 14 % of female patients (OR = 3, 95 % CI 0.5–18.2) [15]. At present, it remains unclear whether a potential gender-specific elimination of 5FU may have some impact on the interplay between *DPYD* and 5FU toxicity.

Since U accumulates in plasma of patients with low systemic *DPYD* activity, the UH_2/U ratio in plasma has been proposed as a metric for *DPYD* activity and as a predictor of 5FU toxicity [29]. Gamelin and colleagues showed that in 152 cancer patients receiving 5FU, a low UH_2/U ratio of <1.8 was associated with higher plasma concentration of 5FU and an increased risk of 5FU toxicity [16]. In fact, toxic adverse events were observed only in patients with a baseline UH_2/U ratio <1.8. Similarly, Ciccolini and colleagues reported that 71 % of 80 patients with severe 5FU toxicity showed an increased U/UH2 ratio as compared to a reference population (3.8 vs. 1.4, $p > 0.01$), suggesting that an impaired *DPYD* activity is a major contributor to 5FU toxicity [9]. In the present study, the plasma UH_2/U ratio at baseline was a significant predictor of AUC_{5FU}, but the lowest individual UH_2/U ratio was 2.7, well above the critical threshold of 1.8 as suggested by Gamelin and colleagues. This is in rather good accordance with the very mild toxicity as found in the present study.

A prerequisite for implementing 5FU TDM is the availability of steady-state plasma concentrations from the individual patient, as published dosing algorithms are based on AUC_{5FU} [7, 20]. Despite a short 5FU half-live of approximately 10 min [4], some studies suggested steady-state not being reached before 2–3 h following the start of 5FU infusion, potentially due to the saline pre-filling of the pump tubing [4, 5]. Indeed, the present data simulations suggest that 3 h after the start of the 5FU infusion is the earliest time point for single PK-sampling. To avoid the potential for taking PK-samples when steady-state is not yet reached, end-of-infusion sampling has to be preferred. In this case, 5FU plasma concentration measurement should be performed $\geq$2 h before the predicted end of the 5FU pump to account for some imprecision of (mechanical) drug pumps. At the end of continuous-infusional 5FU, plasma concentrations rapidly decrease within roughly 30 min, why it is very important to assure proper functioning of the drug pump. Most importantly, assumed steady-state 5FU concentrations $<$1 μmol/L strongly suggest an empty or malfunctioning drug pump. For such cases, additional information from the nursing staff concerning the state of the drug pump at the time of PK-sampling is very valuable. While we had no problems with empty 5FU pumps in our small cohort, some samples were accidentally taken at the central venous port, generating falsely high 5FU drug concentrations that had to be discarded. This shows the importance of good timing and repeated instruction of the nursing staff for the correct sampling and handling of the patient's blood for 5FU TDM.

The present study is limited by the small number of patients, the lack of individuals with severely limited 5FU elimination capacity, and the overall mild toxicity in the patient group. The strengths of the study include the extensive PopPK analysis and the fact that all patients were amenable to *DPYD* genotyping and bioanalysis of 5FU, 5FUH2, U and UH2.

In conclusion, gender-specific elimination of 5FU is supported by the present data and may partly explain the gender-specific association between DPYD risk variants and 5FU-specific toxicity.

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Conflict of interest None.

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