Therapeutic Drug Monitoring of 5-fluorouracil: how should we monitor?

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Abstract

Purpose To evaluate the effect of therapeutic drug monitoring (TDM) on 5-fluorouracil (5-FU) exposure and patient safety.
Methods Blood samples were collected from fifteen patients with metastatic colorectal or pancreatic cancer receiving FOLFOX, FOLFIRINOX or FOLFIRI treatment. Specific formulas and a dosing algorithm were utilized for advised dose adjustments. Optimal exposure was defined as an Area Under the Curve (AUC) of 20-30 mg*h/L or dose-limiting toxicity.
Results Therapeutic window was reached within three cycles at an AUC-value of 20.1 mg*h/L (95% CI: 11.5 – 28.8 mg*h/L). Optimal exposure was reached in 77% of patients in the second cycle. The mean absolute difference between one steady state measurement versus a calculated average of two steady state measurements was approximately -30.8 micrograms/L (95% CI: -130.6 – 69.0, p = 0.525). A weak linear correlation was shown between AUC and adverse event grade for neutropenia with a R^2-value of 0.210. Two out of three patients who had experienced grade 3 neutropenia during different chemotherapy cycles, also suffered from severe fatigue reported with grades 2 to 3.
Conclusions Dose-limiting toxicity and patient frailty should be taken into account when advising 5-FU dose adjustments using dosing algorithms based on 5-FU concentration measurements. In this way, we can ensure optimal 5-FU exposure with use of TDM for each individual patient.

Keywords 5-Fluorouracil • Pharmacokinetics • TDM • Toxicity

Introduction

5-Fluorouracil (5-FU), discovered nearly 70 years ago, remains one of the pillars in chemotherapy for the treatment of colorectal (CRC) and pancreatic cancer (PC) (1). Nowadays, it is used as part of a combination regimen such as FOLFOX, FOLFIRI and FOLFIRINOX. Although the application of 5-FU has evolved, initial dosing is still based on body surface area (BSA) (2).

Earlier studies have suggested that there is no correlation between BSA-based dosage and 5-FU exposure (3, 4). Therapeutic drug monitoring (TDM) has pointed out high intra- and inter-individual pharmacokinetic (PK) variability when using this dosing strategy. A target window of area under the curve (AUC) values between 20-30 mg*h/l appears to be associated with optimal 5-FU exposure (3, 5). This includes a high anti-tumor efficacy and a relatively low risk of developing serious adverse events. According to Beumer et al. (2019), only 25% reaches therapeutic target levels when the initial dose is based on BSA (6). These results have indicated that there are more factors that influence 5-FU exposure, such as age, gender, dihydropyrimidine dehydrogenase (DPD) activity, disease-state, renal- and hepatic function (7-9).

Various dosing algorithms have been developed in order to reach therapeutic window within three or four dose cycles (4, 10). For this study, another dosing algorithm had been developed for the Personalized Prediction and Regulation of 5-FU Exposure (PERFU). The dosing algorithm was adapted in an effort to increase the amount of patients reaching therapeutic window within two dose cycles. The PERFU study opts for an optimal 5-FU exposure, defined as an AUC between 20-30 mg*h/l or dose-limiting toxicity. The primary aim is to evaluate the effect of TDM on mean time to therapeutic range (TTR) in patients with metastatic colorectal and pancreatic cancer receiving FOLFOX, FOLFIRINOX or FOLFIRI treatment. Also, this study aims to investigate how the PERFU dosing algorithm influences TTR and optimal exposure. These aims will be supported by results from the inter- and intra-patient pharmacokinetic (PK) variability from the PERFU study population.

Another major concern in 5-FU chemotherapy is patient safety. Monitoring 5-FU is suggested to lower the incidence of serious adverse events (SAE) in comparison to regular 5-FU chemotherapy without monitoring (11, 12). However, the exact prediction of 5-FU related SAEs in terms of toxicity grades still remains vague (13). In our study, we want to determine the relation between 5-FU AUC and toxicity and elucidate further on the prediction of toxicity throughout the complete grading scale.

Furthermore, in order to optimize the sampling strategy to determine 5-FU exposure, it is necessary to choose specific blood sampling times (10, 14). Therefore, the effect of multiple measurements versus one measurement at steady state on 5-FU AUC calculations and dosage advices is also evaluated.

Materials and methods

Study design and population

The PERFU-study is a monocenter intervention study, carried out in the Amsterdam University Medical Center (Amsterdam UMC), location Vrije Universiteit (VU). Patients that received FOLFOX, FOLFIRI or FOLFIRINOX for the treatment of colorectal or pancreatic cancer were recruited for this study. To be eligible for inclusion, patients had to be over 18 years, able and willing to give written informed consent and to allow for additional blood sampling by venipuncture for 5-FU analysis. Exclusion criteria include the presence of a known substance abuse or addiction, psychotic disorder and/or other diseases that could influence the patients' safety or interfere with the study. In addition, patients were excluded if they were unable or not willing to undergo additional blood sampling for 5-FU analysis.

Treatment

Standard 5-FU chemotherapy according to the following schedule: 5-FU bolus 400 mg/m2 short-time infusion in 4 minutes and continuous infusion 2400 mg/m2 over 46 hours. Initial dosing of 5-FU is based on BSA, *DPYD*-genotyping or DPD-phenotyping. *DYPD*-genotyping is based on activity scores (AS). Patients with an AS 2.0 were recommended to receive standard 5-FU dosage; 1.5 and 1.0 to get 50% dose reduction of 5-FU; 0.5 should receive 5-FU dosing based on DPD-phenotyping and 0.0 should not receive 5-FU based chemotherapy (15). In this study, 5-FU dosing in subsequent cycles following the first cycle will be based on 5-FU AUC.

Adverse events

Reporting of adverse events was based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE version 5.0) (16). After each cycle, the toxicity grade was assessed for anemia, neutropenia, thrombocytopenia, neurotoxicity, cardiotoxicity, stomatitis, mucositis, diarrhea, hand-foot syndrome, nausea, fatigue and hepatic impairment. The grading range for toxicity started at 0 to 5, with grades 3 and 4 defined as severe toxicity and grade 5 defined as death relating to adverse events. The time between cycles was 14 days, according to protocol. Routine blood sampling was performed at day 14 to evaluate lab values for the next chemotherapy cycle. When patients did not meet criteria for starting the next cycle of chemotherapy, the cycle was postponed.

Dosing algorithm and formula

The dosing advice that is given, is based on a dosing algorithm specifically designed for this study (table 1). In order to reach therapeutic window within two cycles and preferably an AUC between 23-27 mg*h/l, the dosing algorithm has been adapted in comparison to existing algorithms. In case of dose-limiting toxicity, doses were not further increased.

5-FU AUC (mg*h/L)	Recommended change in 5-FU dose for next cycle
≥40	30% reduction
37-39	30% reduction
34-36	20% reduction
31-33	10% reduction
28-30	5% reduction
23-27	No change in dose
20-22	5% increase
17-19	10% increase
14-16	20% increase
11-13	30% increase
<11	40% increase

Table 1: PERFU-dosing algorithm

Calculations regarding 5-FU AUC have been carried out by using the formula *AUC = average Css* (concentration at steady state) *x t*. A*verage Css* represents the average concentration at steady state, which was calculated by using the mean concentration measured from two time samples at steady state. In case one blood sample was taken at steady state, the AUC was calculated from that sample. The *t* represents the total time of continuous infusion, which is 46 hours in all regimens.

SAMPLE COLLECTION

Before start of chemotherapy, blood samples were collected for DPD-phenotyping. During the first six cycles of 5-FU chemotherapy, blood samples were collected at specific time points. In the first cycle, multiple samples are taken 5 minutes after the start of short-time bolus infusion. After the start of 5-FU continuous infusion, samples were taken after 30 minutes, two hours and 45 hours. Lastly, blood is drawn 30 minutes after the end of continuous infusion.

For subsequent cycles, only two blood samples have been measured, namely at two hours and at 45 hours after start of continuous infusion. In order to prevent 5-FU breakdown and allow for later processing, the DPD-inhibitor gimeracil was added to all the blood sample tubes.

SAMPLE ANALYSIS

Blood samples for DPD-phenotyping were analyzed at the Laboratory Genetic Metabolic Diseases (LGMZ) of Amsterdam UMC, location Academisch Medisch Centrum (AMC).

The analyses of 5-FU plasma concentrations took place at the pharmacy lab of the Amsterdam UMC, location AMC. In order to determine the concentrations, a validated tandem liquid chromatography-mass spectrometry method (LC-MS/MS) was used. Furthermore, the internal standard, stable-isotope-labeled 5-FU 1,3-$^{15}N_2$-5FU was applied in the 5-FU-analysis.

DATA ANALYSIS

Statistical analyses in this study have been performed using SPSS (IBM SPSS Statistics 26). Figures were made using Graphpad Prism 9. A p-value of 0.05 was considered statistically significant. Descriptive statistics were applied for the patient characteristics. Normality was evaluated using Shapiro-Wilk and Kolmogorov-Smirnov among different cycles. A one-way Analysis of Variance (ANOVA) including post-hoc multiple comparisons were carried out to test for statistical differences between groups expressed as cycles. Paired samples t-test was carried out to assess absolute difference in steady state measurements and to assess AUCs and dosage advices. Pearson correlation coefficient and linear correlations were carried out to test for associations between variables.

Results

PATIENT CHARACTERISTICS

From February 2020 until July 2022, 25 patients have been informed about the PERFU-study and asked to participate. Of the previously mentioned patients, 16 have given informed consent and have been enrolled in the study. One patient, number 16, has been identified as a screen failure. Therefore, no data has been collected of this patient and has also been excluded from the data-analysis. Reasons for not enrolling were mainly contributed to practical considerations such as additional visits and longer stay for the study.

Patient characteristics	*Patients (n = 15)*
Age (years)	
Mean ± SD	63.5 ± 13.2
Range	29 – 82
Gender	
Female	7 (46,7%)
Male	8 (53,3%)
BSA (m²)	
Mean ± SD	1,86 m^2
Range	1.43 – 2.26
Type of cancer	
Colorectal	6 (40%)
Pancreas	9 (60%)
Type of chemotherapy	
FOLFOX	3 (20%)
FOLFIRI	3 (20%)
FOLFIRINOX	9 (60%)
DPYD-genotyping (Activity Score)	
2.0	13 (86,7%)
1.5	1 (6,7%)
1.0	0
0.5	0
0	0
Missing	1 (6,7%)
DPD-phenotyping (nmol/mg/h)	
Mean ± SD	8.34 ± 2.66
Range	3.40 – 12.50
5.9 – 14.0	10 (66.7%)
< 5.9	3 (20%)
Missing	2 (13.3%)

Table 2: Patient characteristics of the PERFU study population

The reference for normal DPD-enzyme activity conforming to DPD-phenotype is set at 5.9 – 14.0 nmol/mg/h (17). Three patients were accounted as DPD-deficient according to DPD-phenotype status (table 1). One patient was identified as DPD-deficient based on DPYD-genotype, namely with an activity score of 1.5. Accordingly, the DPD-phenotype of this patient was found to be 3.40 nmol/mg/h.

One patient had received a liver transplantation in the past. Because DPYD-genotype and DPD-phenotype information from blood is not indicative for the transplanted liver (18), DPYD-genotype and DPD-phenotype for this patient have been excluded.

TIME TO THERAPEUTIC RANGE

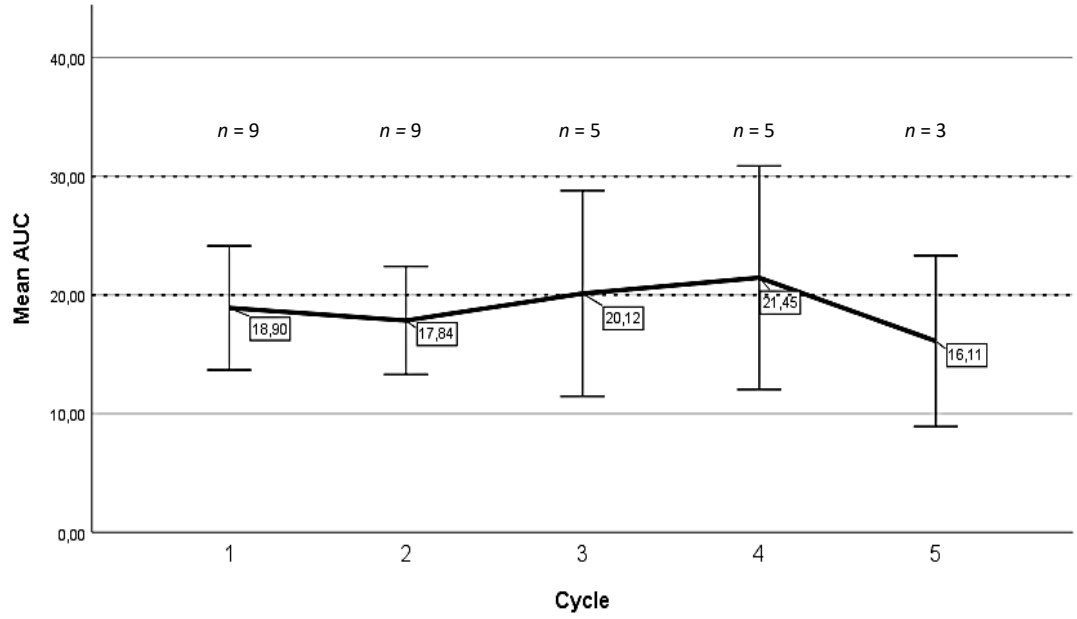

Figure 1: Mean AUC per cycle for patients receiving FOLFOX, FOLFIRI or FOLFIRINOX with a minimum of two completed cycles
*Dotted lines represent the 5-FU therapeutic window of an AUC between 20 and 30 mg*h/l*
Error bars: 95% CI; n = amount of patients

The interpolation line of mean 5-FU AUC from cycles one to five (fig. 1) indicates a mean TTR of three cycles with a mean 5-FU AUC of 20.12 mg*h/L (95% CI: 11.45 – 28.79).

A one-way ANOVA (Appendix A, table A1) revealed that the difference in AUC between cycles one to five was not statistically significant (F-value = 0.435, p = 0,782). Correspondingly, a post-hoc multiple comparisons analysis (Appendix A, table A2) indicated that there is no statistically significant difference in mean 5-FU AUC between cycle one, based on BSA-dosing, compared to the TDM-based cycles two until five: p = 0.992; p = 0.993; p = 0.907; p = 0.930, respectively. The mean 5-FU AUC in the second cycle of 17.84 mg*h/L (95% CI: 14.49 – 21.79) was found to be significantly lower than the aimed mean 5-FU AUC of 25 mg*h/L within two cycles using the PERFU-dosing algorithm (t-value = -3.6, p = 0.007).

Kolmogorov-Smirnov and Shapiro-Wilk tests of normality have yielded non-significant p-values for cycles one to four: 0.200 and 0.743; 0.059 and 0.086; 0.200 and 0.150; 0.200 and 0.191, respectively. For cycle five, only the Shapiro-Wilk test could be computed which yielded a p-value of 0.779.

For one patient, data had been collected until the 12th cycle whilst other patients had a maximum amount of collected data up until cycle 5. Information on this specific patient regarding AUC after the sixth cycle was excluded for the calculation of mean TTR for all patients receiving FOLFOX, FOLFIRI or FOLFIRINOX.

Dose-limiting toxicity occurred in approximately 40% of patients who had AUCs below target range after the first cycle (fig. 2). The 5-FU dose had not been changed for these patients regardless of the 5-FU AUC measured and the dosing advice provided. Optimal exposure had been reached in 77% of patients in the second cycle. One out of five patients, which made up 20% of the amount of patients in cycle 3, did not reach optimal exposure in the third cycle (fig. 2 and Appendix A, fig. A1). This patient was marked as DPD-deficient with a DPD-genotype of 1.5. All patients in cycle five reached optimal exposure.

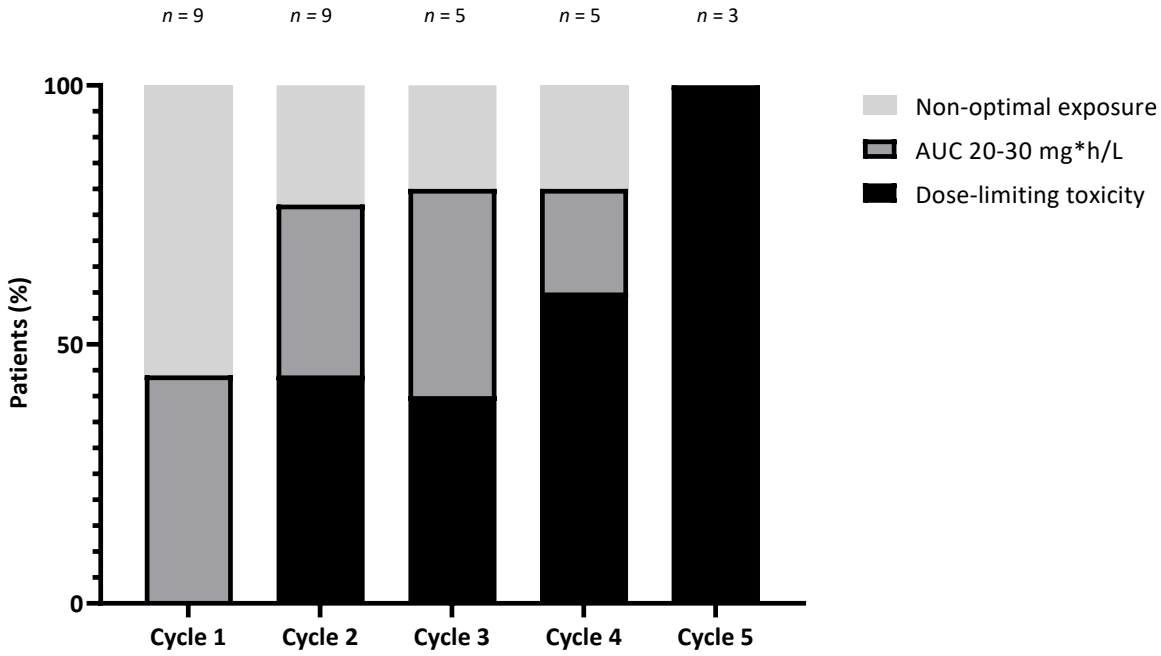

Figure 2: Optimal 5-FU exposure across patients in cycles one to five

INTRA- AND INTER-PATIENT PHARMACOKINETIC VARIABILITY

Mean inter-patient and intra-patient variability was found to be 30% and 21%, respectively (table 3A and 3B). The percentage coefficient of variance (CoV) for the intra-patient variability ranged from 1% to 38%, with diverse lengths of follow-up in cycles among patients (table 3B). Inter-patient variability in clearance showed no statistically significant results (Appendix C, table C1).

Data from patients with at least two completed cycles had been included for the calculation of pharmacokinetic variability.

Cycle	*Mean (L/h)*	*95% CI for Mean*	*CoV (%)*
1	213	179 – 248	33
2	248	203 – 294	24
3	227	127 – 327	35
4	214	137 – 292	29
5	277	68 – 486	30

Table 3A: Inter-patient pharmacokinetic variability in clearance (L/h)

Patient	*Mean (L/h)*	*Range (L/h)*	*CoV (%)*	*Follow-up (cycles)*
001	284	233 – 335	25	2
005	263	178 – 364	26	5
006	185	144 – 226	31	2
007	263	228 – 339	20	4
008	260	156 – 345	22	11
011	166	135 – 197	16	5
012	167	136 – 185	13	4
013	260	259 – 262	1	2
015	263	192 – 333	38	2

Table 3B: Intra-patient pharmacokinetic variability in clearance (L/h)

EFFECT OF LIMITED SAMPLING AT STEADY STATE

No statistically significant result was found for the mean difference between the Css-measurements of 5-FU at two hours versus 45 hours after start of continuous infusion (fig. 3). On average, the Css-measurement at 2 hours after start of the 5-FU continuous cassette was found to be lower with 31 ug/L (Appendix B, table B1).

The effect of sample collection time on the dosage advice based on single Css-measurements versus an average calculated from two Css-measurements was not found to be statistically significant (p = 0.172). The mean absolute difference in dosage advice is 3,7% (95% CI of the Difference: -1.8% – 9.1%).

The mean AUCs calculated from single Css-measurements collected at two hours after start of continuous infusion compared to the mean AUC retrieved from 5-FU concentrations

measured at both two and 45 hours also showed no statistically significant difference (95% CI: -2.95 – 1.63; $p = 0.554$).

Data that were included were all 5-FU concentrations measured at two and 46 hours after start of continuous infusion. Measured 5-FU concentrations and calculated AUCs based on single Css-measurements were excluded for the paired samples test.

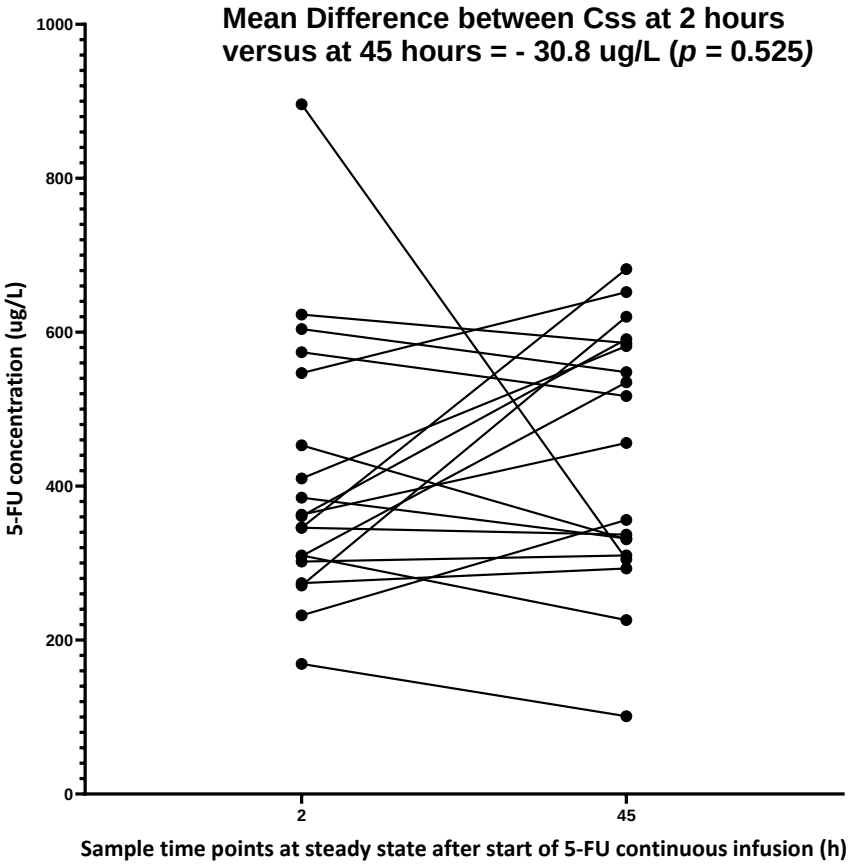

Figure 3: Difference in Css-measurements within the same cycle (19 sample pairs in 14 patients)

ADVERSE EVENTS

A weak linear correlation was shown between AUC and adverse event grade for neutropenia with an R^2-value of 0.210 (fig. 4). On average, 58% of reported cases with a 5-FU AUC of above 25 mg*h/L experienced grade 3 or 4 neutropenia. Grades 0, 1 or 2 neutropenia were observed in 80% of the registered events were observed at a 5-FU AUC of less than 25 mg*h/L. (Appendix D, table D1). Two out of three patients who had experienced grade 3 neutropenia during different chemotherapy cycles, also experienced severe fatigue reported with grades 2 to 3.

In order to test for a correlation between 5-FU AUC and toxicity grade, only adverse events that occurred in all grades were included. For this analysis, neutropenia was reported in all grades ranging from 0 to 4.

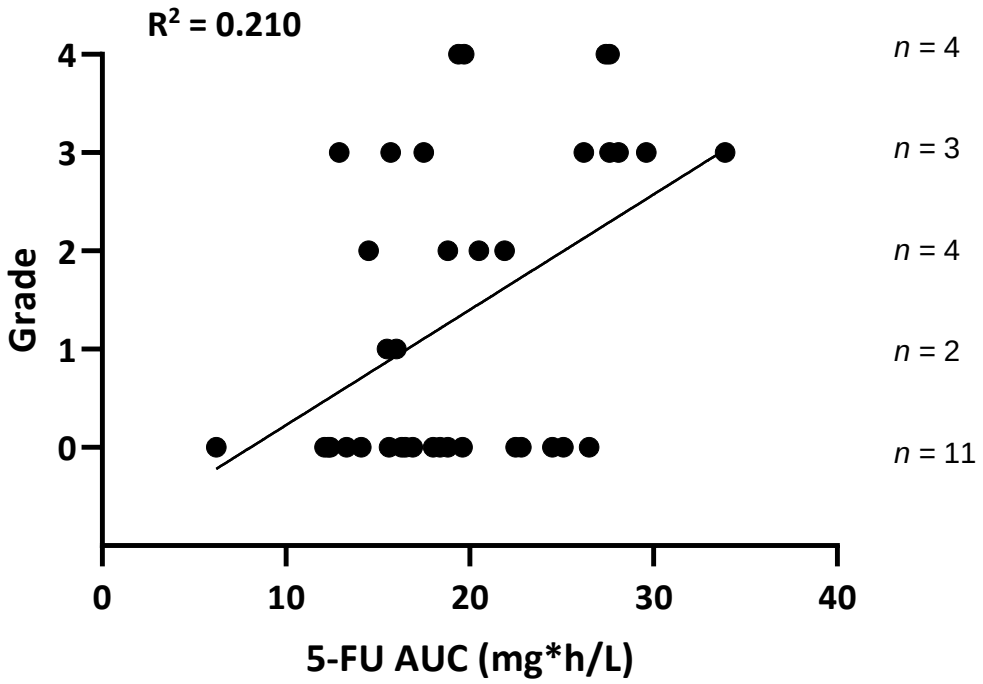

Figure 4: Correlation between 5-FU AUC and toxicity grade for neutropenia
n = amount of patients

Discussion

Therapeutic drug monitoring of 5-FU reveals that 5-FU exposure can be optimized throughout duration of therapy. Our results suggest a mean TTR of 5-FU within three cycles, though optimal exposure was reached in the majority of individual patients within two cycles. Including dose-limiting toxicity as a prerequisite for reaching optimal 5-FU exposure is essential as we aim for patient safety. In clinical practice, 5-FU dosage of both bolus and continuous infusion is never increased and is rather decreased, especially when patients develop adverse events (19). Our results indicate that the mean TTR cannot be extrapolated to the complete study population. Additionally, the time to therapeutic range does not ensure patients stay in therapeutic range, resulting in distinct individual development of 5-FU exposure.

The PERFU dosing algorithm is ineffective in terms of reaching the therapeutic target within two cycles. However, not all dose adaptation advices had been followed by oncologists. For cycle 2, only three out of nine patients received a dose increase, after which two patients reached therapeutic target. Also, four out of nine of patients started with a lower 5-FU start dosage because of previous observed toxicity with similar therapies or due to DPD-status. Of these four patients, two did not reach therapeutic target in the first cycle, which could have influenced the outcome. In addition, the amount of patients over time decreased as they were lost to follow-up. Another limitation is that only patients were included for this analysis if they had been monitored for a minimum of two cycles, which comprises nine out of fifteen included patients. Further investigation is needed to evaluate the efficacy of the PERFU dosing algorithm.

Remarkably, a lower mean 5-FU AUC was observed during the second cycle compared to the first cycle. Though not statistically significant, this finding has not been reported elsewhere in literature where 5-FU plasma concentrations were monitored using dosing algorithms (6, 20). Hypotheses regarding changes in clearance have been formulated such as auto-induction of DPD-enzymes. Yet, literature states autoregulation has only been observed as auto-inhibition (21). Additionally, changes in hepatic flow due to disease state do not seem to influence the 5-FU exposure (22). Another explanation for the lower AUCs measured in the second cycle is that plasma matrices could cause interference with the internal standard. The use of stable-isotope labeled rejects this hypothesis (23).

In our study, no statistically significant difference for the inter-patient pharmacokinetic variability in clearance in cycles one and two were found. A major limitation is the amount of patients included over time, which is the reason no statistical analysis could be carried out for intra-patient pharmacokinetic variability. Also, the amount of data differs per patient, as four out of nine patients had 5-FU monitored for only two cycles. This does not make the intra-patient variability fully comparable. Besides, a relatively weak correlation has been found between 5-FU AUC and absolute dose of 5-FU continuous infusion given (Appendix E, table E1). Furthermore, estimated population kinetics have demonstrated a 5-FU clearance of 256 L/h (24), which largely corresponds with the mean clearance in our study population of 235 L/h. The inter-patient pharmacokinetic variability in our study was found to be approximately 10% lower than previously mentioned in literature (6).

Furthermore, not only time to therapeutic range should be evaluated, but also how patients' 5-FU exposure develops over time. To wit, one DPD-deficient patient with an AS of 1.5 showed an 5-FU AUC of 6.2 mg*h/L almost reached therapeutic window after three cycles. This finding suggests the possibility of under-exposure in DPD-deficient patients and a possible valuable contribution of TDM in these patients (25). In addition, defining DPD-deficiency is of utmost importance for assessing the risk at adverse events. Nowadays, *DPYD*-genotyping for specific sequence variations is the only clinically validated predictive biomarker for 5-FU toxicity (26).

In our study, DPD-phenotyping was also carried out which revealed two borderline DPD-deficient patients with an AS of 2.0. One of these patients had an AUC above 25 mg*h/L during all cycles and even above 30 mg*h/L following dose reduction of 5-FU. DPD-phenotyping still needs prospective clinical validation in order to be used in pre-therapeutic screening (26), though it could be useful in combination with DYPD-genotyping (27). Also, it might aid in studying pharmacokinetic variability in clearance throughout cycles as individual 5-FU exposure seems to vary during therapy. In this case, DPD-phenotyping could be carried out during therapy and not only before the start of therapy. The variability of DPD-clearance can eventually be modelled in various simulations beforehand to optimize 5-FU dosing using model-informed precision dosing (MIPD).

Our results show that the intra-patient Css-measurements of 5-FU from multiple samples collected at steady state within the same cycle do not differ from each other significantly. The relative standard deviation between the Css-samples approximates 15%, which is deemed adequate. This is because the accepted variation for incurred sample reanalysis are ought to be within 20% of the original sample values (28). This slight variation can be explained by circadian rhythms of 5-FU or blood sample handling (29, 30). Previous studies suggest less stable 5-FU concentrations measured at steady state at two hours compared to 22 and 45 hours after start of continuous infusion (10). Limiting blood sample collection to one time point would decrease patient burden and would make TDM of 5-FU more feasible for implementing in clinical practice.

Although previous findings associate higher 5-FU exposure with worse toxicities (13), our results only show a weak correlation on this subject regarding neutropenia. The amount of data for each grade was not equally comparable, therefore leaving solid statements unattainable. In addition, no other SAEs could be assessed due to low incidence of side effects. It should also be noted that no delayed dose response in neutrophil count had been incorporated into this analysis. The time in lowest neutrophil count (T_{nadir}) appears to be around day 9 and day 14 for 5-FU (24, 31). Therefore, the measured neutrophil value as part of routine blood sampling before the next chemotherapy cycle on day 14 could overlook earlier neutrophil toxicity and result in underreporting or lower grading of neutropenia. The measured 5-FU AUC was linked to the toxicity caused by the administered 5-FU dose from was measured from the 5-FU dose that was administered in the previous cycle that caused the toxicity. Still, the incidence of grades 3 and 4 toxicity was more prevalent in patients with 5-FU AUCs measured above 25 mg*h/L. Furthermore, severe neutropenia is often the limiting factor in chemotherapy leading to dose reductions and delays. Consequently, this could lead to decreased patient survival (32). Tuning the aimed 5-FU AUC to AUCs associated with lower risk of neutropenia is therefore crucial for therapy optimization. Our study shows that incidence of neutropenia is markedly lower when the 5-FU AUC stays below 25 mg*h/L. As our study population received combination regimens, it cannot be ruled out that the observed toxicity for neutropenia was possibly related to oxaliplatin or irinotecan (33).

One patient developed grade 3 neutropenia, along with grade 3 diarrhea and grade 2 acute kidney injury after a 30% dose increase of 5-FU. Though the patient reached therapeutic window, namely with an AUC of 27.6 mg*h/L, the adverse events markedly affected the clinical status of the patient. This has led to cessation of systemic therapy and eventually caused grade 5 toxicity. Henceforth, validated 5-FU dosing algorithms solely relying on AUCs could disregard the clinical status of the patient and influence patient safety. Another patient with grade 3 and 4 neutropenia experienced grade 2 fatigue, which worsened during the course of chemotherapy. Frailty of patients should be considered taking into account before dose adjustments, especially dose increase, are advised. This is particularly important as frail patients tend to be underexposed (34).

Conclusion

The mean time to therapeutic range of 5-FU varies greatly among patients, which is explained by differences in 5-FU start dosages, the occurrence of dose-limiting toxicity and inter- and intra-patient variability observed when monitoring 5-FU. Following dosing algorithms based on 5-FU AUCs might therefore not yield the desirable 5-FU exposure regarding therapeutic target. Also, in order to ensure patient safety, dose-limiting toxicity and patient frailty should be taken into account. More research on how to exactly monitor 5-FU is essential in order to optimize and individualize 5-FU exposure with retaining both efficacy and safety. In this way, model-informed precision dosing for patients receiving 5-FU can be realized.

Acknowledgements

Many thanks to my supervisor Pierre Bet and interim-supervisor Daan Huntjens for the guidance I received during the study. I would also sincerely like to thank the patients that participated in the study. Also, I owe a great amount of gratitude towards the nurses Wilma, Anouschka, Sylvie, Max, Jacqueline, Jilda and many others that helped me with the sample collection. I am also grateful for the help of the research analysts Marloes van der Meer, Dennis van der Laan and Miranda Seegers for analyzing the 5-FU samples. I am also thankful to oncologists Kathelijn Versteeg, Tineke Buffart, Heinz-Josef Klümpen, Hanneke Wilmink and nurse practitioner Mariëtte Weterman for helping me with patient inclusions and toxicity assessments. Lastly, I would like to thank Imke Bartelink and my fellow master students, Habibe Yilmaz, Julia van den Nieuwendijk, Shiewanie Raddjoe, Mick van der Plas, Jemayro Hupsel, Chi Fong Loo, Yingxue Li and PhD-student Medhat Saïd with whom I worked together throughout this research period and discussed my results with during the weekly research meeting.

Appendix A

Data output for time to therapeutic range (TTR) and individual 5-FU exposure

ANOVA

AUC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	73,247	4	18,312	,435	,782
Within Groups	1093,373	26	42,053		
Total	1166,620	30			

Table A1: One-way ANOVA of mean 5-FU AUCs between cycles one to five

Multiple Comparisons

Dependent Variable: AUC

Dunnett t (2-sided)[a]

		Mean Difference			95% Confidence Interval	
(I) Cycle	(J) Cycle	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
2	1	-1,05556	3,05697	,992	-9,1027	6,9916
3	1	1,22000	3,61706	,993	-8,3015	10,7415
4	1	2,55000	3,61706	,907	-6,9715	12,0715
5	1	-2,78667	4,32321	,930	-14,1671	8,5937

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table A2: Post-hoc multiple comparisons test between the first cycle, based on BSA-dosing of 5-FU and the subsequent cycles, based on 5-FU AUC

One-Sample Test

	Test Value = 25					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
AUC	-3,619	8	,007	-7,15556	-11,7149	-2,5962

Table A3: One-sample T-test for 5-FU AUCs in cycle two with a test value of 25 mg*h/L

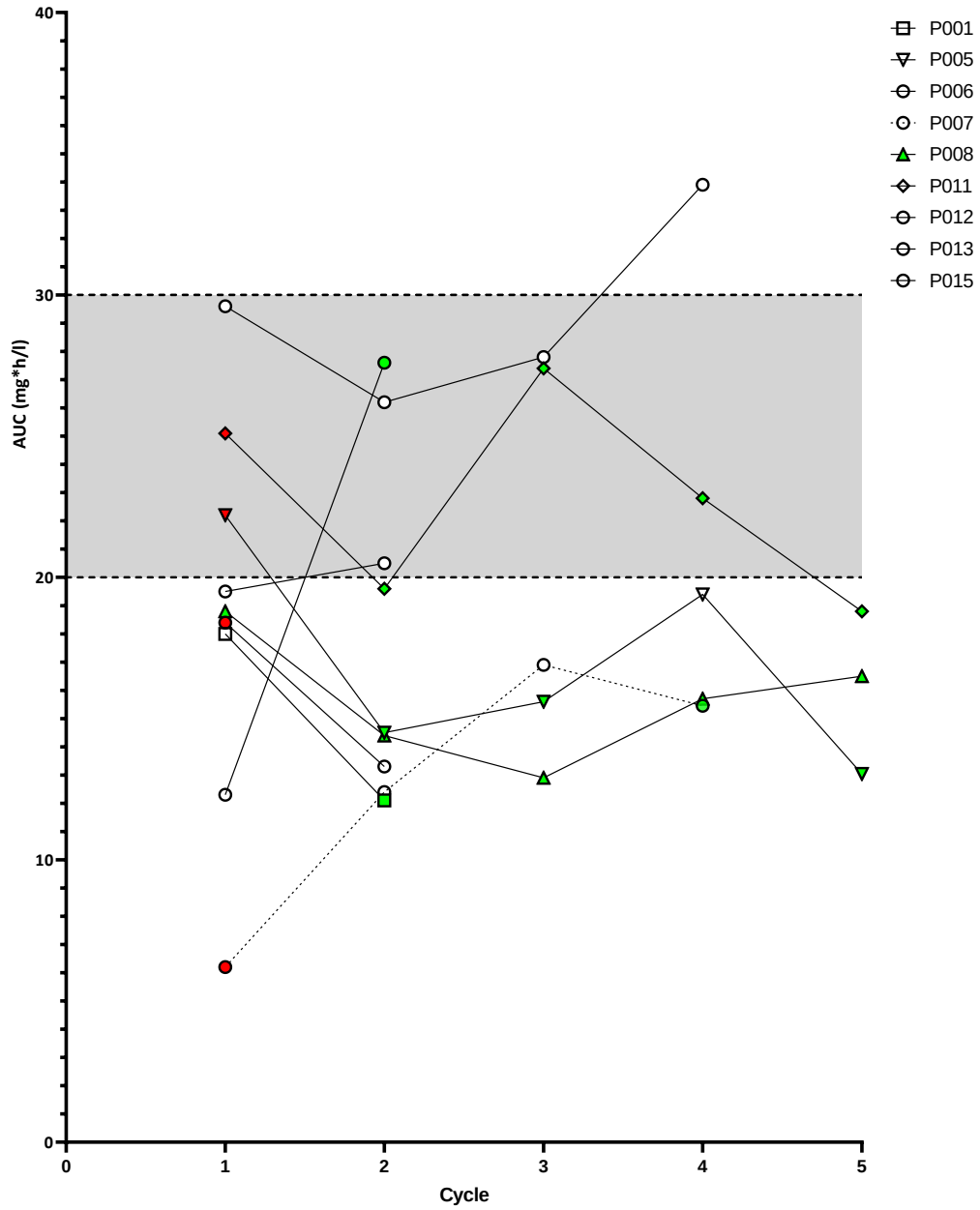

Figure A1: Individual 5-FU exposure of 9 patients in cycles one to five
Red figures: indicate a lower start dosage
Green figures: indicate dose-limiting toxicity as a result of toxicity that occurred in the previous cycle

Appendix B

Data output of the paired samples t-test for 5-FU measurements at steady state

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	Css1* – Css2**	-30,789 ug/L	207,124	47,518	-130,620	69,041	-,648	18	,525
Pair 2	AUC calculated from Css1 – AUC calculated from av.Css***	-,65789 mg*h/L	4,75001	1,08973	-2,94733	1,63154	-,604	18	,554
Pair 3	Dosage advice based on AUC calculated from one Css-measurement – Dosage advice based on average AUC	3,684 %	11,284	2,589	-1,755	9,123	1,423	18	,172

Table B1: Results of a paired samples t-test for comparing the effect of single steady state measurements versus two steady state measurements (sample pairs = 19, *n* = 14)

**Css1: Concentration at steady state in ug/L at 2 hours after start of continuous infusion*

***Css2: Concentration at steady state in ug/L at 45 hours after start of continuous infusion*

****Av.Css: Average concentration at steady state calculated from Css1 and Css2*

Appendix C

Data output of the inter-patient pharmacokinetic variability in clearance

Multiple Comparisons

Dependent Variable: Clearance

			Mean Difference			95% Confidence Interval	
	(I) Cycle	(J) Cycle	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	1	2	-21,11675	32,97134	,967	-117,6717	75,4382
		3	,15791	39,01222	1,000	-114,0875	114,4033
		4	12,81000	39,01222	,997	-101,4354	127,0554
		5	-49,67040	46,62852	,822	-186,2197	86,8789
	2	1	21,11675	32,97134	,967	-75,4382	117,6717
		3	21,27466	39,01222	,982	-92,9707	135,5200
		4	33,92676	39,01222	,905	-80,3186	148,1721
		5	-28,55365	46,62852	,972	-165,1030	107,9957
	3	1	-,15791	39,01222	1,000	-114,4033	114,0875
		2	-21,27466	39,01222	,982	-135,5200	92,9707
		4	12,65210	44,23570	,998	-116,8900	142,1942
		5	-49,82831	51,07898	,864	-199,4106	99,7540
	4	1	-12,81000	39,01222	,997	-127,0554	101,4354
		2	-33,92676	39,01222	,905	-148,1721	80,3186
		3	-12,65210	44,23570	,998	-142,1942	116,8900
		5	-62,48040	51,07898	,738	-212,0627	87,1019
	5	1	49,67040	46,62852	,822	-86,8789	186,2197
		2	28,55365	46,62852	,972	-107,9957	165,1030
		3	49,82831	51,07898	,864	-99,7540	199,4106
		4	62,48040	51,07898	,738	-87,1019	212,0627
Bonferroni	1	2	-21,11675	32,97134	1,000	-122,2369	80,0034
		3	,15791	39,01222	1,000	-119,4890	119,8048
		4	12,81000	39,01222	1,000	-106,8369	132,4569
		5	-49,67040	46,62852	1,000	-192,6758	93,3350
	2	1	21,11675	32,97134	1,000	-80,0034	122,2369
		3	21,27466	39,01222	1,000	-98,3723	140,9216
		4	33,92676	39,01222	1,000	-85,7202	153,5737
		5	-28,55365	46,62852	1,000	-171,5591	114,4518
	3	1	-,15791	39,01222	1,000	-119,8048	119,4890
		2	-21,27466	39,01222	1,000	-140,9216	98,3723
		4	12,65210	44,23570	1,000	-123,0148	148,3190
		5	-49,82831	51,07898	1,000	-206,4829	106,8263
	4	1	-12,81000	39,01222	1,000	-132,4569	106,8369

	2	-33,92676	39,01222	1,000	-153,5737	85,7202
	3	-12,65210	44,23570	1,000	-148,3190	123,0148
	5	-62,48040	51,07898	1,000	-219,1350	94,1742
5	1	49,67040	46,62852	1,000	-93,3350	192,6758
	2	28,55365	46,62852	1,000	-114,4518	171,5591
	3	49,82831	51,07898	1,000	-106,8263	206,4829
	4	62,48040	51,07898	1,000	-94,1742	219,1350

Table C1: Multi-comparisons tests, Tukey-HSD and Bonferroni, for the inter-patient clearance from cycles one to five.

Appendix D

Descriptive statistics for the occurrence of grades 0 to 4 neutropenia above and below a 5-FU AUC of 25 mg*h/L

Count

		AUC (mg*h/L)		
		<25	>25	Total
Grade	0	17	2	19
	1	2	0	2
	2	4	0	4
	3	3	5	8
	4	2	2	4
Total		28	9	37

Table D1: Occurrence of toxicity at an AUC below 25 mg*h/L versus an AUC above 25 mg*h/L

Appendix E

Correlations between 5-FU AUC and dose of 5-FU continuous infusion

Correlations

		AUC	Dose
AUC	Pearson Correlation	1	,541**
	Sig. (2-tailed)		,000
	N	43	43
Dose	Pearson Correlation	,541**	1
	Sig. (2-tailed)	,000	
	N	43	43

**. Correlation is significant at the 0.01 level (2-tailed).

Table E1: Pearson Correlation's test between 5-FU AUC and dose of continuous infusion

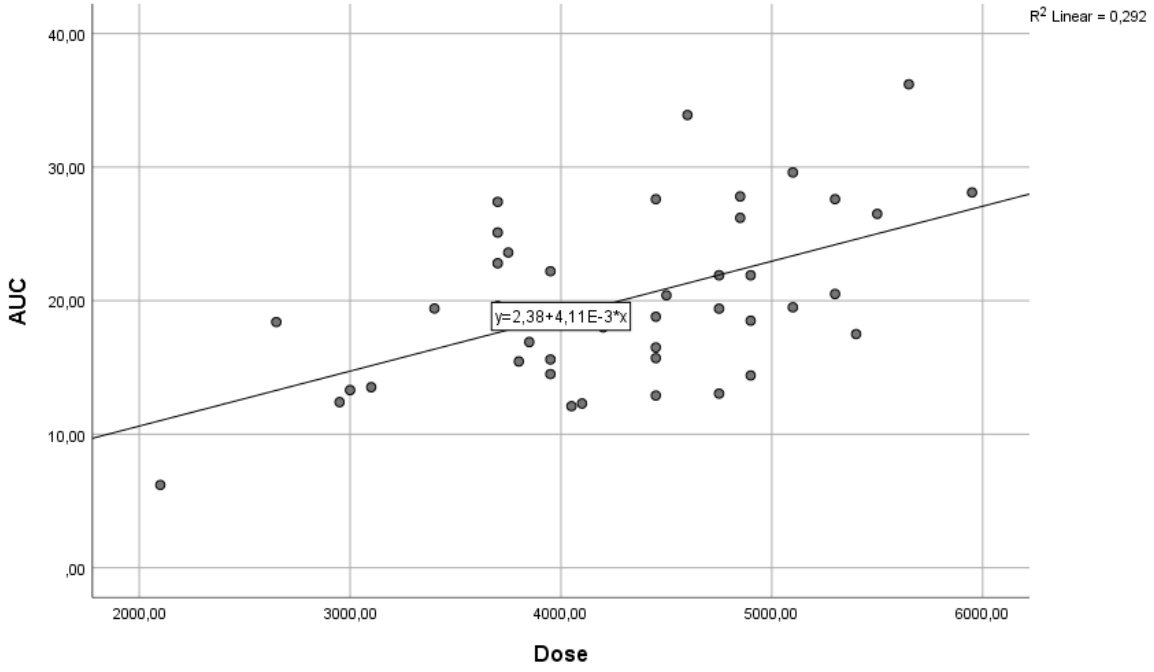

Figure E1: Linear correlation between 5-FU AUC and dose of continuous infusion

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