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# INDIVIDUALIZED PHARMACOKINETICALLY GUIDED DOSAGE ADJUSTMENT OF FLUOROURACIL USING POPULATION PHARMACOKINETIC MODELLING

Diploma thesis

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Bonn, 2014 David Čechlovský

UNIVERZITA KARLOVA V PRAZE
FARMACEUTICKÁ FAKULTA V HRADCI KRÁLOVÉ
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# INDIVIDUALIZOVANÁ FARMAKOKINETICKY VEDENÁ ÚPRAVA DÁVKOVÁNÍ FLUOROURACILU S POUŽITÍM POPULAČNÍHO FARMAKOKINETICKÉHO MODELU

Diplomová práce

Vedoucí diplomové práce: Prof. RNDr. Jiří Vlček, CSc.

Bonn, 2014 David Čechlovský

„Hereby I declare that this diploma thesis is a piece of individual writing and that I used only the sources and references mentioned in the thesis. To my knowledge, this work has not been conducted before and has not been submitted for the award of any degree before this.“

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Hradec Králové, 31.08.2014 David Čechlovský

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# CONTENT

## ABSTRACT

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**Title of diploma thesis:** Individualized pharmacokinetically guided dosage adjustment of fluorouracil (5-FU) using population pharmacokinetic modelling.

**Objectives:** To develop a method to increase the efficacy and tolerability of fluorouracil (5-FU) with pharmacokinetically-guided dose adjustment based on a target AUC.

**Methods:** Blood samples were collected from 90 patients with the diagnosis of colorectal carcinoma treated with fluorouracil (5-FU) administered at various infusion durations. Several versions of compartmental pharmacokinetic models were fitted to the plasma concentration data, using nonlinear mixed effect modelling (NONMEM). Different error models were evaluated. The potential effect of patient covariates was evaluated using a stepwise method. Model evaluation was performed by using the bootstrap method.

**Results:** The one-compartment linear model was chosen as a base model as it was successful in fitting to the data collected..The final model contained Additive Residual Error. A covariate BSA>CL and IIV on CL were significantly correlated to the pharmacokinetic parameters. The mean parameters´ estimates were: CL (L/h), 214; V (L), 30.2; ADR (mg/L), 0.112; BSA>CL, 0.993; CL/Var CL(%), 48.8 and IOV (%), 28.8. The bootstrap resampling method confirmed the stability of the final model.

**Conclusions:** The final model accurately described the pharmacokinetics of fluorouracil (5-FU) administered by infusions of various duration. User friendly tool shall be developed in order to help with dose adjustment in the clinical practice, to improve therapeutic outcomes along with decreasing occurrence of adverse events.

## ABSTRAKT

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**Název diplomové práce:** Individualizovaná farmakokineticky vedená úprava dávkování fluorouracilu (5-FU) s použitím populačního farmakokinetického modelu.

**Cíle:** Vyvinout metodu, která by s pomocí farmakokineticky vedené úpravy dávkování zaměřené na cílovou AUC, zvýšila účinnost a bezpečnost fluorouracilu (5-FU).

**Metody:** Odebráno bylo 90 vzorků krve od pacientů s diagnózou kolorektální rakoviny, léčené fluoruracilem (5-FU) podávaným v různě dlouhých infuzích. Několik verzí farmakokinetických modelů využívajících kompartmentové modely bylo přizpůsobeno pro dané koncentrace plazmy s použitím "nonlinear mixed effect modelling" (NONMEM). Byly prozkoumány různé modely vyhodnocující chyby. "Stepwise" metoda byla použita k vyhodnocení potenciálního efektu náhodných veličin individuálních pro každého pacienta. Vyhodnocení modelu bylo vykonáno pomocí "bootstrap" metody.

**Výsledky:** Jako základní model byl vybrán jedno-kompartmentový lineární model, který byl úspěšný v přizpůsobení se sesbíraným datům. Konečný model obsahoval "Additive Residual Error". Náhodné veličiny BSA>CL a IIV on CL významně korelovaly s farmakokinetickými parametry. Střední hodnoty odhadovaných parametrů byly: CL (L/h), 214; V (L), 30.2; ADR (mg/L), 0.112; BSA>CL, 0.993; CL/Var CL(%), 48.8 a IOV (%), 28.8. "Bootstrap resampling" metoda potvrdila stabilitu konečného modelu.

**Závěr:** Konečný model popisuje přesně farmakokinetiku fluorouracilu (5-FU) podávaného v různě dlouhých infuzích. Měl by být vyvinut jednoduchý, uživatelsky přátelský nástroj, který by zároveň v klinické praxi pomáhal upravit dávkování za účelem zlepšení léčebných výsledků a snížení nežádoucích příhod.

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# ABBREVIATIONS

| | |
|---|---|
| 5-FU | Fluorouracil |
| AUC | Area under the Concentration-Time Curve |
| $AUC_{t-\infty}$ | AUC from a certain time point to infinity |
| BSA | Body Surface Area |
| CL | Clearance |
| Css | Concentration Steady State |
| CVI | Continuous Intravenous Infusion |
| DPD | Dihydropyrimidine Dehydrogenase |
| EOI | End of Infusion |
| GOF | Goodness-of-fit |
| HPLC | High Performance Liquid Chromatography |
| IA | Immunoassay |
| $K_{EL}$ | Elimination rate constant |
| ME | Median Prediction Error |
| MPE | Mean Prediction Error |
| MSE | Mean Squared Prediction Error |
| MM | Michaelis-Menten |
| NCA | Non-compartmental |
| NONMEM | Non-Linear Mixed-Effects-Modelling |
| NONMEM Lim | NONMEM using data from Limited Sampling |
| OFV | Objective Function Value |
| PE | Prediction Error |
| PK | Pharmacokinetics |
| $R^2$ | Pairwise Correlation Coefficient |
| RE | Rectangle Equation |
| RMSE | Root Mean Squared Prediction Error |
| SCM | Stepwise Covariate Modelling |
| SD | Standard Deviation |
| $V_D$ | Volume of Distribution |
| ΔOFV | Difference in OFV |

# 1. Introduction

## 1.1. Individualized pharmacokinetic chemotherapy

The word chemotherapy was first used in the early 1900´s and it defines the process, when chemical compounds are used to treat diseases. Paul Ehrlich, the famous German chemist named this process, whilst treating syphilis with arsenics. (DeVita and Chu 2008; Morrison WB, 2010). From infectious diseases it slowly shifted to cancers. Firstly only hematologic cancers could be treated (antifolates, thiopurines). However, approximately 50 years later in 1957 there was a major breakthrough, with the drug fluorouracil (5-FU) which became the first drug to treat solid tumours (which had been so far treated only surgically). Retrospectively this agent represents the very first example of targeted therapy, which has now become the focus of great attention in the current cancer drug development, although the target in this case was a biochemical pathway and not a molecular target. (DeVita and Chu, 2008). Later the combination of surgery and adjuvant chemotherapy has been found very efficient.

Hutchnison (2011) wrote in Nature Reviews a very concise sentence: "Each cancer is as individual as the patient and continually evolves, and responses to therapies are equally varied." These words perfectly emphasize the importance of an individual therapy approach. Although the idea of personalized medicine has been introduced decades ago, only recently has the frequency of using this approach accelerated, mainly in oncology. Recent research shows the major shift between classic anticancer treatment and cancer targeted therapy embodies the transfer of drugs used from non-specific and toxic killing to genetically specific suppressing toward cancer. (Yan 2012) None the less the current therapies will continue to be used, until new more efficient and safer drugs are developed. Thus there is a need to re-evaluate current dosing methods. For cancer drugs one of the most common is the body surface area (BSA)-based dosing approach, which was adopted from preclinical animal studies, where the dose was extrapolated depending on the animal's BSA. The primary intention of BSA-based dosing was to decrease the total variability in pharmacokinetics (e.g. clearance, drug exposure) amongst patients (Felici et al, 2002; Kaestner and Sewell, 2007).

Joerger (2014) has stated in his article that there is no personalized anticancer treatment without individualized dosing. He claims that BSA-based chemotherapy dosing does not provide any improvement of the substantial pharmacokinetic variability

of individual drugs. Regarding the majority of anticancer drugs it is emphasized, that current dosing strategies present a risk of under- or over-dosing for each individual patient. Gamelin et al. (2008) presented an individual fluorouracil dose adjustment based on the area under the concentration-time curve, which led to increased efficacy and decreased toxicity of 5-FU administered by infusion. This may be perceived as a good example of a pharmacokinetic approach using therapeutic drug monitoring (TDM) to adjust dosing to achieve better therapy outcomes.

The ideal goal in chemotherapy is to administer the right dose, of the right drug, at the right time for the right patient. The choice of drug dose, which is aimed to be within the therapeutic levels, is based on the likely pharmacokinetics of an individual. In the case of the loading dose, the main determinant is the volume of distribution ($V_D$). On the other hand for the maintenance dose the major determinant is the clearance (CL). The problem with correct dosing arises from the large number of covariates, which are diverse for each individual, e.g. renal function, hepatic metabolic function, pharmacogenetics, sex, age, weight, pharmacokinetic drug interactions, etc. (Begg and Chin, 2011)

In several cases the BSA-based dosing might not be suitable, therefore scientists need to find new paths for dosing. Individualized pharmacokinetic chemotherapy might be a more effective strategy. As already mentioned, there are many covariates to be identified by each individual. Due to recent scientific progress humankind is able to understand some of the pharmacokinetic and pharmacodynamic processes in the human body, which helps to discover pharmacogenetic differences in drug response and tolerability. This might be used to adjust dosing, secure drug efficacy and drug safety for each individual according to their genetic variations. (Howland, 2012; Ma and Lu, 2011) Moreover, Joerger (2014) sees the future of individualized chemotherapy dosing in genotyping combined with TDM. Apart from genetics the variable sex is still being ignored in many clinical trials. Miller (2014) recommended that both sex (biological factors) and gender (psychosocial and culture factors) should always be carefully investigated when using the approach of individualized medicine with a view to achieve better outcomes.

## 1.2. Model Building

A pharmacokinetic model is a relatively simple mathematical scheme containing different equations to represent complex physiologic spaces or processes in reality (Concordet et al, 2004). The first models were used to characterize the pharmacokinetics of a specific drug (absorption, distribution, etc.). A nomenclature was developed by DiStefano and Landaw (1984) to break down pharmacokinetic models. Generally there are two types: models of data and models of systems. Models of data, also known as empirical models, are useful when there is a need to make conclusions of the data, which are generated from a physical process, which is not well understood. Models of systems, also known as mechanistic models, involve using the principles of physics and physiology. This means, that these models are based on theory and therefore it is needed to be certain about the theory. Later the pharmacodynamic models were added and that lead to more complex models. A scientific term ”pharmacometrics” began to be used. According to Williams and Ette (2010) pharmacometrics could be defined as “the science of developing and applying mathematical and statistical methods to characterize, understand and predicts a drug´s pharmacokinetic, pharmacodynamic, and biomarker-outcomes behaviour”.

Pharmacokinetics (PK) describes the dynamic drug concentration-time courses in various body fluids resulting from administration of a certain drug dose. Pharmacodynamics (PD) describes the observed effect resulting from certain drug concentration. The rationale for PK/PD modelling is to link pharmacokinetics and pharmacodynamics in order to establish and evaluate dose-concentration-response relationships and subsequently describe and predict the effect-time courses resulting from a drug dose. (Meibohm and Derendorf, 1997) Adding the word “population” means quantifying the effect of the drug on a population of patients to be able to quantify, explain and predict how the variability of the drug plasma concentration acts on the variability of the drug effect. A PK/PD population model can also be used to enable optimization (individualization) of dosage regimens. (Concordet et al, 2004) The most commonly used software for PK/PD analysis is NONMEM (the acronym for nonlinear mixed-effects modelling). Power lies in the ability to accommodate patient data as they arise in the course of routine clinical therapy, where data is typically sparse and obtained at unstructured times. Analysts usually use goodness-of-fit (GOF) plots, which offer a visual comparison between the data and fitted distributions. They provide

an overall picture of the errors in a way that a GOF statistic cannot and allow the analyst the best fitting distribution in a more qualitative and intuitive way.

In 1980s there was an article published by Box (1976), where a simple rule has been stated. Quoting: "All models are wrong, some are useful.". When trying to describe the exact process of a drug in the body, the developer of a model may choose from a variety of models but none of them are the 'right' one. It is essential to be aware, that there are not any good or bad, right or wrong models. They can only be useful or less useful. Models should be judged by three criterions (Rescigno et al. 1987). The first is retro diction, which describes the ability on conformation between the model predictions and original data. The second is prediction, which shows the ability to predict future situations. The third is understanding, which shall increase understanding of a specific system. Of course recently there have been more criteria that are requested to accept a model. For example, the principle of parsimony (Domingos 1999) became a fundamental philosophy of modelling. In brief this philosophy emphasizes the importance to choose a model, which is as simple as possible, instead of an unnecessarily complicated one.

One of the sympathetic model development processes was designed by Mesterton-Gibbons (1989). Everything is encoded in an abbreviation ABC. "A" stands for assume. "B" stands for borrow. Lastly, "C" stands for criticize. Of course this approach is very simple and it establishes the basics to work with. Therefore there are many more formal and detailed proposals, which could be used. (Box and Hill 1967, Chatfield 1988, Bonate 2010). Another good example could be the hints for successful model development presented by Cross and Moscardiny (1985).

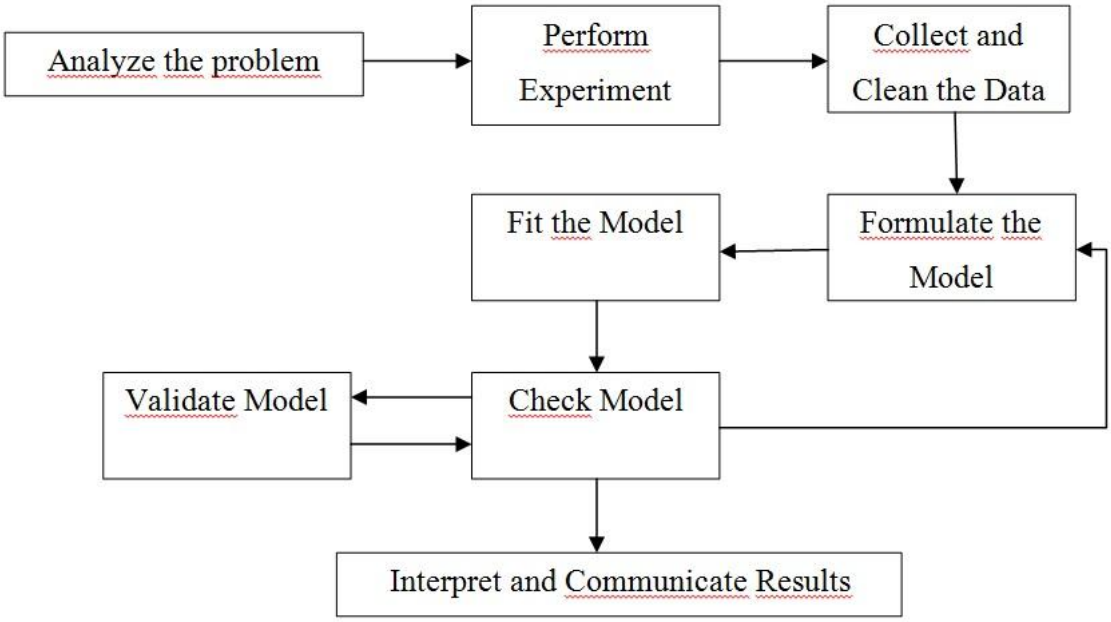


*Fig. 1. The model development process based on Bonate´ suggestion (2010)*

Validation of the model is the last step of the process (Fig.1). Bonate (2010) gives an interesting summary of questions, which shall be asked by the developer: What is the quality of the data? How trustworthy are the model parameters for different populations? What does the goodness-of-fit plot look like? How reasonable are the assumptions? According to Hodges (1991) models without validation can be helpful. These models are not to be used in practice, but they can guide one through the thinking process and so give them answers, which we would not have without this otherwise useless model. It is very important to be aware of the impact, which the model can have (Bonate 2010). In many cases the models are used in clinical environment, thus a useless model may lead to in effective treatment or severe toxicity. Every modeller shall incorporate the value of ethics into his/her work and therefore not try to manipulate the data in order to improve results.

## 1.3. Bayesian Estimation

Thomas Bayes was a mathematician living in the 18$^{th}$ century. He developed a new kind of statistics, which bears his name - the Bayesian statistics. Unfortunately, the importance of his work was recognized only two centuries later. (Bonate, 2011) The Bayesian theory was developed to improve forecasting accuracy by a combination of subjectivity and new information. Likewise a doctor may make a prior diagnosis based firstly on symptoms (subjective) and secondly on the laboratory tests (new

information). Then with both results the doctor might make a new diagnostic "forecast" using the Bayesian estimation, which provides a method to compare both estimations of probability for predicting the disease. This might be easily transferred in developing a drug dosage regimen for a patient. For example, the first dose is based on population pharmacokinetic data, and later the plasma concentrations of the administrated drugs are observed. Combining individual concentration and population PK parameters leads us to the estimation of individual PK parameters and new dose adjustment. (Shargel and Yu, 1992) To conclude, the Bayesians sometimes base their answers on their subjective belief, which is one point of criticism. Nevertheless, the FDA has allowed the use of Bayesian methods in its official Guidance to Industry in 2006, which caused increased credibility. Additionally in a philosophical sense it might be argued, that ignoring all a prior evidence is also non-objective. (Bonate, 2011; Ette and Williams, 2007) Few parameters need to be identified to estimate AUC: a) population mean of PK parameters; b) inter-individual variability of PK parameters c) residual error. If these certain parameters are identified, then the Bayesian method will provide an unbiased estimation of the AUC. (Tsuruta, Fukumoto et al; 2012)

According to Ette and Williams (2007) the fully-Bayesian analyses uses graphical model assessments such as predictions versus observations, weighted residuals versus time and weighted residuals versus predictions. To summarize, Bayesian methods provide an attractive framework to be used for population PK/PD models. The Bayesian approach estimates pharmacokinetic parameters (e.g., CL, Kel and Vd) that will be most consistent with serum levels predicted by both the population model and the actual measured serum levels. To achieve that end, the least squares method based on the Bayesian algorithm estimates the parameters, which minimize the following function:

$$WLS = \sum_{i=1}^{n} \frac{(C_i - \int(t_i, P))^2}{\sigma_i^2} + \sum_{j=1}^{m} \frac{(\bar{p}_j - p_j)^2}{{\omega_j}^2}$$

*Fig. 2. Bayesian function used to estimate the parameters*

*WLS - Weighted Least Squares; n – number of data points, m – number of parameters, C – measured serum level, $\int(t_i, P)$ - predicted serum level from population model, σ - standard deviation of serum level data, $\bar{p}$ – population parameter, p – estimated parameter, ω - standard deviation of population parameter*

## 1.4. Limited Sampling

What is the purpose of blood sampling by each patient? The aim is to control the drug concentration (influenced by individual differences in pharmacokinetics) to ensure the target concentration required for efficacy and to avoid toxicity of fluorouracil. This can be reached by dose adjustment based on measured drug concentration. (Tsuruta, Fukumoto et al 2012)

Sallas (1995) mentioned in his work that trials characterizing the pharmacokinetic profiles of the drug require a full concentration-time profile consisting of 12 or more blood-sampling times. The whole area under the concentration-time curve (AUC) needs to be represented and therefore the samples at specific time points are necessary to have informative data (e.g. by decreasing part of AUC to catch information about elimination). In the case of 5-FU administrated by infusion, there are three main parts.

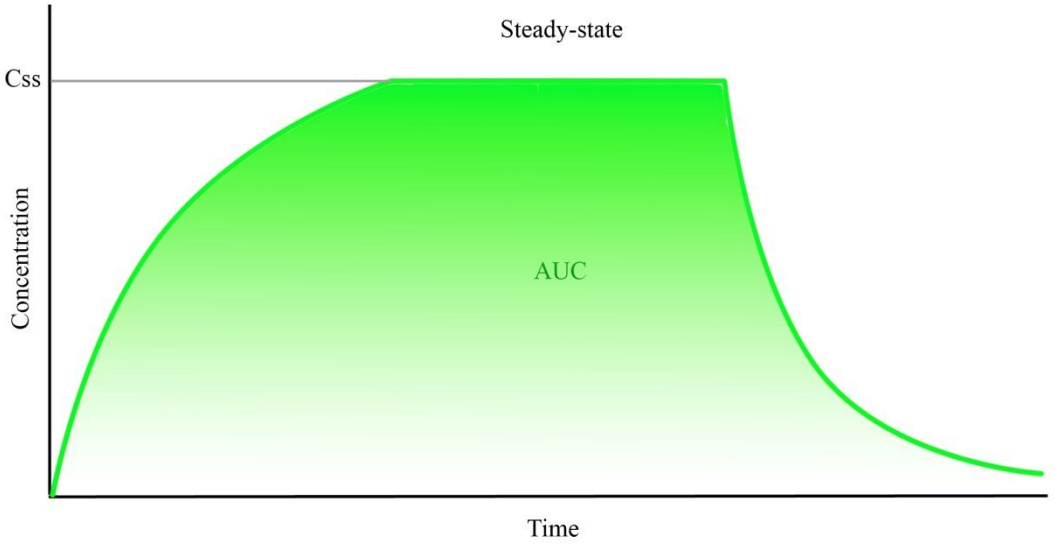


*Fig.3. Expected 5-FU AUC profile after CVI administration (own production)*

Firstly the concentration increases with the beginning of administration. Secondly, when the maximum concentration is reached, this is also called the steady state concentration (Css).Lastly, as the end concentration decreases after end of infusion (EOI), this is also called wash-out (Fig.2). Of course in practice taking 12 samples to describe the individual pharmacokinetics would be very uncomfortable and unethical for each patient. Thus, few precise sampling times need to be chosen (usually 3 or less).

There have been over 100 limited sampling strategies developed to reduce the number of blood samples required to estimate the AUC. The most common strategies are multiple linear regression, trapeziodal rule (where many samples are needed) with exponential curve approximation and Bayesian analysis. (Tsuruta, Fukumoto et al 2012). One of them is emphasized by Hashimoto (2009). It is the popular and useful Bayesian analysis approach, which predicts pharmacokinetic parameters from 1 or 2 blood samplings.

## 1.5. Dose adjustment calculators

Physicians became aware of the need to adjust the dose of a drug individually a long time ago, the first criteria being body-weight. However, as blood volume and body weight are not correlative a new approach using body surface area (BSA), which ratio is constant to blood volume, came to practice (Morrison WB, 2010). As Warmerdam (1997) correctly pointed out, the tailor-made dosing strategies based on pharmacokinetic characteristics of the individual patient could contribute substantially to improve current chemotherapeutic treatment by adjusting the drug dose in each patient to ensure optimal therapeutic outcomes. It is the NONMEM, which is used to calculate PK parameters. As doctors do not possess enough time to learn to work and control the NONMEM programme, it is upon the scientists and developers to create a user-friendly tool, which might be simply and quickly used in clinical settings using well-known and spread software. Excel® may be considered suitable as it has already been proposed in the published dosing tools for etoposide, tacrolimus, busulphan, docetaxel and paclitaxel. (Lustig et al. 1997; Wallin, Karlssson et al. 2009; Wallin, Fasth et al. 2009) Further investigations in the future on the suitable tools shall be executed.

## 1.6. Introduction to the drug

Fluorouracil (5-FU) is still increasingly used in cancer chemotherapy despite being a very old anticancer drug (Etienne et al. 1998). In 1957, the scientist Charles Heidelberger and his colleagues identified a unique biochemical feature of rat hepatome metabolism in that there was a greater uptake of uracil relative to normal tissue. Based on this observation they targeted this biochemical pathway by attaching a fluorine atom to the carbon-5 position of the uracil pyrimidine base, which resulted in the synthesis of the fluoropyrimidine 5-fluorouracil (Fig.3). (DeVita and Chu, 2008; Labianca et al.

1997). 5-FU is typically used to treat solid tumours of the gastrointestinal tract (e.g. advanced colorectal cancer), breast, head (e.g. carcinoma of the oral cavity) and neck. (Etienne et al. 1998; Meyerhardt and Mayer 2005)

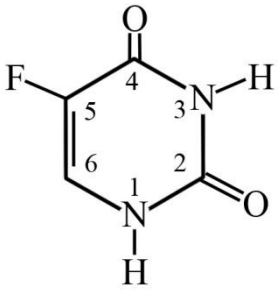


*Fig. 4. Chemical structure of 5-FU*

The routes of administration currently used are oral (using a prodrug capecitabine),.intravenous bolus and continuous intravenous infusion for up to 5 days. It is usually administered with so-called 5-FU biomodulators such as folinic acid or levamisol (Etienne et al. 1998; Porta-Oltra et al. 2004; van Kuilenburg et al. 2012). 5-FU acts as a pro-drug, which needs intracellular activation to exert its effects. There is a gap between 5-FU blood concentrations and drug effects on the target cell, therefore cell toxicity is rarely associated with blood drug concentrations. However, the existence of such a correlation has been proofed. (Etienne et al. 1998) 5-FU is well known for its high likelihood of developing severe toxicity. Amongst the most common adverse effects belong grade III/IV neutropenia (26%), stomatitis (14%) and diarrhoea (13%). Cardiac toxicities including chest pain, tightness of the chest, dyspnoea, and cardiogenic shock have also occurred. Although the toxicity has been reasoned by formation of fluoropyrimidine nucleotides, the catabolic route and genetics both play an important role.

### 1.6.1. Pharmacogenetics

Many studies (Johnson et al., 1997; Wei et al., 1998; Meinsma et al., 1995) have proved that each chemical compound in the human body is naturally metabolized and inactivated by one or more enzymes. In the case of fluorouracil, dihydropyrimidine dehydrogenase (DPD) is responsible for its metabolism. Gonzalez F.J. and P. Fernandez-Salguero (1995) described that DPD is encoded by the human

dihidropyrimidine dehydrogenase gene (DPYD). The catabolism composes of three steps leading to pyrimidine bases uracil and thymine.

Wei X et. al (1996) provided an evidence that 50% of the normal level of DPD activity in cancer patients is sufficient to trigger the development of severe degrees of toxicity with 5-FU. This may be one of the reasons why they suggested that the screening of cancer patients prior to treatment could significantly reduce 5-FU toxic effects. Later Wei et. al (1998) estimated that approximately 3% of the global population have partial DPD enzyme deficiency. It is important to mention that there are different mutations of DPYD which result in decreased DPD activity. Amstutz et. al. (2009) conducted research indicating, that there are many haplotype candidates located outside the coding sequence, which may be predicted. Further studies of non-coding polymorphisms shall ensure a more comprehensive insight into the 5-FU pharmacogenetics. Another example, Kristensen et. al (2010) stated that c1896 T>C, although it is much less frequently mentioned in the literature than IVS14+1 G>A, had a high prevalence in the population of Danish Caucasian patients (5.5%). Genetics differ in humankind across the world and there have been few studies discussing this issue. (Wei X. et al. 1998, Hamdy et al. 2002 and Ridge S.A. et al. 1998). An example for Egyptian population is comparable to Caucasian populations with some differences from Asians and African Americans. Zhang X. et al. (2013) have recently conducted a study on Chinese population evaluating 5 different single nucleotide polymorphisms (SNPs). A significant correlation between lower activity of DPD and side effects of 5-FU treatment has been again confirmed and emphasized upon. Looking back, it would have been possible to detect SNPs in 13% of patients and to predict sever toxic effects of 5-FU. Marsh et al. (2001) and Pullarkat et al. (2001) found out, that another enzyme thymidylate synthase is known to metabolize 5-FU, however there was no information in our patient data and therefore no attention was given to this enzyme.

The pharmacogenetics has been investigated, but not incorporated into the final model due to a result, which has been achieved by Su-arpa Ploylearmsaeng et al. (2007) - very low amount of mutations in the DPYD gene in the study population and statistical insignificance. Including cofactor as DPD genotype led to significantly decreased OFV ($p< 0.05$). One disadvantage of this is that the confidence intervals for estimations overlapped with values between the groups.

## 1.6.2. Clinical pharmacokinetics

### 1.6.2.1. Absorption and distribution

It is impossible to predict 5-FU plasma concentrations after oral administration, because the bioavailability significantly differs (0-80%). (Iyer and Ratain, 1999).This may be caused by the DPD enzyme located in the intestines and liver. The only common oral administration is of the prodrug capecitabine, which is used as adjuvant treatment for stage III colon cancer (van Kuilenburg et al. 2012).

If the 5-FU is administrated by i.v. bolus or infusion, it is distributed in tissue and extracellular fluid, including intestinal mucosa, bone marrow, liver, brain, cerebral spinal fluid, and neoplastic tissue. Reported volumes of distribution ($V_d$) range from 13 to 18 L (Iyer and Ratain, 1999).

### 1.6.2.2. Review of previous published plasma pharmacokinetics

Depending on the dose and time-length of infusion (i.v. bolus or CVI) the pharmacokinetic profile of 5-FU differs. Few chosen parameters from different published 5-FU PK models are compared in the table 1 and 2. The plasma half-life ($t_{1/2}$) of 5-FU is very short, about 6-22 min. The value of clearance of 5-FU is bigger by CVI and increases with lower dose rates. Depending on the genetics and other attributes the clearance differs significantly among individuals. Iyer and Ratian (1999) suggest that elimination of 5-FU is nonlinear and following pharmacokinetic characteristics are noted with increasing doses of 5-FU: an increase bioavailability, area under the plasma concentration-time curve (AUC), and plasma $t_{1/2}$ and a decrease in hepatic extraction ration and total CL.

*Tab. 1. Pharmacokinetic parameters of 5-FU given by i.v. bolus*

| Investigator | Dose ($mg/m^2/d$) | *n* | $t_{1/2}$ (min) | CL (L/h) | $C_{max}$ (µM) | $V_d$ (L) |
|---|---|---|---|---|---|---|
| van Kuilenburg et al. 2012 | 300 | 10 | 7,68±2,58 | 70,4±6,8 | - | 34,1±4,9 |
| van Kuilenburg et al. 2012 (DPD) | 300 | 26 | 16,08±7 | 68,4±7,3 | - | 36,1±8,0 |
| Di Paulo et al., 2001* | 370 | 110 | 21,6 ±3 | 87,19±5,05 | 285,9±31 | 35,8±3,6 |
| Casale et al., 2004* | 400 | 18 | 20 | 52,09 | 45,8 | 32,55 |
| van Kuilenburg et al. 2012 | 450 | 15 | 10,86±2,46 | 63,6±9,0 | - | 32,2±8,1 |
| van Kuilenburg et al. 2012 (DPD) | 450 | 19 | 18,63+6,18 | 65,9±4,6 | - | 37,6±9,0 |
| Zhu et al., 2004* | 500 | 22 | 10,6±1,3 | 58,65±23,46 | 456,6±199,1 | 13,2±5,3 |
| Larsson et al., 1996* | 500 | 82 | 15 | 122,17 | 341±34 | 18,05 |
| Malothu et al., 2010 | 500-900 | 44 | - | 72,3±4,99 | - | 12±1,49 |

*DPD = DPD-deficient patients, $t_{1/2}$ = half-life, CL = Clearance, $C_{max}$ = peak plasma concentration, $V_d$ = volume of distribution*

**Data from these publications were taken from the table from Su-arpa Ploylearmsaeng et al. (2007)*

*Tab. 2. Pharmacokinetic parameters of 5-FU given by continuous infusion*

| Investigator | Duration of infusion (h) | n | Daily Dose ($mg/m^2$) | CL (L/h) | $C_{ss}$ (µM) | $V_d$ (L) |
|---|---|---|---|---|---|---|
| Porta et al., 2013 | 36,86±11,08 | 44 | 417±37,96 | 66,8 | - | 15,1 |
| Erlichman et al. 1986* | 120 | 15 | 1250-2250 | 182,6-245,8 | 3,4±0,4 | - |
| Grem et al., 1993a* | 120 | 24 | 64-200 | 311,1±33,66 | 0,30±0,04 | - |
| Fleming et al., 1992* | 120 | 57 | 1000 | 257,35±69,8 | 2,1 | - |
| Bressolle et al., 1999 | Bol+22** | 104 | 600 | 99,2 | - | 17,9 |
| Mueller et al., 2013 | Bol+46** | 31 | 2400 | 159±20,06 | 2,55 | 54,9 |
| Yoshida et al., 1990* | Protracted | 19 | 190-600 | 207,37 | 1,15±0,15 | - |
| Jodrell et al., 2001* | Protracted | 58 | 300 | - | 0,23-0,2 | 18,0±3,3 |

*CL = Clearance, $C_{ss}$ = steady-state plasma concentration, $V_d$ = volume of distribution*

**Data from these publications were taken from the table from Su-arpa Ploylearmsaeng et al. (2007)*

***Prior the infusion, there has been bolus dose administered.*

# 2. Aim and objectives

## 2.1. Aim

It is well known many anticancer agents, including fluorouracil, have narrow therapeutic windows between efficacy and toxicity. (Tsuruta, Fukumoto et al 2012) A solution to decrease the number of accidents (over- and under-dosing) and improve efficacy and safety might be in 5-FU monitoring and PK-guided dose adjustment. This guideline could ensure that doses remain within the AUC target range, which is defined for 5-FU in literature (Gamelin et al 2008; Kaldate et al 2012) between approximately 20-30 mg·h/L.

## 2.2. Objectives

1. To build a PK model for 5-FU to estimate 5-FU AUC.

2. To define limited sampling strategy that adequately predicts individual AUCs to increase the efficacy and tolerability of fluorouracil (5-FU).

3. To ensure quality, the AUC estimated with the PK model will be compared to the AUC calculated with a rectangle equation in terms of bias and precision.

# 3. Methods

## 3.1. Experimental Design

### 3.1.1. Study populations

The 5-FU PK analysis was performed with data from several studies. All studies have been approved by the Ethics Committees of competent stakeholders and were conducted according to the Declaration of Helsinki in its amended version of Edinburgh, Scotland, 2000 and national and international legal stipulations and guidelines. Only patients treated with 5-FU were allowed to take part in this study. All patients were informed about the procedures and the aims of the study both verbally and in written form. They were accepted only after giving written informed consent to participate.

Patients were recruited in six studies from various oncology departments. Hospital in Cologne has provided 24 patients, hospital in Bonn has investigated 21 patients, hospital in Essen has conducted study with 27 patients. Recently we have received new patients´ results from Bonn (8), Cologne (1) and Berlin (9). All together there were 90 patients with 181 dosing events, including 6 bolus doses. From those, 54 patients received more than one cycle of 5-FU with a maximum of 6 cycles. Together 175 cycles were reported. Population was represented by 34 females and 56 males in age ranging from 29 till 82 years with a median of 61 years. The BSA minimum was 1.40 $m^2$, BSA median was 1.85 $m^2$ and BSA maximum was 2.36 $m^2$. Usually patients were treated with other medication, which we have included in the table as co-medication. In 32 patients there was no other medication or the information was not provided. In 58 patients the co-medication ranged from 1 to 12 medicaments.

*Tab.3. Patient characteristics*

| **Patient characteristics (n = 90)** | | |
|---|---|---|
| **Variable** | Median | Range |
| Males / females | - | 56 / 34 |
| Age (y) | 61 | 29 - 82 |
| BSA ($m^2$) | 1,85 | 1,4 - 2,36 |
| Infusion duration (h) | 46 | 18,18 - 168 |
| 5-FU dose ($mg/m^2$) | 2400 | 536 - 5024 |
| HPLC / IA | - | 24 / 66 |

| | **Study 1 (n=24)** | | **Study 2 (n=21)** | | **Study 3 (n=27)** | | **Study 4 (n=8)** | | **Study 5 (n=1)** | | **Study 6 (n=9)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | Median value | Range | Median value | Range | Median value | Range | Median value | Range | Median value | Range | Median value | Range |
| Males/females | - | 19 / 5 | - | 11 / 10 | - | 17/10 | - | 3/5 | - | 1/0 | - | 5/4 |
| Age (y) | 60,5 | 37-71 | 63 | 44-82 | 60 | 29-76 | 70 | 50-82 | - | 53 | 66 | 45-75 |
| BSA ($m^2$) | 1,91 | 1,48-2,36 | 1,78 | 1,54-2,24 | 1,86 | 1,53-2,29 | 1,70 | 1,48-2,18 | - | 2,05 | 1,85 | 1,40-2,13 |
| Infusion duration (h) | 118,6 | 115-122 | 23 | 18-93 | 46 | 46-46 | 24 | 20,9-24,1 | - | 24 | 46,9 | 23,6-168 |
| 5-FU dose ($mg/m^2$) | 4525 | 3150-5025 | 2000 | 1200-4000 | 2400 | 1800-2600 | 2000 | 1800-2000 | - | 536 | 1200 | 615-2250 |
| HPLC / IA | - | 24/0 | - | 0/21 | - | 0/27 | - | 0/8 | - | 0/1 | - | 0/9 |

*HPLC / IA = Method of Measurement*

### 3.1.2. Sampling Scheme

5-FU was administered in four different infusion times (tab. 4).

*Tab.4. Median dose of 5-FU per infusion duration*

| Infusion duration (h) | Median dose (mg/m$^2$) | Number of cycles |
|---|---|---|
| 24 | 2000 | 73 |
| 48 | 2400 | 70 |
| 96 | 4000 | 7 |
| 120 | 5000 | 25 |

For the whole population the minimum dose administered was 537 mg/m$^2$, median dose was 2400 mg/m$^2$ and maximum dose was 5000 mg/m$^2$. Total number of samplings was 591 reaching from 1 up to maximum of 13 samplings per 1 cycle/patient. For each patient there was at least one sample of 5-FU during steady state concentration.

### 3.1.3. Bioanalytical methods

To measure the concentration of the 5-FU in the plasma there were two major bioanalytical methods used. Depending on the laboratory department the patients´ 5-FU samples were analyzed either with HPLC (High Performance Liquid Chromatography) with a limit of quantification (LLOQ) 0.005 ug/L or with the Saladax My5-FU™ immunoassay (IA) having a LLOQ of 0.085 ug/ml method. With HPLC assay there were 24 patients´ samples analyzed and with IA there were 66 patients´ samples analyzed (Tab. 3.)

## 3.2. Data preparation methods

### 3.2.1. Data preparation method

For detection of outliers in the dataset of 5-FU concentrations an outlier Dixon´s test was conducted. An outlier in this case was defined as an outlier with significance higher than $p<0.05$. We analyzed samplings for outliers only in $C_{SS}$ time points for each patient individually. $C_{SS}$ was defined as time range: 2 hours after beginning of infusion until EOI. First a two sided Dixon's outlier test was conducted. In case the significance for an outlier was in the interval $0.05<p<0.1$, then the one sided test was conducted to confirm significance $p<0.05$. One sided test allows choosing whether the outlier shall be higher than median or lower. This increases the significance.

Further outlier detection was based on assumption that in reality the $C_{SS}$ shall be the highest measured concentration. We determined the median from all $C_{SS}$ samplings for each patient. All concentrations measured in the interval before 2 hours after start of infusion and in the interval after EOI to infinity higher than determined median of each individual patient $C_{SS}$ were deleted as outliers.

## 3.3. Model development

All dataset of 5-FU concentrations from 6 different studies were incorporated into one dataset and used to develop the model

### 3.3.1. Software for pharmacokinetic analysis

The pharmacokinetic analysis was performed using the nonlinear mixed-effects modelling software NONMEM®, Version 7.2 (ICON Development Solutions, Ellicott City, MD, USA). Piraña® (version 2.8.1 for Windows, Pirana Software & Consulting BV, www.pirana-software.com) was used as a powerful modelling surface for NONMEM®, PsN®, R Studio® and Xpose®. NONMEM was used to describe the pharmacokinetics and variability with compartmental population pharmacokinetic modelling of 5-FU. Influence of variability (interindividual and residual) as well as covariates (sex, age, BSA) on 5-FU clearance and volume of distribution were investigated. Fitting of 5-FU concentrations was performed with the "first-order conditional estimates" algorithm, taking interaction between the parameters into account. Additional analysis and statistics were done with GraphPad Prism® (version 5.01 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com) to visualize the results from NONMEM.

### 3.3.2. Model building approach

The model was built in several steps:

- Step 1: Building a basic model without covariates including development of a structural pharmacokinetic model for 5-FU.
- Step 2: Building the final model including all relevant covariates.
- Step 3: Model Assessment

The following criteria were taken into account for model evaluation:

a) value of objective function (ΔOFV)

The goodness-of-fit (GOF) of the model to the data was evaluated based on changing in the minimum value of objective function (OFV). ΔOFV of >3.84 was required to indicate that the model with change of one parameter with the lowest OFV was associated with the better model ($p < 0.05$) and ΔOFV of >10.6 was required to indicate better model with change of two parameters. The log likelihood ratio was used to simultaneously estimate population values of fixed-effects parameters (e.g. CL, $V_D$) and values of random effects parameters (interindividual and residual variability).

b) The plausibility of estimated parameters and their 95% confidence intervals

c) Quality of goodness-of-fit plots

GOF plots included the following: (1) observed vs. predicted concentrations, (2) predicted concentrations vs. weighted residuals (deviations of predictions from observed concentrations) and (3) time vs. weighted residuals. Distribution of predicted concentrations shall be close to observed concentrations and weighted residuals shall be equally distributed to indicate a better fit.

### 3.3.3. Compartment models tested

There were four compartment models investigated in our analysis:

- One-compartment, linear elimination
- One-compartment, MM (Michaelis-Menten) elimination
- Two-compartment, linear elimination
- Two compartment, MM elimination

### 3.3.4. Residual error models

*Tab.5. Four error models investigated*

| Error model type | Equation |
|---|---|
| Additive | $Y = IPRED + EPS$ |
| Proportional | $Y = IPRED * (1 + EPS)$ |
| Combination (Add.+Prop.) | $Y = IPRED * (1 + EPS_1) + EPS_2$ |
| Exponential | $Y = IPRED^{exp(EPS)}$ |

*IPRED = Individual predicted concentration; EPS ($\varepsilon$) = Residual variability*

For the different 5-FU detection methods there were different error models tested. For HPLC the additive error was more precise and for the immunoassay the combination was more precise.

### 3.3.5. Interindividual error models and interocasional variability

An exponential error model was used to describe the IIV. The IIV was tested on two main parameters: clearance and volume of distribution. An exponential error model was used to describe interoccasion variability on 5-FU clearance.

### 3.3.6. Covariate analysis

In Pirana the automatic stepwise covariate modelling (SCM) was used for covariate analysis. The SCM uses a p-value as an indicator of when to halt inclusion or deletion of further covariate coefficients. The test was run with forward inclusion p-value 0.05 and backward elimination p-value 0.01. There were three covariates tested: sex, BSA and age. The categorical covariate (sex) was tested with two valid states (not included and linear) and the continuous covariates (age and BSA) were tested with five valid states (not included; linear; piecewise linear; power and exponential). Each covariate was tested on CL and $V_D$.

### 3.3.7. Model validation

A total of 1000 replicates were generated from the original dataset with the bootstrap resampling technique as an internal evaluation method to confirm the stability of the final population pharmacokinetic model. Population pharmacokinetic parameter estimates for each replicate were obtained and the model stability was evaluated by

visual inspection of the bootstrap distribution of the model parameters. Furthermore the mean parameter estimates and their 95% confidence intervals were obtained from the bootstrap replicates and compared with the estimated population pharmacokinetic parameters obtained from the original dataset.

## 3.4. Verification of the AUC estimated by NONMEM

In order to verify the precision of the AUC estimated by NONMEM a non-compartmental approach to count the AUC was used as a reference. The dataset of 5-FU concentrations from real patients were chosen as the input data. The non-compartmental (NCA) counting of AUC was executed with the trapezoid rule. There were two possible approaches:

a) linear equation to count AUC in all concentrations

b) linear equation to count AUC in increasing concentrations (beginning of infusion + $C_{SS}$) in combination with logarithmic equation to count AUC in decreasing concentrations (after EOI)

After comparing both approaches on several examples of AUC we discovered only small negligible difference in results of these two methods ($\Delta AUC<0.01$). Considering the curve shape, we decided to use only linear trapezoid rule for both increasing and decreasing concentrations. Final equations used to count the non-compartmental AUC:

$$(1)\ AUC_{C_0-C_1} = \frac{(C_0 + C_1) * \Delta t}{2}$$

$$(2)\ AUC_{0-t} = \sum \left(AUC_{C_0-C_1}\right) + \left(AUC_{C_1-C_2}\right) \ldots + \left(AUC_{C_n-C_t}\right)$$

$$(3)\ AUC_{t-\infty} = \frac{C_t}{K_{el}}$$

First equation (1) was used to count AUC between two measured concentrations. $AUC_{C0–C1}$ = AUC between two concentration points. Sum of $c_0$ and $c_1$ (two after each other following concentrations) divided by two and multiplied by the difference of times $\Delta t$ when the concentrations were measured. Second equation (2) was sum of all counted AUCs from the beginning of infusion till the last measured concentration. Third equation (3) counted the AUC from last measured concentration ($c_t$) till infinity by using the rate constant of elimination ($K_{el}$).

In addition the NONMEM Limited sampling and Rectangle Equation methods were compared by 5-FU dose-adjustment algorithm. Target AUC range is 20 – 30 mg·h/L. The recommendations to change the dose depend on the estimated AUC (Tab. 6).

*Tab.6. PK-guided dosing algorithm for 5-FU*

| **Estimated AUC [mg·h/L]** | **Change in dose (mg/m$^2$)** |
|---|---|
| > 40 | ↓ 30% |
| 37-40 | ↓ 25% |
| 33-37 | ↓ 20% |
| 30-33 | ↓ 10% |
| 20-30<br>(normal AUC range) | no change needed |
| 17-20 | ↑ 10% |
| 13-17 | ↑ 20% |
| 8-13 | ↑ 25% |
| < 8 | - repeat previous dose or<br>- if repeated increase by ↑ 30% |

## 3.5. Comparison of different methods to estimate AUC

Four different methods used to estimate AUC were compared in their median, bias and precision in real data (18 patients). The NCA-AUC (trapezoid rule) was used as a reference. Three methods were compared: NONMEM estimated AUC using the whole concentration measurements, NONMEM estimated AUC using only the last concentration measurement before EO ($C_{LAST}$) and the rectangle equation. The rectangle equation (4) used:

$$(4)\ AUC = t_{INF} * C_{LAST}$$

The AUC was counted by multiplying the infusion duration ($t_{INF}$) and last measured concentration before EOI ($C_{LAST}$).

## 3.6. Limited sampling

Due to the ethical reasons it is not possible to intensively collect blood samples to estimate a patient's AUC. Therefore, limited sampling models were inspected in order to choose the best strategy for collecting a minimum of blood samples that can still adequately estimate the 5-FU AUC.

### 3.6.1. Limited Sampling

Various limited sampling strategies were inspected (tab. 7). The maximum number of concentration measurements was set to two samplings; however strategies with only one sampling were included. Limited sampling times were inspected for all infusion durations. As it does not make sense to measure the concentration in the beginning of infusion the first time point was set to 1-hour. During the $C_{SS}$ (defined in our case from two hours after start of infusion until EOI) the concentration shall not change. Therefore another time point for sampling was only set at two hours before EOI. In clinical practice it might happen that the sampling was not taken during the infusion, therefore we also included samplings taken after EOI in the limited sampling analysis.

All limited sampling strategies were compared with the reference "Full simulated data", which contained intensive sampling: 35 samplings per patient (tab 8.). All limited sampling schemes were compared to the reference both visually (using box plots and linear regression graphs) and numerically (using median AUC, bias, precision and $R^2$).

The Prediction Error (PE) was calculated as the difference between predicted value ($AUC_P$) and true value ($AUC_T$) as in Equation (5) (Wu 1995). The Median Prediction Error (ME) was calculated as the value that is half way through the PE data set. Mean Prediction Error (MPE) was executed as in Equation (6) (Sheiner and Beal 1981). Bias, the average difference between the estimator and the true value, was determined by the PE, ME and MPE.

The overall variability of estimation was measured with the Mean Squared Prediction Error (MSE). The overall variability of estimation, in the same units as the parameter being estimated, was calculated with the Root Mean Squared Prediction Error (RMSE). Precision, the standard deviation of the estimator, was determined by the MSE (Equation 7) and RMSE (Equation 8) (Sheiner and Beal 1981). Since the

"true" parameter values were known from the full estimated data set, the precision of parameter estimation from the limited sampling could be quantified. Both the degree of bias and the precision of estimates relative to true values were computed.

(5) $PE = AUC_P - AUC_T$

(6) $MPE = \frac{1}{n}\sum_{i=1}^{n} PE_i$

(7) $MSE = MPE^2 + \frac{1}{n}\sum_{i=1}^{n}(PE_i - MPE)^2$

(8) $RMSE = \sqrt{MSE}$

*Tab.7. Limited sampling strategies*

| One sampling during CVI | Two samplings | Sampling after EOI |
|---|---|---|
| After 1 hour | After 1h & 2h before EOI | 5 min after EOI |
| After 2 hours | After 1h & at EOI | 10 min after EOI |
| 2 hours before EOI | After 1h & 30 min after EOI | 15 min after EOI |
| At EOI | EOI & 30 min after EOI | 20 min after EOI |
| | EOI & 60 min after EOI | 25 min after EOI |
| | | 30 min after EOI |
| | | 60 min after EOI |

*CVI = Continuous Intravenous Infusion, EOI = End of Infusion*

### 3.6.2. Simulated Dataset

As a reference a dataset of 1000 patients was simulated with the final 5-FU PK-model for each infusion duration (24h, 48h, 96h and 120h) separately. Following doses were used: 2000 mg/m$^2$ for 24h; 2400 mg/m$^2$ for 48h; 4000 mg/m$^2$ for 96h; 5000 mg/m$^2$ for 120h. Each simulated patient was created with 35 sampling time points (tab. 8) and information about BSA (1,4 – 2,39 m²), sex (400 females and 600 males) and method of measurement (499 HPLC and 501 IA). Afterwards the simulated concentrations were used as input in the dataset and the data was analyzed as a Bayesian estimation using the POSTHOC function in NONMEM. The results of this Bayesian estimation with the intensive sampling were set as reference.

The limited sampling strategies mentioned in table 8 were tested for each infusion duration separately. All datasets were analyzed with NONMEM as a Bayesian

estimation using the POSTHOC function with the final 5-FU PK model and results (AUC, bias, precision, $R^2$) were compared with the reference.

*Tab.8. Intensive sampling for the different 5-FU infusion durations*

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Infusion 24 hours – Sampling points [h]*** | | | | | | | | | | | |
| 0,25 | 0,5 | 0,75 | 1 | 1,25 | 1,5 | 1,75 | 2 | 3 | 4 | 5 | 6 |
| 7 | 8 | 10 | 12 | 16 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 24,08 | 24,17 | 24,25 | 24,33 | 24,42 | 24,5 | 25 | 25,5 | 26 | 28 | 30 | |
| ***Infusion 46 hours – Sampling points [h]*** | | | | | | | | | | | |
| 0,25 | 0,5 | 0,75 | 1 | 1,25 | 1,5 | 1,75 | 2 | 3 | 4 | 6 | 8 |
| 12 | 18 | 24 | 30 | 36 | 40 | 41 | 42 | 43 | 44 | 45 | 46 |
| 46,08 | 46,17 | 46,25 | 46,33 | 46,42 | 46,5 | 47 | 47,5 | 48 | 50 | 52 | |
| ***Infusion 96 hours – Sampling points [h]*** | | | | | | | | | | | |
| 0,25 | 0,5 | 0,75 | 1 | 1,25 | 1,5 | 1,75 | 2 | 3 | 4 | 6 | 12 |
| 24 | 36 | 48 | 60 | 72 | 84 | 91 | 92 | 93 | 94 | 95 | 96 |
| 96,08 | 96,17 | 96,25 | 96,33 | 96,42 | 96,5 | 97 | 97,5 | 98 | 100 | 102 | |
| ***Infusion 120 hours – Sampling points [h]*** | | | | | | | | | | | |
| 0,25 | 0,5 | 0,75 | 1 | 1,25 | 1,5 | 1,75 | 2 | 3 | 4 | 6 | 12 |
| 24 | 36 | 48 | 60 | 72 | 96 | 115 | 116 | 117 | 118 | 119 | 120 |
| 120,08 | 120,17 | 120,25 | 120,33 | 120,42 | 120,5 | 121 | 121,5 | 122 | 124 | 126 | |

### 3.6.3. Verification of the AUC by Limited Sampling

In order to confirm the precision of the NONMEM estimates of the limited sampling strategies the AUC values were used to test the accuracy of prediction. For the whole simulated dataset of 5-FU concentrations with four different infusion durations the AUC estimates 5 minutes after EOI were compared between the NONMEM Limited Sampling and rectangle equation. As reference the NONMEM Full sampling AUCs were used.

# 4. Results

## 4.1. Data accounting

The pharmacokinetic analysis was carried out for patients treated with 5-FU, who were collected in six different studies (Tab. 3). Statistical differences between mean doses (±SD) of 5-FU administrated in different studies were found: 4400±596.2 for 120h-infusion (study 1); 1942±456,8 for 24/46h-infusion (study 2); 2372±154,7 for 46h-infusion (study 3); 1971±57.40 for 24h-infusion (study 4); 536,6±0 for 24h-infusion (study 5); 1306±636 for 24/46h-infusion (study 6). These differences are mainly due to the duration of the infusion and difference in chemotherapy protocols administered. The 5-FU plasma concentrations decreased rapidly when the infusion administration was stopped due to short half life of 5-FU.

### 4.1.1. Data preparation

For detection of outliers in the dataset of 5-FU concentrations the Dixon´s test detected 3 outliers in the $C_{SS}$ with significance lower than $p<0.05$ and therefore these outliers have been excluded from the final dataset used to develop the PK/PD model.

## 4.2. Model description

The population pharmacokinetic model was developed based on 591 plasma concentrations of 5-FU obtained from 90 patients. The open one-compartmental pharmacokinetic base model without covariates with linear elimination (subroutine ADVAN1 TRANS2 in NONMEM) was successfully fitted to the data. The additive residual error was chosen as the final residual error model. The exponential error model to describe the interindividual variability on clearance was added.The mean population pharmacokinetic parameter estimates for 5-FU obtained from this 5-FU model were clearance (CL) 219 L/h and volume of distribution ($V_D$) 30,5 L (Tab. 9). Interoccassional variability on cycles in base model was 29.2 % (Tab. 8). The screening of three covariates (sex, age and BSA) on clearance and volume of distribution was conducted with the automatic stepwise covariate modelling (SCM) of Pirana. Only BSA on CL was a significant ($p<0.01$) covariate and was added in the final model. The final equation (9) for the CL including BSA as covariate was:

$$(9)\ \mathrm{CL}\ [\mathrm{L/h}] = 214 * [1 + 0{,}933 * (\mathrm{BSA} - 1.80)]$$

The final model including covariates provided the mean population pharmacokinetic parameter estimates for 5-FU and their 95% confidence intervals (95% CI). Estimates obtained from this 5-FU model were clearance (CL) 214 L/h (195,9-235,1) and volume of distribution ($V_D$) 30,2 L (20,1-47,2) (Tab. 9). Comparing the base model with the final model with BSA as covariate resulted in a decrease of IIV on CL from 52,2% to 48,8% (Tab. 9) Fixed and random effect estimates from the original dataset fall in the 95% confidence interval obtained from the bootstrap replicates, indicating that the developed model is stable.

*Tab.9. Population pharmacokinetics of 5-FU (base and final model)*

| | **Base Model** | **Final Model** | |
|---|---|---|---|
| **Model Parameters** | Estimate | Estimate | CI |
| ***Cl [L/h]*** | 219 | 214 | 195,9-235,1 |
| ***$V_D$ [L]*** | 30,5 | 30,2 | 20,1-47,2 |
| ***Add. Res Error [mg/L]*** | 0,112 | 0,112 | 0,097-0,128 |
| ***BSA on Cl*** | - | 0,993 | 0,36-1,43 |
| ***IIV Cl [%]*** | 52,2 | 48,8 | 28,96 - 64,53 |
| ***IOV on CL C1 [%]*** | 29,2 | 28,8 | 13,75 - 55,71 |
| ***IOV on CL C2 [%]*** | 29,2 | 28,8 | 13,75 - 55,71 |
| ***IOV on CL C3 [%]*** | 29,2 | 28,8 | 13,75 - 55,71 |
| ***IOV on CL C4 [%]*** | 29,2 | 28,8 | 13,75 - 55,71 |
| ***IOV on CL C5 [%]*** | 29,2 | 28,8 | 13,75 - 55,71 |
| ***IOV on CL C6 [%]*** | 29,2 | 28,8 | 13,75 - 55,71 |

*CL = Clearance; $V_D$ = Volume of distribution; BSA = Body surface Area; IOV = interoccassional variability; CI = 95% Confidence Interval; IIV = interindividual variability*

### 4.2.1. Goodness-of-fit plots

The appropriateness of the base and final model was evaluated graphically by goodness of fit (GOF) plots. The scatter plot of the individual predicted versus observed 5-FU plasma concentrations indicates that the model adequately describes the real concentration time data (Fig. 5). Population prediction indicated the model had tendency to overestimate 5-FU plasma concentrations. (Fig. 6) For 5-FU concentrations and on the weighted residuals vs. predicted concentrations plots (Fig. 7 and 8) did not show distributions suggesting systematic deviations. The GOF plots showed the

reasonable fit obtained with the final model. The additional error model yielded the lowest objective function value and the best residual plots (Fig. 7).

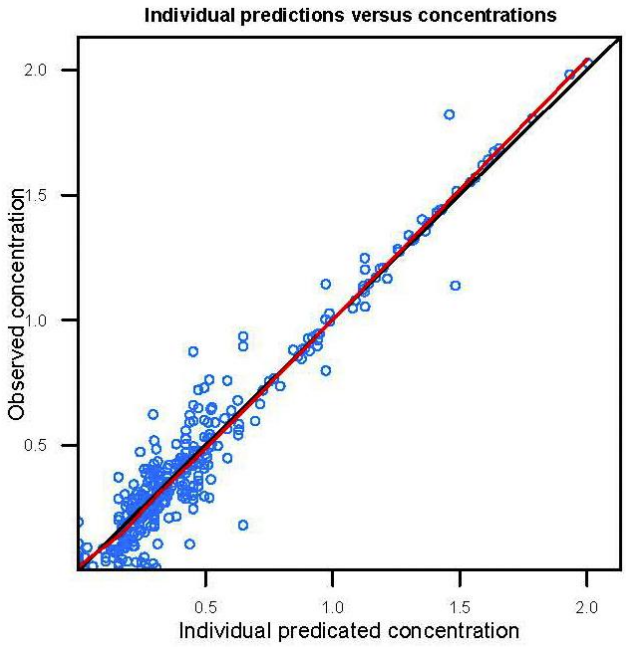


*Fig. 5. Observed vs. individual predicted concentrations*

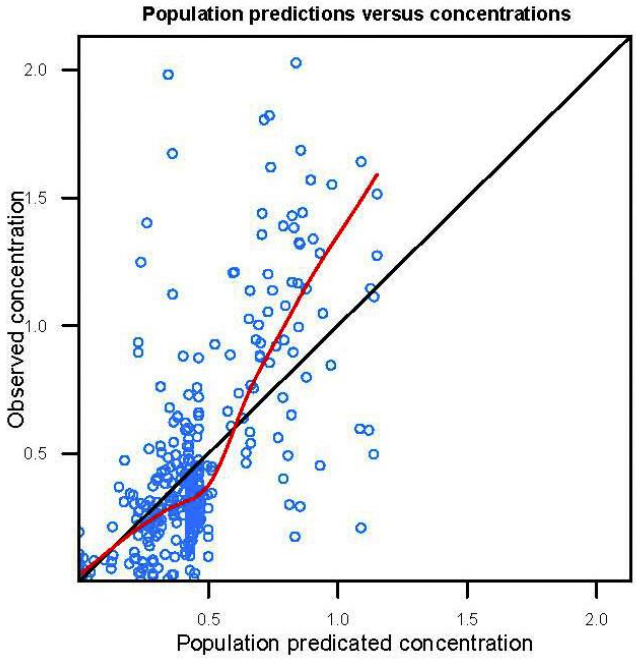


*Fig. 6. Observed vs. population predicted concentrations*

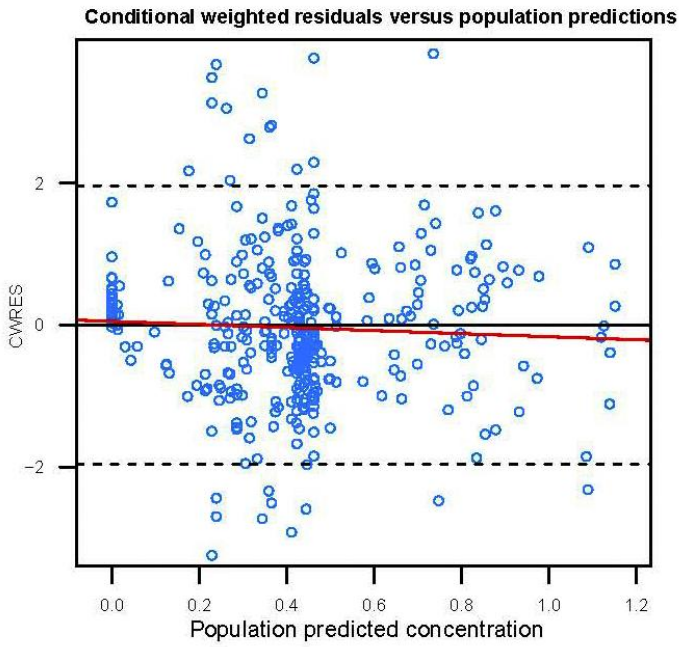


*Fig. 7. Population predicted concentrations vs. weighted residuals*

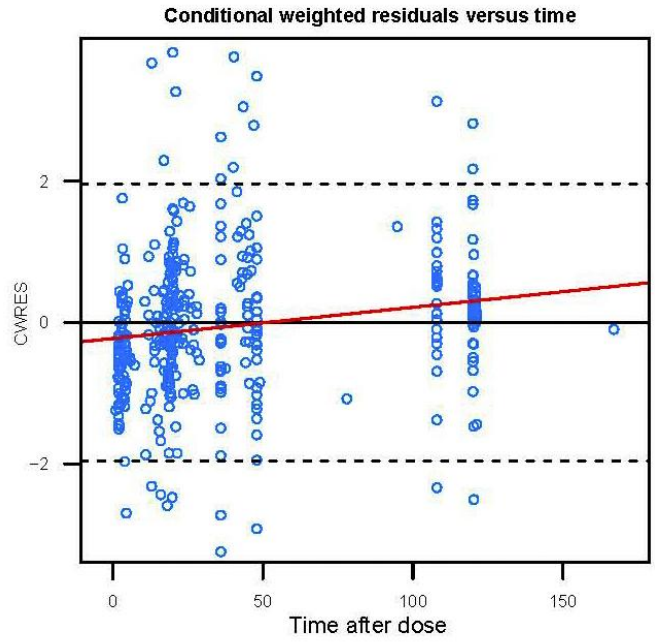


*Fig. 8. Time vs. weighted residuals*

## 4.3. Verification of the NONMEM estimated AUC

The 5-FU concentration data were used to validate the process of NONMEM estimating the AUC correctly and to compare the correctness of the rectangle equation. From the data, 18 patients showed enough blood samplings to estimate the AUC using the NCA approach (trapezoid rule). The AUC estimates by NCA, used as reference, were compared with the AUC estimates obtained from NONMEM using all of the concentrations from the 18 patients (NONMEM Full), AUC from NONMEM using the last measured concentration before EOI (NONMEM Lim) and AUC from the rectangle equation (Tab. 10) using the last measured concentration before EOI (Rectangle Eq). The criteria for comparison were the median AUC, bias, precision and linear regression.

*Tab. 10. Validation of AUC estimation*

| | | NCA-AUC | NONMEM Full | NONMEM Lim | Rectangle Eq |
|---|---|---|---|---|---|
| **Median AUC [mg·h/L]** | | 29,74 | 35,82 | 43,81 | 46,04 |
| **5th Percentile [mg·h/L]** | | 17,26 | 21,49 | 27,98 | 25,20 |
| **95th Percentile [mg·h/L]** | | 70,50 | 76,89 | 98,55 | 106,4 |
| **Bias absolute** | MPE [mg·h/L] | | 6,71 | 13,21 | 14,94 |
| | ME [mg·h/L] | | 6,06 | 12,34 | 15,26 |
| **Bias relativ** | MPE [%] | | 21,90% | 42,45% | 47,29% |
| | ME [%] | | 21,77% | 39,51% | 44,71% |
| **Precision absolute** | RMSE [mg·h/L] | | 7,14 | 15,03 | 17,67 |
| | Absolute ME [mg·h/L] | | 6,06 | 12,34 | 15,26 |
| **Precision relative** | RMSE [%] | | 22,72% | 47,66% | 55,34% |
| | Absolute ME[%] | | 21,77% | 39,51% | 44,71% |
| **$R^2$** | | **1,00** | **0,9767** | **0,9090** | **0,8770** |

*ME = Median prediction error; MPE = Mean prediction error; RMSE = Root mean squared prediction error*

The validation results were cross-checked graphically using the box- and GOF plots (Fig. 9 and 10). For the whole data set (90 patients) the GOF plot to compare AUC estimates between NONMEM Limited Sampling (NONMEM Lim) and Rectangle Equation (RE) method was developed (Fig 11). Each patient, respectively cycle, was

represented with one blood sample, respectively drug concentration. The concentration value was used in two different ways (NONMEM Lim and RE) to count the AUC. Results of AUCs were graphed in GOF plots. Majority of the AUCs were similarly estimated, but there were several cases which need to be emphasized upon. The six estimates closest to the X axis showed that the AUC estimates differs significantly (Tab. 11; Fig. 11). The six measured concentrations before EOI were very low probably because of an analytical/measurement error. With rectangle equation the AUC is dramatically underestimated. With NONMEM estimation there is the shrinkage effect. That means that the PK parameters tend to the population mean, when there is no informative data available as in these cases. Therefore using NONMEM gives the estimation ration of safety as the shrinkage effect recognize outliers as measurement errors.

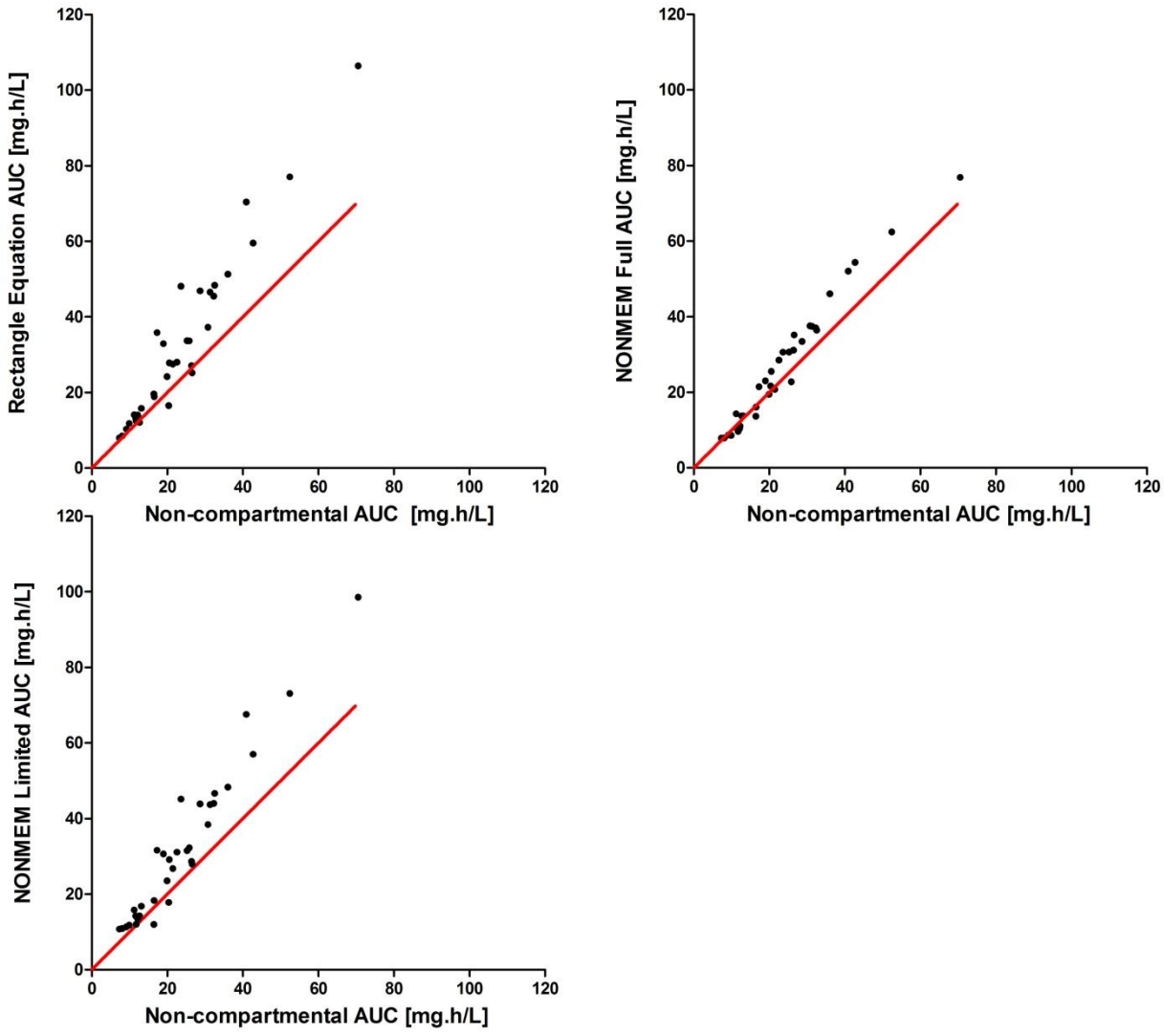


*Fig. 9. GOF plots: Validation of AUC estimates for 18 patients*

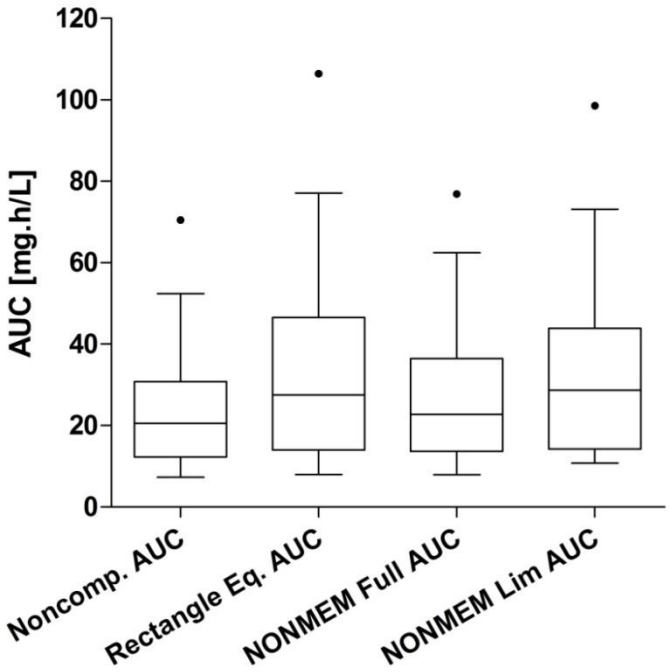


*Fig. 10. Tukey box Plots: Validation of AUC estimates for 18 patients*
*Noncomp. AUC (using Trapezoid rule); Rectangle Eq. AUC (using Rectangle Equation), NONMEM Full AUC (using NONMEM with full sampling data), NONMEM Lim AUC (using NONMEM with limited sampling data)*

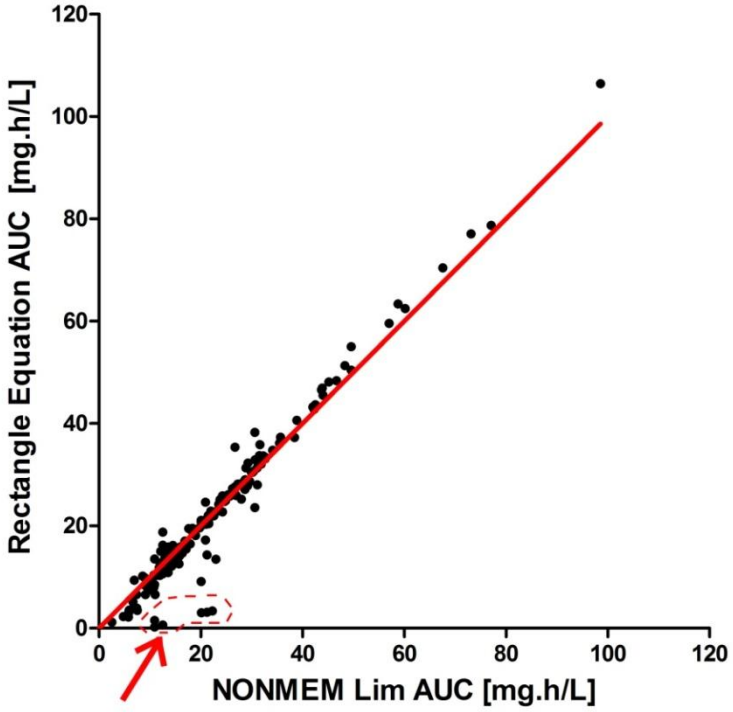


*Fig. 11. GOF Plot: AUC comparison of Rectangle equation and NONMEM Lim (limited sampling data) for all 90 patients*

*Tab. 11. AUC comparison of Rectangle equation and NONMEM Lim (limited sampling data) from all 90 patients – Special cases*

| Patient ID | Concentration [µmol/L] | AUC Rectangle Eq [µmol·h/L] | AUC NONMEM Lim [µmol·h/L] |
|---|---|---|---|
| **1** | 0,027 | 3,12 | 21,21 |
| **27** | 0,028 | 3,37 | 22,29 |
| **28** | 0,025 | 3,03 | 20,11 |
| **40 (Cyc2)** | 0,005 | 0,22 | 10,94 |
| **40 (Cyc3)** | 0,034 | 1,50 | 10,94 |
| **100** | 0,013 | 0,60 | 12,54 |

### 4.3.1. Dosing algorithm

The 5-FU concentration data from real data (n=90, cycles=175) were used to count the AUC by two different approaches: The rectangle equation (RE) and the limited sampling NONMEM approach. Results of AUC from both methods were compared with the target AUC value (20-30 mg·h/L) and if different, the dose adjustment recommendation in percentage was chosen from the variety: -30%, -20%, -10%, 0%, 10%, 20%, +30%. To evaluate the methods, the sum of same adjustments was compared with sum of differences in adjustments. Differences were divided into significant if $AUC_{NONMEM} - AUC_{RE} > 1{,}5$ and just difference in case it was lower. Final summary of dose adjustment recommendations was compiled in the Tab. 12.

*Tab. 12. AUC adjustment comparison between Rectangle equation and NONMEM Limited Sampling for real patients (n=90)*

| | Same adjustment: | Significant difference in AUC ( > 1,5 mg·h/L): | Difference in AUC (< 1,5 mg·h/L): |
|---|---|---|---|
| **AUC over 30:** | 31 | 5 | 0 |
| **AUC 20-30:** | 45 | 12 | 1 |
| **AUC under 20:** | 55 | 12 | 14 |
| | **131** | **29 (15,57%)** | **15 (8,57%)** |
| **Sum:** | **131 (74,86%)** | **44 ( 25,14%)** | |

## 4.4. Limited Sampling Strategy

The 5-FU concentration data with 1000 simulated patients were used to compare adequate estimation of AUC in different limited sampling strategies. The reference was the intensive sampling Bayesian estimation data. The criteria of adequate AUC estimation of the limited sampling strategies were the median AUC, bias, precision and linear regression. From one-sampling strategies the "2 hours" and "2 hour before EOI" were appointed as the most eligible one-sampling strategies as they had similar results, which was caused by the fact, that the $C_{SS}$ in simulated data set was at the same level in both cases. The two-sampling strategies "1 hour & 2 hour before EOI" and "1h & EOI" were appointed as the most accurate. The strategies were investigated for each infusion duration (24, 46, 96 and 120 hours) separately and expressed graphically (Fig. 12, 13) as well as numerically (Tab. 13). As it is possible to see from the box plots (Fig 12), the AUC estimates did not vary significantly if the sampling was taken in or very close to $C_{SS}$ time range. Therefore, the model was considered as very robust. The samplings before the start of $C_{SS}$ and especially after EOI showed significantly worse results than the recommended sampling strategies. To outline the differences, the results from the 24h-infusion can be found in the appendix. Other infusion durations are not included as they showed similar deviations as the 24h-infusion.

Due to clinical practice tendencies the one-sampling strategy with the time point at 2 hours before EOI was chosen as most suitable. Therefore, it would be recommended for the physicians to use this time point to obtain the blood sample by patients treated with 5-FU infusions. Further investigation in transforming this decision into practice is needed.

*Tab.13. Limited sampling strategies comparison for all infusion durations*

| | | Ref 24h-Inf | 2 h bef EOI | 1h & 2h bef EOI | Ref 46h-Inf | 2 h bef EOI | 1h & 2h bef EOI | Ref 96h-Inf | 2 h bef EOI | 1h & 2h bef EOI | Ref 120h-Inf | 2 h bef EOI | 1h & 2h bef EOI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Median AUC [mg·h/L]** | | 16,70 | 16,69 | 16,68 | 20,03 | 20,05 | 20,05 | 33,38 | 33,43 | 33,41 | 41,72 | 41,79 | 41,77 |
| **5th Percentile [mg·h/L]** | | 7,157 | 8,811 | 8,139 | 8,811 | 12,08 | 10,91 | 14,99 | 21,59 | 19,36 | 18,74 | 26,99 | 24,20 |
| **95th Percentile [mg·h/L]** | | 39,47 | 38,93 | 39,16 | 47,32 | 45,68 | 46,39 | 78,81 | 74,58 | 76,38 | 98,51 | 93,23 | 95,47 |
| **Bias absolute** | MPE [mg·h/L] | - | 0,22 | 0,12 | - | 0,34 | 0,24 | - | 0,51 | 0,42 | - | 0,64 | 0,53 |
| | ME [mg·h/L] | - | 0,01 | 0,00 | - | 0,03 | 0,01 | - | 0,06 | 0,04 | - | 0,08 | 0,04 |
| **Bias relativ** | MPE [%] | - | 4,09% | 2,39% | - | 6,52% | 4,18% | - | 7,57% | 5,12% | - | 7,57% | 5,12% |
| | ME [%] | - | 0,06% | 0,03% | - | 0,15% | 0,08% | - | 0,20% | 0,11% | - | 0,20% | 0,11% |
| **Precision absolute** | RMSE [mg·h/L] | - | 0,74 | 0,43 | - | 1,61 | 0,99 | - | 3,52 | 2,21 | - | 4,40 | 2,76 |
| | Absolute ME [mg·h/L] | - | 0,41 | 0,21 | - | 1,15 | 0,62 | - | 2,79 | 1,54 | - | 3,48 | 1,92 |
| **Precision relative** | RMSE [%] | - | 10,70% | 6,50% | - | 16,64% | 10,74% | - | 19,66% | 13,08% | - | 19,66% | 13,08% |
| | Absolute ME[%] | - | 1,53% | 0,79% | - | 3,71% | 1,98% | - | 5,58% | 3,04% | - | 5,57% | 3,04% |
| **$R^2$** | - | 1,00 | 0,9979 | 0,9993 | 1,00 | 0,9932 | 0,9977 | 1,00 | 0,9884 | 0,9961 | 1,00 | 0,9884 | 0,9961 |

*ME = Median prediction error; MPE = Mean prediction error; RMSE = Root mean squared prediction error*

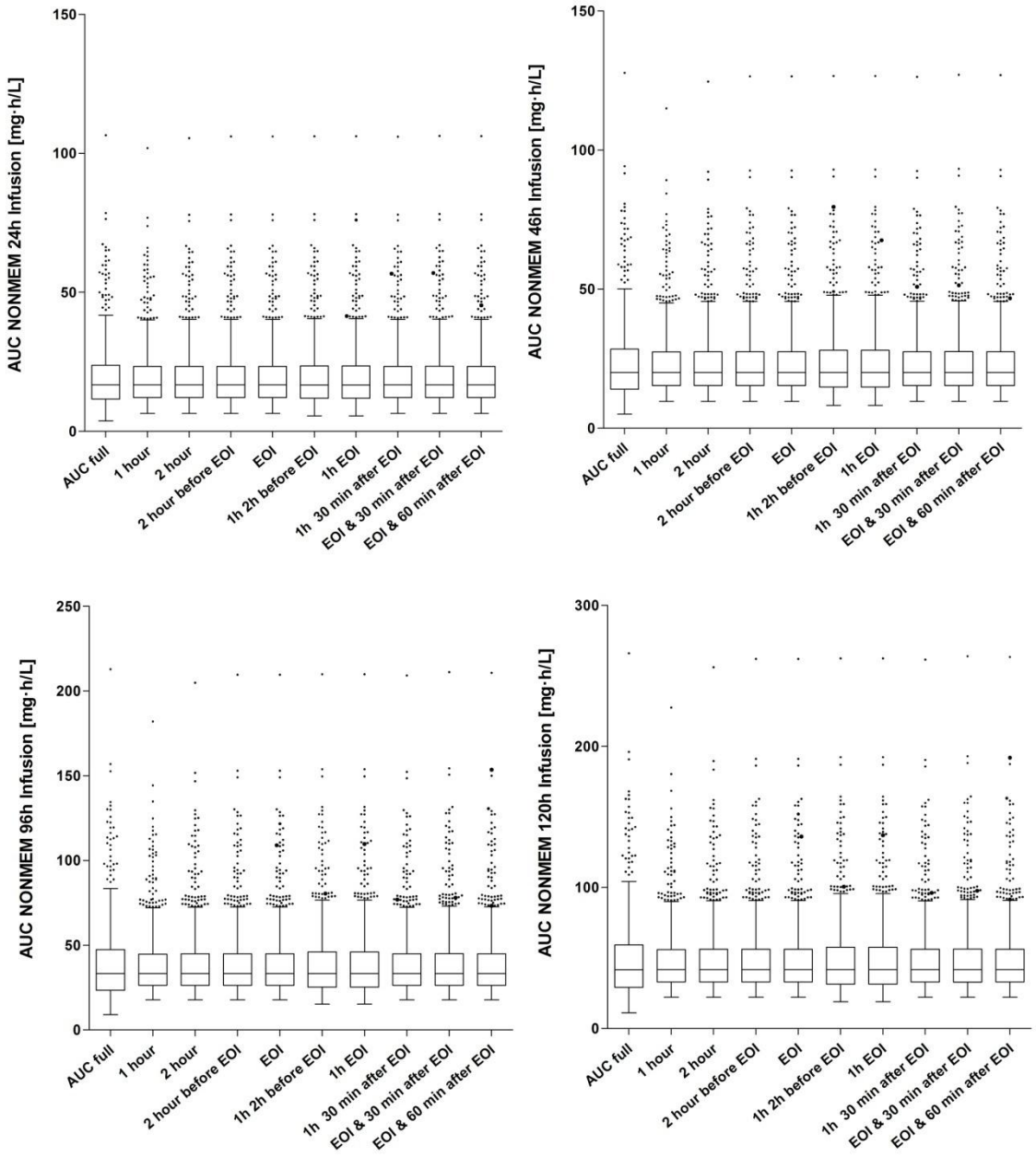


*Fig. 12. Tukey box plots: Limited sampling strategies*

*AUC Full – using full data set; 1h 2h before EOI (2 samplings) – 1 hour & 2 hours before EOI, 1h EOI (2 samplings) – 1 hour & EOI, 1h 30 min after EOI (2 samplings) – 1 hour & 30 min after EOI*

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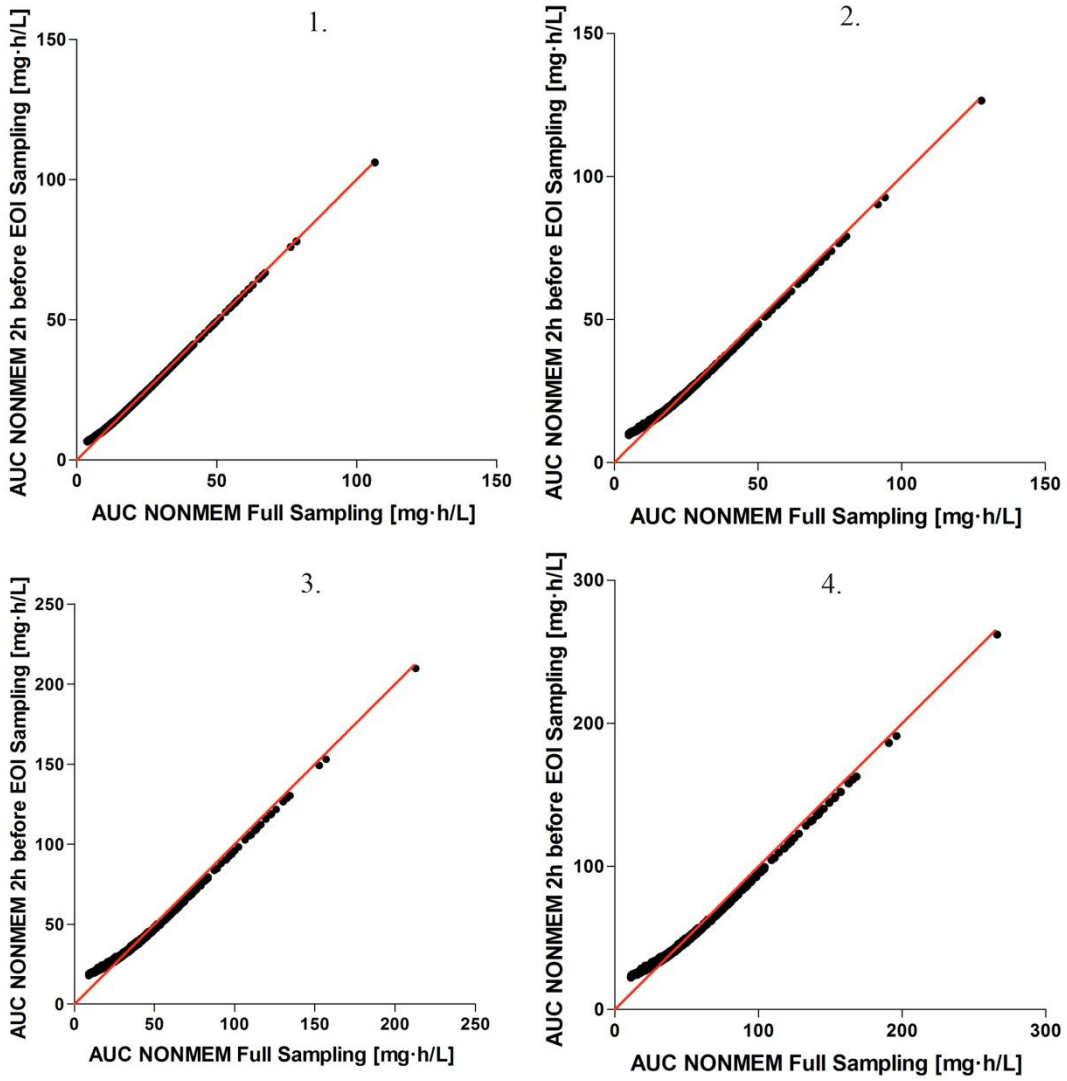


*Fig. 13. GOF plots: Limited sampling 2 hours before EOI strategy*

*1. Plot for the infusion with 24 hours duration; 2. Plot for the infusion with 46 hours duration; 3. Plot for the infusion with 96 hours duration; 4. Plot for the infusion with 120 hours duration*

The plots for all infusion duration times for the strategy "2 hours before EOI" showed an acceptable linear regression value ($R^2$>0.9884) and therefore the limited sampling strategy was chosen sufficient to predict the AUC correctly.

# 5. Discussion

The concept of individualization of drug doses based on population pharmacokinetics in cancer chemotherapy has been applied for a number of agents – even for 5-FU (Gamelin et al. 2008, Kaldate et al. 2012). The principal aim of population pharmacokinetic analysis is to count variability in a population of patients. A better understanding of the intra- and inter-individual variability associated with pharmacokinetic and pharmacodynamic behaviour of therapeutic agents can lead to higher efficacy and safer drug use (Malothu et al. 2010).

In this diploma thesis, the analysis of 5-FU pharmacokinetics in 90 patients was performed in order to create a tool to establish individual dosage adjustments. In literature there are many publications with different 5-FU compartmental models used: one-compartmental model (Malothu et al. 2010; Bressolle et al. 1999, Climente et al. 2002, Etienne et al. 1998), two-compartmental model (Mueller et al. 2013, Porta et al. 2004), and a multi-compartmental model (Kuilenburg et al 2012). From the one-compartmental models, Malothu et al (2010) and Climente (2002) used first-order elimination. Etienne et al (1998) described the model with zero-order (linear) elimination, however, the proportional error model was chosen. Only Bressolle et al. (1999) used an experimental model similar to ours, the linear elimination and additive residual error. To evaluate the pharmacokinetic parameters, a comparison was made between CL and $V_D$ (Porta et al. 2013; Bressolle et al 1999; Mueller et al. 2013; etc.) values with other references (Tab. 1, 2). Comparing the closest models (one-compartment, linear elimination) with our study: Bressolle et al (1999) published the CL value 99,2 [L/h] and $V_D$ 17,9 [L]. Etienne et al. (1998) published only CL with value 235 [L/h]. Our results: CL 214 [L/h] and $V_D$ 17,9 [L] plausibly relate to these two as well as to other references. Therefore the model can be considered as useful, however further investigation on other compartmental models shall be executed. Interestingly, Porto et al. (2004) investigated both the one-compartmental and two-compartmental model for 5-FU. In contrast to the present study, where the two compartmental model was not better, their two-compartmental pharmacokinetic model described the patient data significantly better than the one-compartmental model, as the reduction of the ΔOFV obtained was statistically significant. In our case it may be caused by the data, where there was not enough information available at start and after the end of infusion. Additive residual error chosen in our 5-FU PK model was also

used by Malothu et al. (2010); as well as Climente et al. (2002). On the other hand the proportional error model was used by Etienne et al. (1998), Bressole et al. (1999) and Mueller et al. (2013). Surprisingly, there are different error model types used in previously published models, however this does not influence the quality of the model as the patient populations differ significantly from each other.

Age, gender, genetic differences, weight, disease states, concomitant medication and others are well known factors influencing the relationship between dose and steady state level of 5-FU. In our final model only BSA on CL was significant ($p<0.01$) enough to be included as a covariate. Some publications showed no covariates found (Malothu et al. 2010; Porta et al. 2004) and in contrast others showed the following factors as significant: age (Etienne 1998), DPYD mutation (Kuilenburg et al. 2012), gender (Bressolle et al. 1999; Mueller 2013), and weight/ideal body weight (Climente et al. 2002). Surprisingly, Climente et al. (2002) and Etienne et al. (1998) suggests the covariate BSA is not the best predictor of 5-FU CL because BSA fails to standardize the marked inter-patient variation in PK in most cytotoxic drugs. Several interdependent authors correspondingly confirmed that BSA is not significant covariate. The routine use of body surface area for dose calculation of anticancer drugs, at least for 5-FU, should be therefore re-evaluated. The dosing schemes used for 5-FU are routinely normalized by BSA. Approvingly, in the current model the body size was affecting the pharmacokinetic parameters of 5-FU. This situation is not new in the oncology area where different antineoplastic dosing schemes are normalized by body size variables, even when no relationship between body size variables and pharmacokinetic and/or pharmacodynamic parameters have been found. Although BSA is not normally correlated with CL, in our case, BSA was a significant covariate and therefore was incorporated into the final model. Consideration to use a range of fixed doses independent of BSA and based on drug elimination shall be used for adjustments (Porta et al. 2004). Although these conclusions were made in 5-FU short intravenous infusion (less than one hour) they might not be applicable to this case where infusions last from 24 to 121 hours. Depending on the patient population size used for development of the models the covariates may differ resulting in a discrepancy among previously published models. Based on the previous statements further investigation on bigger population sample shall be executed in order to identify the final covariates.

The final limited sampling strategy was chosen to be 2 hours before EOI, because the blood sample taken during the $C_{SS}$ allows adequate prediction of the AUC.

This decision is not to be final, as there are no practical results from clinical routine and therefore it is recommended to reinvestigate the limited sampling strategy to suit the majority of physicians using the PK model approach. For i.v. bolus doses a different approach is necessary as there is no $C_{SS}$ reached during the infusion duration (e.g. 2 min). Kuilenburg et al. (2012) presented the strategy to obtain the sample at 30 or 60 min after the beginning of infusion. Overall, the aim for infusions is to take the blood sample during $C_{SS}$ because it allows adequate prediction of the AUC. In our study, one sample for prediction of the AUC was sufficient.

Comparing the AUC estimated by NONMEM and by Rectangle Equation led to interesting results (tab. 12). Approximately 75% (n = 131) of the AUC estimations were in the same dose adjustment interval. The other 25% (n = 44) were different. One of the reasons was that the AUC estimations were on the boundaries of neighbouring AUC interval change dose recommendations (n = 15). This difference in dose adjustment could be neglected as the change in dose correspondingly decreases or increases and proper interpretation of results ultimately lies with the physician. The second reason was regular differences in estimation of AUC (n = 23). The third reason was several (n = 6) by Rectangle Equation underestimated AUC values (0,22-3,37 [µmol·h/L]), which were in contrast classified by NONMEM as measurement errors (Tab. 11). This clarified that using NONMEM gives the estimation rationale of safety, as the shrinkage effect recognizes outliers as measurement errors.

In conclusion, the pharmacokinetics of 5-FU administered in various intravenous infusion durations were accurately described by the one-compartmental linear model presented, which also provided stable parameters´ estimates. Thus, this model may be used as prior information to get the Bayesian estimate of the patients´ pharmacokinetic parameters. This methodology might be useful to decrease the variability in patients´ 5-FU exposure by dose optimization. The optimization of 5-FU dosing clearly indicates this solution may provide further improvement in chemotherapy treatments for cancer. However the model whose use has high consequences (dosage adjustment, respectively patient´s safety), such as our model, require a great degree of validation. Poorly predictive models may lead to a sub therapeutic response or worse, severe toxicity or death. (Bonate 2011) Therefore, further investigation needs to be executed before this model can be utilized to ensure optimal and individualized 5-FU therapy.

# 6. Conclusion

The aim of this study was to build a PK model for 5-FU to estimate the 5-FU AUC that could be used as a dose adjustment tool to increase the efficacy and tolerability of the medication. Different limited sampling strategies were evaluated.

Ninety patients with the diagnosis of colorectal carcinoma treated with fluorouracil (5-FU) administered at various infusion durations were entered into the study. Several versions of compartmental pharmacokinetic models were fitted to the plasma concentration data, using nonlinear mixed effect modelling (NONMEM). Different error models and the potential effect of patient covariates were evaluated.

The one-compartment linear model was found to best describe the data collected. The final model contained Additive Residual Error. A covariate BSA>CL and IIV on CL were significantly correlated to the pharmacokinetic parameters. The mean parameters´ estimates were: CL 214 [L/h]; V 30,2 [L]; ADR 0,112 [mg/L]; BSA>CL 0,993; CL/Var CL 48,8% and IOV 28,8%. The final model accurately described the pharmacokinetics of fluorouracil (5-FU) administered by infusion.

The study population size might be limiting a limiting factor in properly describing the actual behaviour of 5-FU in the population. Furthermore this model cannot be used in clinical setting as the majority of physicians do not possess the knowledge to use PK-model.

In conclusion the present study provides pharmacokinetic data on 5-FU infusion in various infusion lengths. Properties of the model shall be further investigated, as there are dissimilarities among published models. As a consideration to the future, a study with large patient population should be executed in order to establish a more robust model. Moreover, an appropriate user-friendly tool shall be developed to enable individual pharmacokinetic dose adaptation of fluorouracil in clinical routine to improve therapeutic outcomes along with decreasing occurrence of adverse events.

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# 8. Appendices

## 8.1. Imputed Data

*Tab. 14. Patient data*
*Dur = Duration of infusion; Sex: 0 = female, 1 = male; BSA = Body surface area; Com = Co-medication; Assay = Method of measurement (0= HPLC, 1= immunoassay)*

| #ID | Dose(mg) | Rate (mg/h) | Dur (h) | Sex | Age | BSA | Assay | Com |
|---|---|---|---|---|---|---|---|---|
| | | | **Study population number 1 (24 patients)** | | | | | |
| 1 | 8804,07 | 76,173 | 115,58 | 1 | 68 | 1,79 | 0 | 1 |
| 2 | 6342,96 | 53,932 | 117,61 | 1 | 59 | 1,99 | 0 | 1 |
| 3 | 8856,94 | 74,359 | 119,11 | 1 | 37 | 1,91 | 0 | 0 |
| 4 | 8855,17 | 75,312 | 117,58 | 1 | 46 | 1,98 | 0 | 0 |
| 5 | 7535,4 | 63,44 | 118,78 | 0 | 58 | 1,5 | 0 | 1 |
| 7 | 9004,36 | 75,268 | 119,63 | 1 | 41 | 1,91 | 0 | 1 |
| 9 | 7848,13 | 65,581 | 119,67 | 0 | 50 | 1,59 | 0 | 0 |
| 12 | 7907,88 | 65,97 | 119,87 | 1 | 64 | 1,61 | 0 | 0 |
| 13 | 8929,91 | 74,571 | 119,75 | 1 | 62 | 2,36 | 0 | 0 |
| 14 | 8940,83 | 75,879 | 117,83 | 1 | 50 | 2,12 | 0 | 0 |
| 15 | 7944,23 | 67,899 | 117 | 0 | 60 | 1,59 | 0 | 0 |
| 16 | 8640,33 | 72,81 | 118,67 | 1 | 51 | 1,91 | 0 | 0 |
| 17 | 4688,15 | 39,93 | 117,41 | 0 | 70 | 1,48 | 0 | 0 |
| 18 | 8801,76 | 75,209 | 117,03 | 1 | 61 | 1,99 | 0 | 1 |
| 20 | 8843,28 | 74,608 | 118,53 | 1 | 66 | 1,85 | 0 | 1 |
| 21 | 8860,5 | 75,229 | 117,78 | 1 | 71 | 2,12 | 0 | 0 |
| 22 | 8828,39 | 74,652 | 118,26 | 1 | 66 | 1,95 | 0 | 1 |
| 23 | 8844,7 | 74,4 | 118,88 | 1 | 68 | 1,81 | 0 | 1 |
| 24 | 8877,42 | 74,55 | 119,08 | 1 | 64 | 2,17 | 0 | 0 |
| 25 | 5816,28 | 49,029 | 118,63 | 1 | 70 | 1,8 | 0 | 1 |
| 26 | 8742,91 | 74,3 | 117,67 | 1 | 52 | 1,79 | 0 | 0 |
| 27 | 8368,03 | 69,479 | 120,44 | 0 | 40 | 1,68 | 0 | 0 |
| 28 | 9157,61 | 75,44 | 121,39 | 1 | 51 | 2,05 | 0 | 1 |
| 29 | 7645,05 | 63,719 | 119,98 | 1 | 66 | 2,09 | 0 | 0 |

**Study population number 1 (21 patients)**

| #ID | *Dose(mg)* | *Rate (mg/h)* | *Dur (h)* | *Sex* | *Age* | *BSA* | *Assay* | *Com* |
|---|---|---|---|---|---|---|---|---|
| 31 | 4800 | 105,495 | 45,5 | 1 | 63 | 2,22 | 1 | 2 |
| 32 | 3400 | 154,545 | 22 | 1 | 82 | 1,71 | 1 | 3 |
| 33 | 6320 | 68,696 | 92 | 0 | 56 | 1,58 | 1 | 4 |
| 34 | 7600 | 86,364 | 88 | 1 | 44 | 1,91 | 1 | 4 |
| 35 | 4280 | 190,222 | 22,5 | 1 | 55 | 2,14 | 1 | 5 |
| 38 | 4000 | 173,913 | 23 | 1 | 62 | 2,1 | 1 | 5 |
| 39 | 7400 | 84,091 | 88 | 1 | 74 | 1,85 | 1 | 1 |
| 40 | 2000 | 45,455 | 44 | 0 | 73 | 1,68 | 1 | 3 |
| 41 | 3780 | 164,348 | 23 | 1 | 78 | 1,89 | 1 | 3 |
| 42 | 3220 | 149,767 | 21,5 | 0 | 62 | 1,61 | 1 | 7 |
| 43 | 4020 | 186,977 | 21,5 | 1 | 60 | 2,01 | 1 | 3 |
| 44 | 3140 | 142,727 | 22 | 0 | 69 | 1,57 | 1 | 8 |
| 47 | 5670 | 121,935 | 46,5 | 0 | 48 | 1,89 | 1 | 7 |
| 48 | 3160 | 137,391 | 23 | 0 | 67 | 1,58 | 1 | 3 |
| 49 | 4000 | 177,778 | 22,5 | 1 | 64 | 2,24 | 1 | 3 |
| 51 | 3080 | 128,12 | 24,04 | 0 | 73 | 1,54 | 1 | 3 |
| 53 | 2088 | 49,714 | 42 | 1 | 65 | 1,74 | 1 | 3 |
| 54 | 4160 | 189,091 | 22 | 0 | 78 | 1,6 | 1 | 10 |
| 55 | 2460 | 58,571 | 42 | 1 | 52 | 2,07 | 1 | 3 |
| 56 | 3560 | 195,82 | 18,18 | 0 | 50 | 1,78 | 1 | 11 |
| 57 | 6960 | 74,823 | 93,02 | 0 | 60 | 1,74 | 1 | 12 |

**Study population number 3 (27 patients)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 85 | 4300 | 93,478 | 46 | 1 | 53 | 1,79 | 1 | 3 |
| 86 | 3900 | 84,783 | 46 | 1 | 60 | 1,625 | 1 | 3 |
| 87 | 4680 | 101,739 | 46 | 0 | 60 | 1,95 | 1 | 3 |
| 89 | 4460 | 96,957 | 46 | 1 | 72 | 1,86 | 1 | 3 |
| 91 | 4800 | 104,348 | 46 | 1 | 60 | 2 | 1 | 3 |
| 92 | 4200 | 91,304 | 46 | 0 | 60 | 1,75 | 1 | 3 |
| 93 | 4800 | 104,348 | 46 | 1 | 60 | 2 | 1 | 3 |

| #ID | *Dose(mg)* | *Rate (mg/h)* | *Dur (h)* | *Sex* | *Age* | *BSA* | *Assay* | *Com* |
|---|---|---|---|---|---|---|---|---|
| 94 | 3420 | 74,348 | 46 | 0 | 58 | 1,9 | 1 | 3 |
| 95 | 4350 | 94,565 | 46 | 1 | 65 | 1,81 | 1 | 3 |
| 96 | 3360 | 73,043 | 46 | 1 | 76 | 1,53 | 1 | 3 |
| 97 | 4500 | 97,826 | 46 | 1 | 72 | 1,87 | 1 | 3 |
| 100 | 4500 | 97,826 | 46 | 0 | 29 | 1,83 | 1 | 3 |
| 102 | 4200 | 91,304 | 46 | 1 | 56 | 1,75 | 1 | 3 |
| 103 | 4800 | 104,348 | 46 | 0 | 56 | 2 | 1 | 3 |
| 104 | 5950 | 129,348 | 46 | 1 | 52 | 2,29 | 1 | 3 |
| 106 | 4000 | 86,957 | 46 | 0 | 55 | 1,67 | 1 | 3 |
| 107 | 4460 | 96,957 | 46 | 1 | 57 | 1,86 | 1 | 3 |
| 108 | 4320 | 93,913 | 46 | 1 | 45 | 1,8 | 1 | 3 |
| 110 | 4300 | 93,478 | 46 | 1 | 74 | 1,79 | 1 | 3 |
| 111 | 4000 | 86,957 | 46 | 0 | 55 | 1,67 | 1 | 3 |
| 112 | 5000 | 108,696 | 46 | 1 | 68 | 2,08 | 1 | 3 |
| 114 | 4800 | 104,348 | 46 | 1 | 54 | 1,85 | 1 | 3 |
| 115 | 3600 | 78,261 | 46 | 0 | 72 | 1,8 | 1 | 3 |
| 116 | 5000 | 108,696 | 46 | 1 | 43 | 2,08 | 1 | 3 |
| 117 | 4750 | 103,261 | 46 | 1 | 36 | 2 | 1 | 3 |
| 118 | 4600 | 100 | 46 | 0 | 63 | 1,91 | 1 | 3 |
| 119 | 4600 | 100 | 46 | 0 | 63 | 1,91 | 1 | 3 |
| **Study population number 4 (8 patients)** | | | | | | | | |
| 301 | 3180 | 132,5 | 24 | 1 | 74 | 1,59 | 1 | |
| 302 | 3220 | 134,17 | 24 | 0 | 54 | 1,61 | 1 | 0 |
| 303 | 3640 | 174,02 | 20,917 | 1 | 72 | 1,82 | 1 | 0 |
| 304 | 2960 | 141,51 | 20,917 | 0 | 54 | 1,48 | 1 | 0 |
| 305 | 3340 | 157,79 | 21,167 | 0 | 82 | 1,67 | 1 | 0 |
| 306 | 3400 | 141,18 | 24,083 | 0 | 72 | 1,739 | 1 | 0 |
| 307 | 4000 | 166,67 | 24 | 1 | 50 | 2,18 | 1 | 0 |
| 308 | 3840 | 160 | 24 | 0 | 68 | 1,94 | 1 | 0 |

**Study population number 5 (1 patient)**

| #ID | *Dose(mg)* | *Rate (mg/h)* | *Dur (h)* | *Sex* | *Age* | *BSA* | *Assay* | *Com* |
|---|---|---|---|---|---|---|---|---|
| 401 | 1100 | 45,83 | 24 | 1 | 53 | 2,05 | 1 | 0 |

**Study population number 6 (9 patients)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 501 | 2244 | 47,83 | 46,917 | 1 | 63 | 1,87 | 1 | 0 |
| 505 | 2205 | 13,13 | 168 | 0 | 45 | 1,4 | 1 | 0 |
| 507 | 1800 | 40,45 | 44,5 | 0 | 54 | 1,5 | 1 | 0 |
| 508 | 1020 | 23,45 | 43,5 | 0 | 69 | 1,635 | 1 | 0 |
| 509 | 3560 | 37,28 | 95,5 | 1 | 75 | 1,938 | 1 | 0 |
| 510 | 3800 | 160,56 | 23,667 | 1 | 72 | 1,852 | 1 | 0 |
| 511 | 2244 | 45,8 | 49 | 1 | 62 | 1,87 | 1 | 0 |
| 512 | 1020 | 23,02 | 44,3 | 0 | 69 | 1,656 | 1 | 0 |
| 515 | 4800 | 102,13 | 47 | 1 | 66 | 2,131 | 1 | 0 |

## 8.2. Sampling Strategies´ Results for 24 hour infusion

*Tab.15. Limited sampling strategies comparison for 24 hour infusion*

| | | Ref 24h-Inf | 1 hour | 2 hours | 2h bef EOI | EOI | 1h, 2h bef EOI | 1h EOI | 1h, 30 min af EOI | 5 min after EOI | 15 min after EOI | 30 min after EOI | 60 min after EOI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Median AUC [mg·h/L]** | | 16,70 | 16,69 | 16,69 | 16,69 | 16,69 | 16,68 | 16,68 | 16,69 | 16,71 | 16,68 | 16,69 | 16,52 |
| **5th Percentile [mg·h/L]** | | 7,157 | 8,811 | 8,811 | 8,811 | 8,811 | 8,139 | 8,139 | 8,811 | 9,754 | 13,29 | 14,87 | 14,81 |
| **95th Percentile [mg·h/L]** | | 39,47 | 38,73 | 38,92 | 38,93 | 38,93 | 39,16 | 39,16 | 38,91 | 38,90 | 38,62 | 37,77 | 19,44 |
| **Bias absolute** | MPE [mg·h/L] | - | 0,16 | 0,21 | 0,22 | 0,22 | 0,12 | 0,12 | 0,21 | 0,39 | 1,08 | 0,71 | -1,94 |
| | ME [mg·h/L] | - | 0,01 | 0,01 | 0,01 | 0,01 | 0,00 | 0,00 | 0,01 | 0,01 | 0,04 | 0,11 | 0,12 |
| **Bias relativ** | MPE [%] | - | 3,96% | 4,08% | 4,09% | 4,09% | 2,39% | 2,39% | 4,08% | 6,65% | 16,28% | 20,85% | 15,05% |
| | ME [%] | - | 0,06% | 0,06% | 0,06% | 0,06% | 0,03% | 0,03% | 0,06% | 0,08% | 0,24% | 0,64% | 0,76% |
| **Precision absolute** | RMSE [mg·h/L] | - | 0,80 | 0,74 | 0,74 | 0,74 | 0,43 | 0,43 | 0,74 | 1,13 | 2,67 | 4,98 | 9,89 |
| | Absolute ME [mg·h/L] | - | 0,45 | 0,41 | 0,41 | 0,41 | 0,21 | 0,21 | 0,41 | 0,48 | 0,99 | 3,36 | 5,39 |
| **Precision relative** | RMSE [%] | - | 10,72% | 10,70% | 10,70% | 10,70% | 6,50% | 6,50% | 10,70% | 17,56% | 38,58% | 55,73% | 63,02% |
| | Absolute ME[%] | - | 1,88% | 1,53% | 1,53% | 1,53% | 0,79% | 0,79% | 1,53% | 1,77% | 4,42% | 17,02% | 33,45% |
| **$R^2$** | | 1,00 | 0,9985 | 0,9980 | 0,9979 | 0,9979 | 0,9993 | 0,9993 | 0,9980 | 0,9944 | 0,9637 | 0,8144 | 0,2353 |

*ME = Median prediction error; MPE = Mean prediction error; RMSE = Root mean squared prediction error*

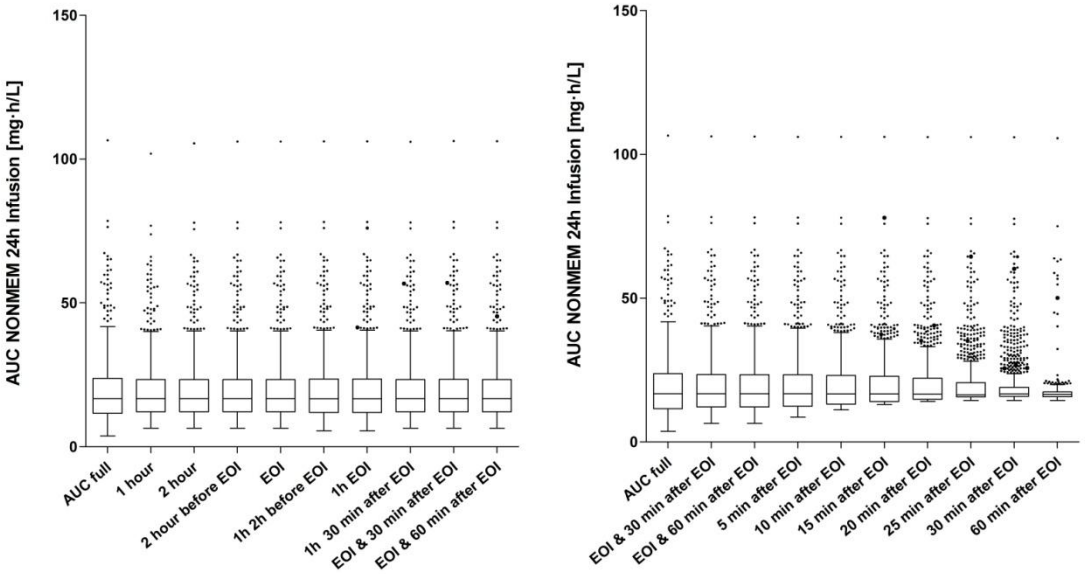


*Fig. 14. AUC Tukey box plots: All sampling strategies*
*AUC Full – using full data set; 1h 2h before EOI (2 samplings) – 1 hour & 2 hours before EOI, 1h EOI (2 samplings) – 1 hour & EOI, 1h 30 min after EOI (2 samplings) – 1 hour & 30 min after EOI*

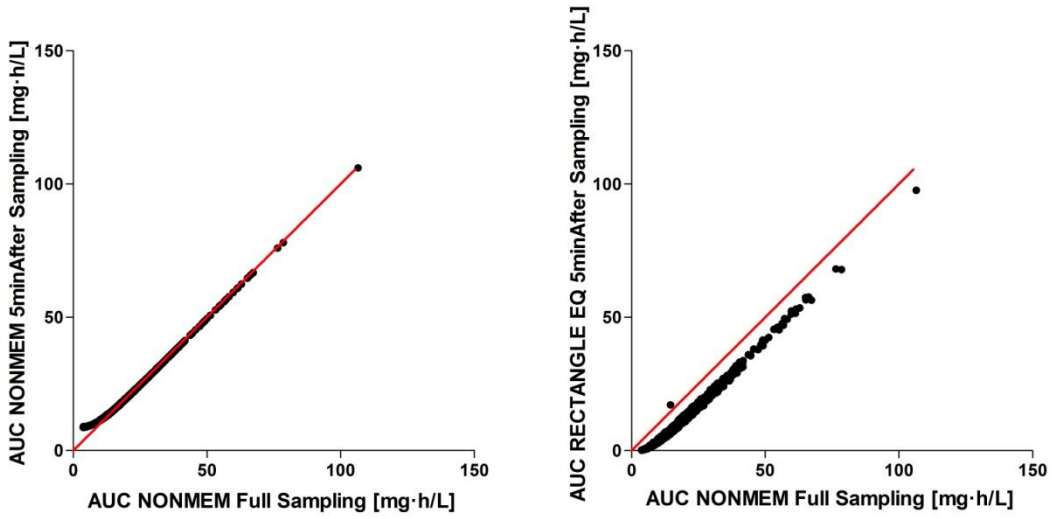


*Fig. 15. AUC calculated with a sampling of 5 minutes after end of infusion compared to AUC calculated with a full sampling.Left figure: AUC calculated with NONMEM using a sampling of 5 minutes after end of infusion; Right figure: AUC calculated with rectangle equation using a sampling of 5 minutes after end of infusion*
*5minAfter – 5 minutes after EOI; Sampling – Limited Sampling dataset*

## 8.3. Analysis Code

```
$PROBLEM 94 FU-PK-Model

$INPUT ID CYC TIME AMT RATE DUR DURA IR DV MDV EVID BSA SEX AGE
ASY STU OCC COMED DOS REGIMEN

$DATA FINAL_Data.csv

$SUBROUTINES ADVAN1 TRANS2

$PK
;STATISTIC MODEL
OCC0=0
OCC1=0
OCC2=0
OCC3=0
OCC4=0
OCC5=0
IF(OCC.EQ.0) OCC0=1
IF(OCC.EQ.1) OCC1=1
IF(OCC.EQ.2) OCC2=1
IF(OCC.EQ.3) OCC3=1
IF(OCC.EQ.4) OCC4=1
IF(OCC.GE.5) OCC5=1
IOV=OCC0*ETA(2)+OCC1*ETA(3)+OCC2*ETA(4)+OCC3*ETA(5)+OCC4*ETA(6
)+OCC5*ETA(7)

;Structural Model
TVCL=THETA(1)*( 1 + THETA(4)*(BSA - 1.80))
CL = TVCL * EXP(ETA(1)+IOV)
TVV=THETA(2)
V  = TVV
S1 = V
AUC  = AMT/CL

$ERROR

IPRED = F+0.000001
IRES = DV-IPRED
W =THETA(3)
Y    = IPRED+W*EPS(1)
DEL  = 0
IF(W.EQ.0) DEL = 1
IWRES = IRES/(W+DEL)
```

```
$THETA
(0, 214) ; Clearance
(0, 30.2) ; Volume of distribution
0.112 ; additive residual error
0.933  ; Covariate BSA on CL

$OMEGA
0.214  ; 1 OM_CL / VARIANCE IN CL
$OMEGA BLOCK(1) 0.0796 ; 3 OM_IOV OF CYC ON CL
$OMEGA BLOCK(1) SAME    ; 4 OM-IOV OF CYC ON CL
$OMEGA BLOCK(1) SAME    ; 5 OM-IOV OF CYC ON CL
$OMEGA BLOCK(1) SAME    ; 6 OM-IOV OF CYC ON CL
$OMEGA BLOCK(1) SAME    ; 7 OM-IOV OF CYC ON CL
$OMEGA BLOCK(1) SAME    ; 8 OM-IOV OF CYC ON CL

$SIGMA
1 FIX

$EST METHOD=1 INTER MAXEVAL=1000 NOABORT SIG=3 PRINT=1 POSTHOC
;$COV SLOW UNCONDITIONAL MATRIX=S

$TABLE ID CYC TIME DV MDV EVID IPRED IWRES CWRES REGIMEN ASY ONEHEADER NOPRINT FILE=sdtab0094
$TABLE ID CYC TIME AMT DV CL V AUC ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ASY ONEHEADER NOPRINT FILE=patab0094
$TABLE ID TIME BSA AGE ONEHEADER NOPRINT FILE=cotab0094
$TABLE ID TIME SEX ONEHEADER NOPRINT FILE=catab0094
```